AACR International Conference

NEW HORIZONS IN CANCER RESEARCH

May 3-5, 2019 | Sheraton Shenzhen Futian Hotel | Shenzhen, China

Conference Cochairs

Elizabeth M. Jaffee, MD
Johns Hopkins Sidney Kimmel
Comprehensive Cancer Center, Baltimore, MD, USA

Hong Wu, MD, PhD
School of Life Science, Peking University,
Beijing, China

Program and Proceedings

AACR American Association for Cancer Research FINDING CURES TOGETHER

PEKING UNIVERSITY 1898

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1811022O

2019 New Horizons in Cancer Research Conference Attendees:

加入AACR，一同在全球攻克癌症

THE ESSENTIAL ASSOCIATION FOR YOU. JOIN TODAY!

AACR Membership

Special Invitation

The American Association for Cancer Research (AACR) is pleased to extend a Special Invitation to **2019 New Horizons in Cancer Research** conference attendees who are not members of the AACR to join the AACR and to work with multidisciplinary specialists from the global cancer community and discuss up-to-date findings to reduce the impact of cancer on the international level. Nonmember individuals who join are eligible for:

- **FREE** AACR membership through December 31, 2019
- No nominators required for submission

Accepting Membership Invitation

To take advantage of this amazing offer, simply submit a completed AACR membership application at the following link: **AACR.org/Membership/NEWHRZ19** and email a copy of the application along with your most recent curriculum vitae and bibliography to membership@aacr.org by **December 15, 2019**. Look for an email from AACR Membership with additional details on this offer.

For more information about AACR membership, please visit the AACR Exhibit Booth at the Ballroom 1 and 2 Foyer, 6F.

Already a Member?

Be sure to visit myAACR.org to pay your membership dues, register for future AACR annual meetings and conferences, take advantage of early access to housing reservations for annual meetings, or update your Membership Profile.

A Message from

Margaret Foti, PhD, MD (hc)
Chief Executive Officer
American Association for Cancer Research (AACR)

Defeating the global scourge of cancer will require a global effort. The AACR is on the front lines of this fight. Our membership spans 120 countries and we have longstanding partnerships with cancer research organizations around the world to help facilitate the innovative international collaborations we need to achieve the scientific breakthroughs that will lead to future cures.

JOIN US IN OUR MISSION. BECOME A MEMBER TODAY!

Please contact AACR Membership with any questions:
215-440-9300 Telephone | 267-765-1078 Fax
membership@aacr.org

615 Chestnut St., 17th Floor | Philadelphia, PA 19106-4404

AACR American Association for Cancer Research®
FINDING CURES TOGETHER®

www.AACR.org/Membership

TABLE OF CONTENTS

PROGRAM COMMITTEE

COCHAIRS

Elizabeth M. Jaffee, MD
Johns Hopkins Sidney Kimmel Comprehensive Cancer Center
Baltimore, MD, USA

Hong Wu, PhD
School of Life Science, Peking University
Beijing, China

MEMBERS

Tak W. Mak, PhD
Campbell Family Institute for Breast Cancer Research, University of Toronto Health Network
Toronto, ON, Canada

Mao Mao, MD, PhD
SeekIn, Inc.
Shenzhen, China

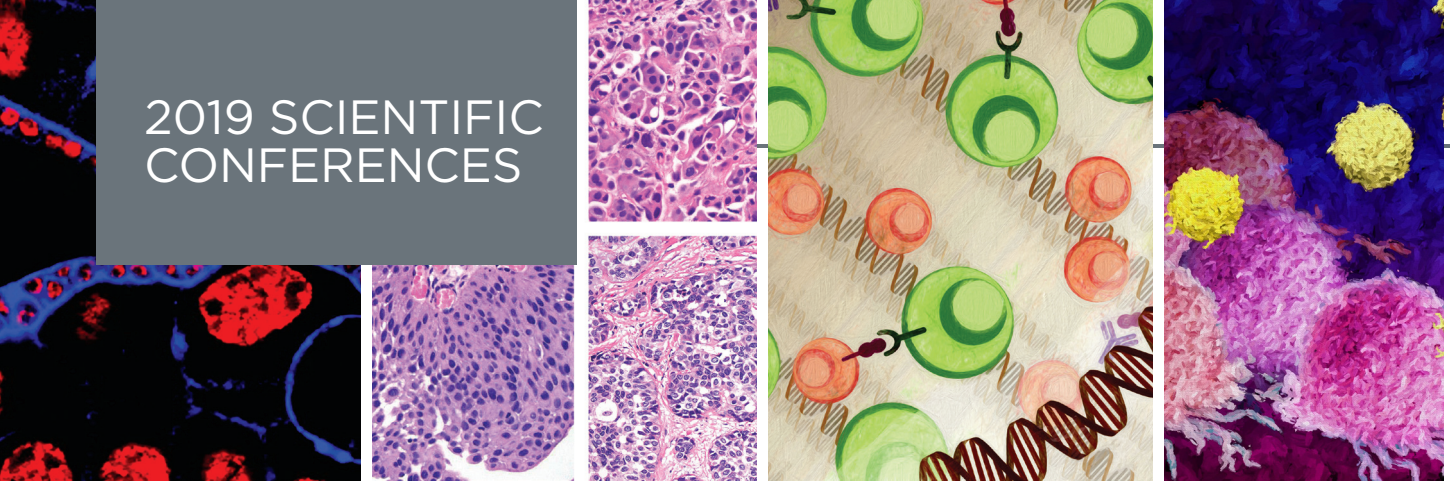
Hongyang Wang, MD, PhD
National Center for Liver Cancer
Shanghai, China

Zhen Yang, PhD
Peking University
Shenzhen, China

ABOUT THE AACR

Founded in 1907, the American Association for Cancer Research (AACR) is the world's oldest and largest professional organization dedicated to advancing cancer research and its mission to prevent and cure cancer. AACR membership includes 38,000 laboratory, translational, and clinical researchers; population scientists; other health care professionals; and cancer advocates residing in 108 countries. The AACR marshals the full spectrum of expertise of the cancer community to accelerate progress in the prevention, biology, diagnosis, and treatment of cancer by annually convening more than 25 conferences and educational workshops, the largest of which is the AACR Annual Meeting with over 21,000 attendees. In addition, the AACR publishes eight peer-reviewed scientific journals and a magazine for cancer survivors, patients, and their caregivers. The AACR funds meritorious research directly as well as in cooperation with numerous cancer organizations. As the Scientific Partner of Stand Up To Cancer, the AACR provides expert peer review, grants administration, and scientific oversight of team science and individual grants in cancer research that have the potential for near-term patient benefit. The AACR actively communicates with legislators and policymakers about the value of cancer research and related biomedical science in saving lives from cancer.

2019 SCIENTIFIC CONFERENCES



The Hippo Pathway: Signaling, Cancer, and Beyond
Conference Cochairs: Fernando Camargo, Anwesha Dey, and Kun-Liang Guan
May 8-11, 2019 | San Diego, CA

Bladder Cancer: Transforming the Field
Conference Cochairs: Charles G. Drake, Jason A. Efstathiou, Donna E. Hansel, Dan Theodorescu, and Ellen C. Zwarthoff
May 18-21, 2019 | Denver, CO

International Conference on Malignant Lymphoma (ICML)
Local Organizing Committee Chair: Michele Ghielmini
June 18-22, 2019 | Lugano, Switzerland

Environmental Carcinogenesis: Potential Pathway to Cancer Prevention
Conference Cochairs: Margaret L. Kripke, Ernest T. Hawk, and Timothy R. Rebbeck
June 22-24, 2019 | Charlotte, NC

Immune Cell Therapies for Cancer: Successes and Challenges of CAR T Cells and Other Forms of Adoptive Therapy
Conference Cochairs: Crystal L. Mackall and Patrick Hwu
July 19-22, 2019 | San Francisco, CA

Pancreatic Cancer: Advances in Science and Clinical Care
Conference Cochairs: Dafna Bar-Sagi, Luis A. Diaz, Elizabeth M. Jaffee, Ben Z. Stanger, and Brian M. Wolpin
September 6-9, 2019 | Boston, MA

Advances in Ovarian Cancer Research
Conference Cochairs: Carol Aghajanian, David D. L. Bowtell, George Coukos, Alan D. D'Andrea, and Karen H. Lu
September 13-16, 2019 | Atlanta, GA

Advances in Pediatric Cancer Research
In association with the AACR Pediatric Cancer Working Group (PCWG)
Conference Cochairs: Crystal Mackall, David Malkin, Stefan Pfister, and Kimberly Stegmaier
September 17-20, 2019 | Montreal, Quebec, Canada

12th AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
Conference Chair: Laura Fejerman
Conference Cochairs: Phyllis Pettit Nassi, Mariana Stern, Clayton Yates, Sandi L. Pruitt, and Smita Bhatia
September 20-23, 2019 | San Francisco, CA

Fifth CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference: Translating Science into Survival
Conference Cochairs: Christoph Huber, Guido Kroemer, Ellen Puré, and Giorgio Trinchieri
September 25-28, 2019 | Paris, France

Cancer Research UK-AACR Joint Conference: Engineering and Physical Sciences in Oncology
Conference Cochairs: Sangeeta N. Bhatia, Kevin M. Brindle, Joe W. Gray, and Molly Stevens
October 15-17, 2019 | London, England

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
Organizing Committee Cochairs: Elizabeth M. Jaffee, James H. Doroshow, and Denis A. Lacombe
Scientific Committee Cochairs: William R. Sellers, James L. Gulley, and Emiliano Calvo
October 26-30, 2019 | Boston, MA

AACR International Conference: Infection and Cancer
Conference Chair: Tak W. Mak
Conference Cochairs: Anthony T. C. Chan, Suet Yi Leung, Douglas R. Lowy, and Andrew X. Zhu
November 8-10, 2019 | Hong Kong

Tumor Immunology and Immunotherapy
Conference Cochairs: Timothy A. Chan, Charles G. Drake, Marcela V. Maus, and Arlene H. Sharpe
November 17-20, 2019 | Boston, MA

San Antonio Breast Cancer Symposium
Codirectors: Carlos L. Arteaga, Virginia G. Kaklamani, and C. Kent Osborne
December 10-14, 2019 | San Antonio, TX

WELCOME

Dear Colleagues,

On behalf of the American Association for Cancer Research (AACR), we have the great pleasure to welcome you to Shenzhen, China, for the AACR New Horizons in Cancer Research Conference.

This year, we are honored that Peking University will be a collaborating partner for the conference. The AACR has presented several successful broad-based conferences in Shanghai over the past few years, and we are very excited to hold this year's program in Shenzhen and appreciate your participation this week.

This year's program has attracted 300 attendees from 15 different countries and features sessions that appeal to a wide audience (genomics, immunotherapy, drug development, etc.) as well as sessions focused on disease sites with high prevalence in the region (lung cancer, nasopharyngeal carcinoma, liver cancer, etc.).

The scientific program features a mix of keynote lectures, plenary sessions, focused concurrent sessions, poster sessions, and satellite symposia. The interactive three-day program features thought leaders from around the globe and will appeal to clinical investigators and translational researchers from all career levels.

We would like to thank the Program Committee Members—Drs. Tak W. Mak (Campbell Family Institute for Breast Cancer Research), Mao Mao (SeekIn, Inc.), Hongyang Wang (National Center for Liver Cancer), and Zhen Yang (Peking University) —for their time and expertise in developing this year's scientific program.

We are confident that you will find this to be an engaging and insightful program that provides a unique opportunity to network with colleagues and forge collaborations that will bring us all closer to our unified mission to prevent and cure cancer.

Sincerely,

Elizabeth M. Jaffee, MD
AACR Past President, Program Committee Cochair
Johns Hopkins Sidney Kimmel
Comprehensive Cancer Center
Baltimore, MD, USA

Hong Wu, MD, PhD
Program Committee Cochair
School of Life Science
Peking University
Beijing, China

PROGRAM COMMITTEE COCHAIRS

Elizabeth M. Jaffee, MD, FAACR

President, AACR (2018-2019)
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Baltimore, MD, USA

Elizabeth M. Jaffee, MD, is deputy director of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins in Baltimore; associate director of the Bloomberg~Kimmel Institute for Cancer Immunotherapy at Johns Hopkins; the Dana and Albert "Cubby" Broccoli Professor of Oncology; Professor of Pathology at the Johns Hopkins University School of Medicine; active staff in oncology at the Johns Hopkins Hospital; Associate Director for Translational Research, Codirector of Gastrointestinal Cancer and Diseases program, and Codirector of the Skip Viragh Center for Pancreas Cancer Clinical Research and Patient Care; and member of the faculty, Graduate Programs in Immunology, Cellular and Molecular Medicine, and Pharmacology, at the Johns Hopkins University School of Medicine.

Jaffee is chair (2016-present) and member (2013-present) of the National Cancer Advisory Board for the National Cancer Institute (NCI) and cochair of the NCI Blue Ribbon Panel for the National Cancer Moonshot Initiative (2016-present).

An active member of the AACR, Jaffee was elected to the Board of Directors (2013-2016). Her contributions to the AACR have been vast and far-reaching. She served as a member of the Organizing Committee of the AACR-LACOG Translational Cancer Medicine Conference in Brazil (2017), and other posts include cochair (2015, 2017) and member (2006) of the Annual Meeting Program Committee; member of the NextGen Transformative Grants Scientific Review Committee (2015-2017); member of the CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference Organizing Committee (2015-2016); member of the Publications Committee (2014-2017); member of the *Cancer Today* Editorial Advisory Board (2014-present); chair (2012-2014) and member (2011, 2012) of the Pancreatic Cancer Action Network-AACR Innovative Grants Scientific Review Committee; deputy editor of the AACR journal *Cancer Immunology Research* (2012-2015); chair (2012-2013) and member (2009-present) of the Cancer Immunology Working Group; member of the AACR-Princess Takamatsu Memorial Lectureship Award Selection Committee (2012); and cochair of the Special Conferences "Tumor Immunology: Multidisciplinary Science Driving Basic and Clinical Advances" (2012) and "Tumor Immunology: Basic and Clinical Advances" (2010).

She has also been a member of the AACR Team Science Award Selection Committee (2011-2012, 2015-2017); member of the Tumor Microenvironment Working Group (2009-present); member of the Pancreatic Cancer Research Grants Scientific Review Committee (2009-2010); member of the AACR-Gertrude B. Elion Cancer Research Award Scientific Review Committee (2009); faculty for the Scientist↔Survivor Program at the Annual Meeting (2009); member of the Science Education Committee (2008-2011); cochair of the Mentored Grants and Research Fellowships Committee (2008); faculty of the educational workshop "Methods in Clinical Cancer Research" (2008, 2009); member of the Annual Meeting Education Committee (2007, 2017); member of the Kirk A. Landon AACR Prize for Basic Cancer Research Selection Committee (2006); member of the Clinical and Translational Cancer Research Committee (2005-2008); faculty of the AACR Educational Workshop "Molecular Biology in Clinical Oncology" (2005); member of the Science Policy and Legislative Affairs Committee (2003-2014); associate editor of Cancer Research (2003-2005); member of the Clinical Cancer Research editorial board (2003-2005); member of the Women in Cancer Research Council; member of the AACR-Women in Cancer Research Charlotte Friend Memorial Lectureship Award Selection Committee (2002, 2007); and a member of the Molecular Cancer Therapeutics editorial board (2001-2004).

Jaffee is the leader of the Stand Up To Cancer (SU2C)-Lustgarten Foundation Dream Team: Transforming Pancreatic Cancer to a Treatable Disease. The AACR is the Scientific Partner of SU2C.

Jaffee has received many honors and awards. She was recognized with the AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Cancer Research (2015), the Johns Hopkins University Office of Women in Science and Medicine Vice Dean's Award (2012), the Spore Program Investigator of the Year from the NCI (2006), the Director's Award for Outstanding Teaching from the Johns Hopkins University School of Medicine (1998, 1999, 2001), a Career Development Award from the National Kidney Foundation (1992), and the Physician-Scientist Award from the National Institutes of Health (1992).

Additionally, Jaffee has been a member of the International Society for Biological Therapy of Cancer's board of directors (2008-2011) and the NCI's board of scientific counselors (2005-2008).

Jaffee's previously held positions include Deputy Director of the Institute for Translational and Clinical Research at Johns Hopkins University School of Medicine (2007-2015); Medical Director of the Johns Hopkins Oncology Center, Cell Processing and Gene Therapy Facility (1992-2014); Codirector of Immunology (2006-2011) and Chair of the Clinical Research Committee (2004-2005) at the Sidney Kimmel Comprehensive Cancer Center; Associate Professor of Pathology (1999-2002), Associate Professor of Oncology (1997-2002), and Assistant Professor of Oncology (1992-1997) at Johns Hopkins University School of Medicine.

Jaffee graduated magna cum laude from Brandeis University in Waltham, Massachusetts, and received her medical degree from New York Medical College in Valhalla. For her postdoctoral training, Jaffee was a senior clinical fellow in oncology at the Johns Hopkins Hospital (1989-1992); senior clinical/research fellow in oncology at the Johns Hopkins University School of Medicine (1989-1992); and research fellow (1988-1989), and intern and resident (1985-1988) in the Department of Medicine at the University of Pittsburgh's Presbyterian-University Hospital. She joined the faculty at Johns Hopkins in 1992.

Hong Wu, MD, PhD

School of Life Science, Peking University
Beijing, China

Dr. Hong Wu is Professor and Dean of School of Life Science, and Senior Investigator of Peking-Tsinghua Center for Life Sciences and Beijing Advanced Innovation Center for Genomics, Peking University.

Before returning to China, Dr. Hong Wu was David Geffen Chair Professor of Molecular Medicine and Director of Institute for Molecular Medicine, David Geffen School of Medicine at UCLA. Dr. Wu received her medical training from Beijing Medical College, China, and PhD degree in Biological Chemistry from Harvard Medical School. After postdoctoral training as a Damon Runyon-Walter Winchell postdoctoral fellow at the Whitehead Institute for Biomedical Research, MIT, she joined UCLA as a faculty member. A major research focus of Dr. Wu's laboratory is to study the molecular mechanism of PTEN tumor suppressor-controlled tumorigenesis. By generating tissue-specific PTEN deficient animal models, Dr. Wu's laboratory elucidated the important role of PTEN in regulating stem cell self-renewal, proliferation, and survival, as well as its roles in controlling the PI3K pathway. These models have been used for preclinical studies of new therapeutic agents and for identifying biomarkers for human cancers. Dr. Wu was an elected fellow of American Association for the Advancement of Science (AAAS) in 2011 and member of European Molecular Biology Organization (EMBO) in 2016.

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Peking University Shenzhen Graduate School

省部共建肿瘤化学基因组学国家重点实验室
State Key Laboratory of Chemical Oncogenomics

深圳湾实验室
Shenzhen Bay Laboratory

GOLD SPONSOR

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The AACR would like to thank the following organizations for their endorsement of this conference:

Media Partners

GENERAL INFORMATION

The Conference will be held at the 5-star facilities of the Sheraton Shenzhen Futian Hotel located in the heart of Shenzhen Central Business District.

Website: sheraton.com/Shenzhen

Telephone: +86-755-8383-8888

Fax: +86-755-8383-8998

Address: Great China International Exchange Square, Fuhua Road, Futian District Shenzhen 518048, P.R. China

CONFERENCE REGISTRATION

Registration will be held in the Hotel Lobby, Level 1, on the following schedule:

Friday, May 3	15:00-20:00
Saturday, May 4	07:30-18:45
Sunday, May 5	07:30-17:00

LOCATION OF OFFICES AND SESSION ROOMS

AACR Office: Song 2, Level 6

Tea Breaks: Ballroom Foyer, Level 6

Conference Registration: Hotel Lobby, Level 1

Exhibits: Ballroom Foyer, Level 6

Plenary Sessions: Ballroom 1 and 2, Level 6

Concurrent Sessions: Ballroom 1 and 2, Ballroom 3, Level 6

Poster Sessions: Han, Level 6

Speaker Preparation Room: Ming, Level 6

CERTIFICATES OF ATTENDANCE AND RECEIPTS

Certificates of attendance and receipts for conference registration fees are available at the conference registration desk beginning Sunday, May 5.

POSTER SESSIONS

Poster Sessions will be held in Han, Level 6, on the following schedule:

Session A

Saturday, May 4 13:00-15:45

Session A presenters must stand by their posters for at least 70 minutes during the session.

Session B

Sunday, May 5 12:00-13:45

Session B presenters must stand by their posters for at least 50 minutes during the session.

Presenters from Session A are encouraged to put up their posters between 11:00-12:00 on Saturday, May 4. Presenters from Session B are encouraged to put up their posters between 10:00-11:45 on Sunday, May 5. Posters should be taken down immediately following the conclusion of the scheduled poster session.

RECEPTIONS AND MEALS

Opening Reception: All registrants are invited to attend the Opening Reception on Friday, May 3, from 19:30 to 21:00 in the Ballroom Foyer, Level 6.

Poster Session: A buffet luncheon will be set up in Han, Level 6, for Poster Sessions A and B on the following schedule:

Saturday, May 4 13:00-15:00

Sunday, May 5 12:00-13:45

Tea Breaks: All breaks will be held in the Ballroom Foyer, Level 6, on the following schedule:

Saturday, May 4 10:00-10:20, 17:15-17:30

Sunday, May 5 10:00-10:30, 15:15-15:30

SPEAKER PREPARATION ROOM

The Speaker Preparation Room is in Ming on Level 6. All speakers are required to check in at this room on the day prior to their presentations. The Speaker Preparation Room will be open on the following schedule:

Friday, May 3 10:00-20:00

Saturday, May 4 07:30-18:45

Sunday, May 5 07:30-17:00

EXHIBITS

The AACR International Conference New Horizons in Cancer Research Exhibit Show will be held in the Ballroom Foyer, Level 6. Representatives from the exhibiting companies will be in the booths during the following times:

Friday, May 3 15:00-21:00

Saturday, May 4 08:00-18:00

Sunday, May 5 08:00-17:00

CHEMISTRY IN CANCER RESEARCH WORKING GROUP (CICR) TOWN HALL MEETING AND LUNCHEON

The CICR Town Hall will be held in Ballroom 3, Level 6, on Sunday, May 5, 12:45-13:45. Come join your fellow researchers for an informative program and lunch. We look forward to seeing you there!

SOCIAL MEDIA

While we encourage your use of social media in and around AACR conferences, we remind you to adhere to the AACR's social media guidelines and accepted social media etiquette. Please be aware of the following guidelines:

- Please refrain from sharing details of any data presented, as this may preclude subsequent publication of the data in a scholarly journal. Follow rules about data sharing.
- While engaging in conversations, please be tolerant of differences of opinion that you may encounter. Keep criticism constructive, and refrain from personal attacks.

MEETING POLICIES AND PROCEDURES

- **Photography and Social Media Policies**
  - **Photography.** Conference attendees may take photographs during oral or poster presentations provided that the photographs are strictly for personal, noncommercial use and are not to be published in any form. Attendees are prohibited from using flash photography or otherwise distracting the presenters or members of the audience.
  - **Social Media.** Conference attendees may share information from presentations on social media provided that they respect the wishes of presenters. Oral presenters may label any or all slides in their presentations with "DO NOT POST." Similarly, poster presenters may label their posters with "DO NOT POST." Attendees must respect the presenters' requests in

these instances and refrain from posting any images from these designated slides or posters on social media.

- In accordance with the Resolution adopted at the 1968 Annual Meeting of the AACR, registrants must refrain from smoking in all meeting rooms. This regulation applies to all session rooms, including the poster area.
- Children under 12 years of age are not permitted in any scientific session or poster session at any time. Children cannot be left unattended or unsupervised.
- Cell phones, pagers, and other electronic devices must be turned off or placed on "silent" mode before entering a session.
- Lost and Found: Attendees may contact the AACR Registration Desk for any lost items.
- Poster presenters are solely responsible for placing their poster on the assigned poster board and removing their poster according to the schedule provided. The AACR cannot be responsible for any posters that are not removed at the designated time. Posters left in the Poster Hall after that time may be discarded.
- Poster presenters should not leave any items at their poster board unattended, including poster tubes, meeting bags, programs, personal items, etc. The AACR is not responsible for any items left in the Poster Hall.

AACR MEMBERSHIP

The AACR has more than 42,000 members in 120 countries and territories around the world. Over 30% of members live outside the United States and Canada, and 20% of the AACR's international members are located in countries with emerging economies. The AACR is a dynamic and vibrant organization that offers its members programs and activities that promote the exchange of timely scientific information, and excellent opportunities to participate more fully in the global conquest of cancer by fostering important relationships and collaborations with cancer scientists internationally. Six categories of membership in the AACR are available to support each aspect of our members' professional development and enhancement in cancer research. The AACR is also eager to support the exchange of knowledge and research with investigators who are located in countries with emerging economies. Significantly reduced membership dues are available for these investigators. Join our mission and apply for AACR membership today!

Special Membership Invitation

The AACR is pleased to extend a special invitation to 2019 New Horizons in Cancer Research conference attendees who are not members to join the AACR and to work with multidisciplinary specialists from the global cancer community and discuss up-to-date findings to reduce the impact of cancer on the international level. Nonmember individuals who join are eligible for free AACR membership through December 31, 2019, and are not required to provide nominators for submission. To take advantage of this offer, simply submit a completed AACR membership application and email a copy of the application along with your most recent curriculum vitae and bibliography to membership@aacr.org by December 15, 2019. For more information about AACR membership, please visit the AACR exhibit booth at Ballroom 1 and 2 Foyer, Level 6, or e-mail membership@aacr.org. Candidates may apply online at AACR.org/Membership/NEWHRZ19.

ASSISTANCE FOR PHYSICALLY CHALLENGED REGISTRANTS

Registrants with special requirements for transportation should notify a member of the AACR staff at the registration desk upon arrival at the conference.

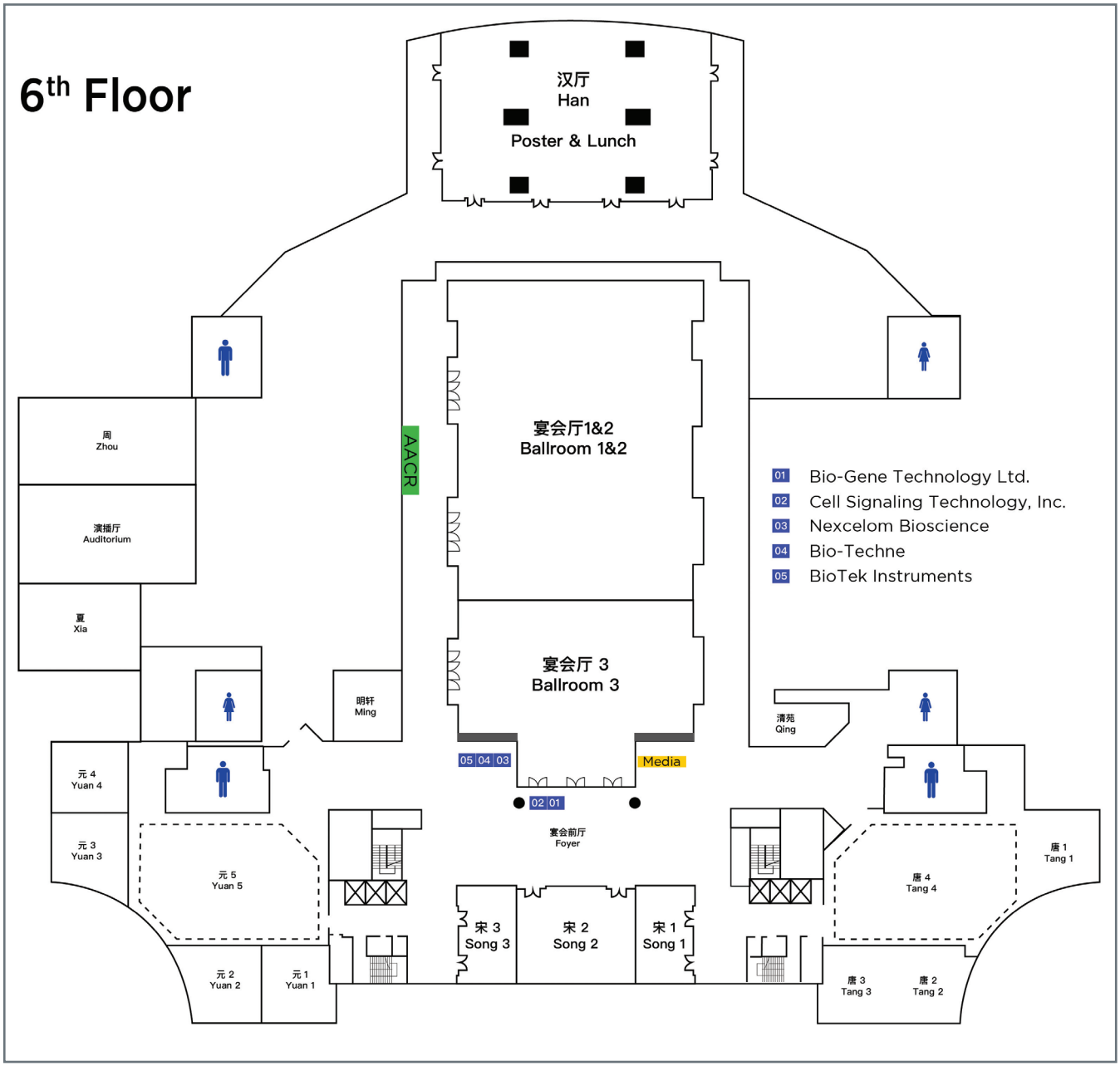
EMERGENCY NUMBERS

First Aid	120
Police	110
Fire Department	119

INTERNET

Free Internet is available in the Hotel Lobby, Level 1. There is limited Internet available in the meeting rooms. To ensure everyone has access, please limit your connection time and only connect one device at a time.

SHERATON SHENZHEN FUTIAN HOTEL FLOOR PLAN



CONFERENCE SCHEDULE

FRIDAY, MAY 3

15:00-20:00 **Registration**
Hotel Lobby, Level 1

18:30-18:45 **Welcome**
Ballroom 1 and 2, Level 6

18:45-19:30 **Keynote Lecture**
Ballroom 1 and 2, Level 6

Session Cochairs:
Elizabeth M. Jaffee, Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA, and **Hong Wu,** School of Life Science, Peking University, Beijing, China

18:45-19:30 **Genome alterations and precision medicine of esophageal squamous-cell carcinoma**
Dongxin Lin, Chinese Academy of Medical Sciences, Beijing, China

19:30-21:00 **Opening Reception**
Ballroom Foyer, Level 6

SATURDAY, MAY 4

8:00-10:00 **Plenary Session 1: Genomics**
Ballroom 1 and 2, Level 6

Session Cochairs:
Mao Mao, SeekIn, Inc., Shenzhen, China, and **Zemin Zhang,** Peking University, Beijing, China

8:00-8:30 **Single-cell analysis of infiltrating immune cells in multiple cancer types**
Zemin Zhang

8:30-9:00 **Genetic basis of immunotherapy efficacy**
Timothy A. Chan, Memorial Sloan Kettering Cancer Center, New York, NY, USA

9:00-9:30 **A comprehensive human gastric cancer organoid biobank that captures molecular diversity and heterogeneity and enables therapeutic screening**
Suet Yi Leung, The University of Hong Kong, Hong Kong

9:30-10:00 **Identifying occult maternal malignancies via low-coverage whole-genome sequencing in 2 million pregnant women**
Mao Mao

10:00-10:20 **Break**

10:20-11:20 **Panel Discussion: FDA Session**
Ballroom 1 and 2, Level 6

Moderator:
Xiaodong Wang, National Institute of Biological Sciences, Beijing, China

Panelists:
Ke Liu, U.S. Food and Drug Administration, Silver Spring, MD, USA

Zengjun Xu, Center for Drug Evaluation, National Medical Products Administration, Beijing, China

11:30-12:45 **Concurrent Sessions 1-2**

CC1: Cancer Epigenetics
Ballroom 1 and 2, Level 6

Session Cochairs:
Nilofer S. Azad, Johns Hopkins Sidney Kimmel Cancer Center, Baltimore, MD, USA, and **Qunying Lei,** Fudan University, Shanghai, China

11:30-11:50 **Nuclear lactate dehydrogenase A senses ROS to produce α-hydroxybutyrate for HPV-induced cervical tumor growth**
Qunying Lei

11:50-12:10 **Epigenome and cistrome-based understanding of prostate cancer susceptibility**
Gonghong Wei, University of Oulu, Oulu, Finland

12:10-12:30 **Harnessing the immunologic modulatory potential of epigenetic therapy**
Nilofer S. Azad

12:30-12:45 **Questions / Panel discussion**

CC2: Lung Cancer
Ballroom 3, Level 6

Session Cochairs:
Bruce Robinson, The University of Western Australia, Perth, Australia, and **Bo Zhu,** Institute of Cancer, Xinqiao Hospital, Third Military Medical University, Chongqing, China

11:30-11:50 **Heterogeneity of tumor immune microenvironment in non-small cell lung carcinoma**
Bo Zhu

11:50-12:10 **Lung cancer plasticity and precision medicine**
Hongbin Ji, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

12:10-12:30 **Neoantigens in pulmonary malignancies—overcoming hurdles to neoantigen immunotherapies**
Bruce Robinson

12:30-12:45 **Questions / Panel discussion**

13:00-15:45 **Poster Session A / Lunch**
Han, Level 6

16:00-17:15 **Concurrent Sessions 3-4**

CC3: Cancer Metabolism
Ballroom 1 and 2, Level 6

Session Cochairs:
Sheng-Cai Lin, Xiamen University School of Life Sciences, Xiamen, China, and **Tak W. Mak,** Campbell Family Institute for Breast Cancer Research, University of Toronto Health Network, Toronto, ON, Canada

16:00-16:20 **Therapeutic implications of altered epigenetics and DNA damage responses in hematologic disorders**
Tak W. Mak

16:20-16:40 **Pkm1 mediates metabolic advantages and promotes cell-autonomous tumor cell growth**
Nobu-hiro Tanuma, Miyagi Cancer Center Research Institute, Natori, Japan

16:40-17:00 **Glucose sensing links to control of AMPK and mTOR**
Sheng-Cai Lin

17:00-17:15 **Questions / Panel discussion**

CC4: Leukemia and Lymphoma
Ballroom 3, Level 6

Session Cochairs:
Xiaojun Huang, Peking University, Beijing, China, and **Eduardo M. Sotomayor,** George Washington University, Washington, DC, USA

16:00-16:20 **Cellular therapy for hematologic malignancy disease in China**
Xiaojun Huang

16:20-16:40 **The evolving landscape of management of multiple myeloma: Immunotherapy and beyond**
James C.S. Chim, Queen Mary Hospital, University of Hong Kong, Hong Kong

16:40-17:00 **Targeted therapy for B-cell lymphomas: Understanding mechanism(s) of resistance to move the field forward**
Eduardo M. Sotomayor

17:00-17:15 **Questions / Panel discussion**

17:15-17:30 **Break**

17:30-18:45 **Concurrent Sessions 5-6**

CC5: New Drug Development
Ballroom 1 and 2, Level 6

Session Cochairs:
Brigette B. Ma, Chinese University of Hong Kong, Hong Kong, and **Zhen Yang,** Peking University, Shenzhen, China

17:30-17:50 **Overcoming endocrine resistance in breast cancer by selective estrogen receptor covalent antagonist**
Ping Zhu, H3 Biomedicine, Cambridge, MA, USA

17:50-18:10 **Novel drug combinations to improve the therapeutic ratio of radiotherapy: leveraging on AI to phenotype profiling**
Melvin L.K. Chua, National Cancer Center Singapore, Singapore

18:10-18:30 **Phase 1 development of new immune-checkpoint inhibitors for solid tumors—lessons learnt and future perspective**
Brigette B. Ma

18:30-18:45 **Questions / Panel discussion**

CC6: Nasopharyngeal Carcinoma
Ballroom 3, Level 6

Session Cochairs:
Anthony T.C. Chan, Chinese University of Hong Kong, Hong Kong, and **Chao-Nan Qian,** Sun Yat-Sen University Cancer Center, Guangzhou, China

17:30-17:50 **From uncovering the mechanisms underlying nasopharyngeal carcinoma metastasis to discovering antimetastasis agents**
Chao-Nan Qian

17:50-18:10 **Pathogenesis and molecular targets of nasopharyngeal carcinoma**
Mu-Sheng Zeng, Sun Yat-Sen University Cancer Center, Guangzong, China

18:10-18:30 **Update on systemic therapy in NPC**
Anthony T.C. Chan

18:30-18:45 **Questions / Panel discussion**

18:45 **Evening Off / Dinner on Own**

SUNDAY, MAY 5

8:00-10:00 **Plenary Session 2: Immunotherapy**
Ballroom 1 and 2, Level 6

Session Cochairs:
Chen Dong, Tsinghua University, Beijing, China, and **Elizabeth M. Jaffee,** Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA

8:00-8:30 **Targeted cellular immunotherapy—lessons learned from acute lymphoblastic leukemia**
Shannon L. Maude, Children's Hospital of Philadelphia, Philadelphia, PA, USA

8:30-9:00 **Turning the heat up on pancreatic cancer: Lessons on overcoming a "cold" immunologic microenvironment**
Elizabeth M. Jaffee

9:00-9:30 **Intrinsic tumor genomic and metabolic factors leading to immunoresistance**
Patrick Hwu, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

9:30-10:00 **New players in cancer immunotherapy—lessons from immune tolerance**
Chen Dong

10:00-10:30 **Break**

10:30-11:45 **Concurrent Sessions 7-8**

CC7: Male-Specific Cancers: New Insights into Advanced Prostate Cancer
Ballroom 3, Level 6

Session Cochairs: Cory Abate-Shen, Columbia University Irving Comprehensive Cancer Center, New York, NY, USA, and **Wei-Qiang Gao,** Shanghai Jiao Tong University, Shanghai, China

10:30-10:50 **Overcome castration-resistant prostate cancer by targeting cancer stem cells, EMT, and immune microenvironment**
Wei-Qiang Gao

10:50-11:10 **Lineage plasticity as a mechanism of resistance in advanced prostate cancer**
Mark A. Rubin, University of Bern, Bern, Switzerland

11:10-11:30 **Investigating prostate cancer metastasis using cross-species systems approaches**
Cory Abate-Shen

11:30-11:45 **Questions / Panel discussion**

CC8: Female-Specific Cancers
Ballroom 1 and 2, Level 6

Session Cochairs:
Mien-Chie Hung, China Medical University, Taichung, Taiwan, and **Hui-Kuan Lin,** Wake Forest University School of Medicine, Winston Salem, NC, USA

10:30-10:50 **New insight for oncogenic Akt signaling and cancer targeting**
Hui-Kuan Lin

10:50-11:10 **Signal transducing long noncoding RNAs regulating cancer biology**
Erwei Song, Sun Yat-Sen University, Guangzhou, China

11:10-11:30 **Marker-guided target therapy, PARP inhibitors, and development of effective immune checkpoint therapy**
Mien-Chie Hung

11:30-11:45 **Questions / Panel discussion**

12:00-13:45 **Poster Session B / Lunch**
Han, Level 6

12:45-13:45 **AACR Chemistry in Cancer Research Working Group (CICR) Town Hall and Luncheon**
Ballroom 3, Level 6

Host:
Zhao-Kui Wan, Royal Society of Chemistry, Lynk Pharmaceuticals, Pudong, Shanghai, China

14:00-15:15 **Concurrent Sessions 9-10**

CC9: New Technologies
Ballroom 1 and 2, Level 6

Session Cochairs:
Janis M. Taube, Johns Hopkins University School of Medicine, Baltimore, MD, USA, and **Fuchou Tang,** Peking University, Beijing, China

14:00-14:20 **Single-cell multiple omics sequencing of human colorectal cancer**
Fuchou Tang

14:20-14:40 **Astronomy accelerates pathology: Multiplex immunofluorescence imaging of the tumor microenvironment**
Janis M. Taube

14:40-14:50 **Short talk from proffered paper**

14:50-15:00 **Short talk from proffered paper**

15:00-15:15 **Questions / Panel discussion**

CC10: Liver Cancer
Ballroom 3, Level 6

Session Cochairs:
Tim F. Greten, National Cancer Institute, Bethesda, MD, USA, and **Hongyang Wang,** National Center for Liver Cancer, Shanghai, China

14:00-14:20 **Involvement of cholesterol in HCC progression**
Hongyang Wang

14:20-14:40 **Translating liver cancer biology: From functional target discovery to academic drug discovery and development**
Lars Zender, University Hospital Tübingen, Tübingen, Germany

14:40-15:00 **Bile acids, the microbiome, and antitumor immunity in HCC**
Tim F. Greten

15:00-15:15 **Questions / Panel discussion**

15:15-15:30 **Break**

15:30-16:45 **Concurrent Sessions 11-12**

CC11: Cancer Prevention
Ballroom 3, Level 6

Session Cochairs:
Adriana Albini, IRCCS MultiMedica, Milan, Italy, and **You-lin Qiao,** Academy of Chinese Medicine, Beijing, China

15:30-15:50 **Precise prevention of cervical cancer in China**
You-lin Qiao

15:50-16:10 **Precision prevention of cancer: From target identification to clinical practice**
Zigang Dong, University of Minnesota Hormel Institute, Austin, MN, USA

16:10-16:30 **Dietary derivatives and repurposed drugs for antiangiogenesis, chemoprevention, and cancer interception**
Adriana Albini

16:30-16:45 **Questions / Panel discussion**

CC12: GI Cancers
Ballroom 1 and 2, Level 6

Session Cochairs:
Jin Gu, Peking University Cancer Hospital, Beijing, China, and **Aida Habtezion,** Stanford University School of Medicine, Palo Alto, CA, USA

15:30-15:50 **Current status of colorectal cancer surgery in China**
Jin Gu

15:50-16:10 **Immune signals in GI inflammation and cancer**
Aida Habtezion

16:10-16:20 **Short talk from proffered paper**

16:20-16:30 **Short talk from proffered paper**

16:30-16:45 **Questions / Panel discussion**

16:45-17:00 **Closing Remarks**
Ballroom 1 and 2, Level 6

17:00 **Departure**

IA05 Identifying occult maternal malignancies via low-coverage whole-genome sequencing in 2 million pregnant women. Mao Mao. SeekIn Inc., Shenzhen, China.

Aneuploidy caused by chromosome instability is a hallmark for most solid tumors. Multiple chromosomal aneuploidies (MCAs) from failed noninvasive prenatal tests (NIPTs) have been reported to be associated with maternal malignancies.

In this study, a cancer risk model called the Cancer Detection Pipeline (CDP) was established by assessing copy number variations from the low-coverage NIPT sequencing data (i.e., 0.1x whole-genome sequencing) of more than 1.93 million pregnant women who underwent NIPTs between January 2016 and December 2017. Of the 639 subjects who tested positive for MCAs in the initial NIPTs, 41 maternal malignant cancer cases were diagnosed with a median follow-up of 399 days (IR, 303-487 days). The 41 cancer patients presented a wide spectrum of cancer types, the most frequent being breast cancer (10 cases), liver cancer (9), and lymphoma (9), at stages II to IV. Application of the CDP model allowed 34 of the 41 (83%) cancer cases to be identified with a positive predictive value (PPV) of 30.1% and specificity of 84.2%. Moreover, combining the CDP model with 8 plasma tumor markers gave PPV of 75.0%.

Taken together, these data suggest that integrating tumor markers into cfDNA (cell-free DNA) analysis is a promising approach for detecting presymptomatic cancers and warrants further investigation in large clinical trials, particularly in populations at high risk of cancer.

IA06 Nuclear lactate dehydrogenase A senses ROS to produce α-hydroxybutyrate for HPV-induced cervical tumor growth. Yuan Liu[1], Ji-Zheng Guo[2], Ying Liu[3], Canhua Huang[1], Qun-Ying Lei[2]. [1]Department of Biotherapy, State Key Laboratory of Biotherapy and Cancer Center, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu, China, [2]Fudan University Shanghai Cancer Center and Cancer Metabolism Laboratory, Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai, China, [3]Department of Pathology, School of Basic Medical Sciences, Shanghai Medical College, Fudan University, Shanghai, China.

It is well known that high-risk human papilloma virus (HR-HPV) infection is strongly associated with cervical cancer, and E7 was identified as one of the key initiators in HPV-mediated carcinogenesis. Here we show that lactate dehydrogenase A (LDHA) preferably locates in the nucleus in HPV16-positive cervical tumors due to E7-induced intracellular reactive oxygen species (ROS) accumulation. Surprisingly, nuclear LDHA gains a noncanonical enzyme activity to produce α-hydroxybutyrate and triggers DOT1L (disruptor of telomeric silencing 1-like)-mediated histone H3K79 hypermethylation, resulting in the activation of antioxidant responses and Wnt signaling pathway. Furthermore, HPV16 E7 knocking-out reduces LDHA nuclear translocation and H3K79 tri-methylation in K14-HPV16 transgenic mouse model. HPV16 E7 level is significantly positively correlated with nuclear LDHA and H3K79 trimethylation in cervical cancer. Collectively, our findings uncover a noncanonical enzyme activity of nuclear LDHA to epigenetically control cellular redox balance and cell proliferation, facilitating HPV-induced cervical cancer development.

IA09 Heterogeneity of tumor immune microenvironment in non-small cell lung carcinoma. Qingzhu Jia, Zhihua Gong, Shuo Huang, Zhongyu Wang, Haixia Long, Bo Zhu. Institute of Cancer, Xinqiao Hospital, Third Military Medical University, Chongqing, China.

Immune checkpoint blockade has delivered unprecedented success in treating non-small cell lung carcinoma in both first-line and second-line treatment to extend overall survival. However, clinical benefits to immunotherapy in the patients with non-small cell lung carcinoma are mixed: clinical response to immunotherapy varies greatly among the patients with different age, tumors with different driver-gene mutations, different anatomic lesion sites, and different loci in the same lesion site. On the other hand, biomarkers based on tumor immune microenvironment such as tumor mutation burden, the expression levels of PD-L1, the extent of T-cell infiltration, and immune signature have been shown to improve efficacy rate. During the last three years, we have employed exome sequencing, transcriptome profilin,g and T-cell repertoire analysis of tumor tissues from the patients in our center, combined with the data analysis from TCGA database, to address the above-mentioned heterogeneity. Our results revealed that immune microenvironment including tumor mutation burden, immune suppressor cell infiltrations, T-cell infiltration, and immune signature differs in patients with different age, driver gene mutations, lesion site, and intratumoral location. Together, these findings suggest that multiple biopsies should be performed in real time to effectively determine tumor immune microenvironment for diagnosis and prognosis.

IA10 Lung cancer plasticity and precision medicine. Hongbin Ji. Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.

Lineage transition in adenocarcinoma (ADC) and squamous cell carcinoma (SCC) of non-small cell lung cancer (NSCLC), as implicated by clinical observation of mixed adenomatous and squamous pathologies in adenosquamous cell carcinoma (Ad-SCC), remains a fundamental yet unsolved

question. We provide in vivo evidence showing Lkb1 loss elicits the ADC-to-SCC transdifferentiation (AST) in three different mouse models. Interestingly, we reveal differential metabolic stress in LKB1-deficient lung ADC and SCC in mouse model, and similar redox heterogeneity in human adenosquamous cell carcinoma (Ad-SCC) with LKB1 deficiency. Mechanistically, pentose phosphate pathway deregulation and inactivation of AMPK-ACC-dependent fatty acid oxidation contribute to stress accumulation independent of autophagy and mTOR activation. In preclinical trials towards cancer metabolism, we find that certain ADC develops drug resistance through squamous transdifferentiation. Recent observations in the clinic further suggest that such histologic transition might be responsible for resistance to tyrosine kinase inhibitor (TKI) therapy and chemotherapy in relapsed EGFR-mutant lung ADC patients. These findings demonstrate that lung cancer plasticity potentially affects therapeutic response and precision medicine through histologic transition.

IA13 Pkm1 mediates metabolic advantages and promotes cell-autonomous tumor cell growth. Nobu-hiro Tanuma. Miyagi Cancer Center Research Institute; Tohoku University, Graduate School of Medicine, Sendai, Japan.

Tumor cells often fail to maximally oxidize glucose, a phenomenon known as the Warburg effect, although the role of this activity in cancer is not fully understood. In the last decade, investigators have been interested in switching of the two isoforms (PKM1 and PKM2) of the glycolytic enzyme PKM, since the discovery that PKM2 is highly expressed in cancer cells and diverts glucose-derived carbons from catabolic to biosynthetic pathways to promote the Warburg effect. However, PKM2 function in tumorigenesis remains controversial, and it is unclear whether PKM2 expression is cancer-promoting or -suppressing.

Relevant to this issue, we recently found that PKM1, rather than PKM2, is tumor-promoting if expressed in mouse models and in some human cancers. Analysis of novel Pkm-mutant mouse lines established that PKM1, rather than PKM2, boosts tumor growth cell-intrinsically. PKM1 activated glucose catabolism without interfering with biosynthetic pathways, conferring malignancy. Importantly, we observed that pulmonary neuroendocrine tumors (NETs), including small-cell lung cancer (SCLC), express PKM1 at high levels and that PKM1 expression is required for SCLC cell proliferation. I will discuss how we can target metabolism of SCLC, one of the deadliest human cancers, for which few druggable mutations have been identified.

References

1. Sato T et al. Mol Cell Oncol 2018;5:e1472054.

2. Morita M et al. Cancer Cell 2018;33:355-67.

IA16 The evolving landscape of management of multiple myeloma: Immunotherapy and beyond. James Chim. Queen Mary Hospital, University of Hong Kong, Hong Kong.

Multiple myeloma (MM) arises from neoplastic proliferation of plasma cells with complications of hypercalcemia, renal failure, anemia, bone pain, and fractures. The advent of novel agents such as proteasome inhibitors (PI) and immunomodulatory agents (IMiD) has markedly improved the complete remission rate and prolonged survival. However, MM remains an incurable disease. Recently, advances in the development of monoclonal antibodies such as daratumumab and elotuzumab have markedly improved progression-free survival (PFS) of relapsed MM. Daratumumab targets CD38 and works by direct induction of apoptosis, Fc-related cytotoxicity, and modulation of the immunosuppressive microenvironment. Efficacy of single-agent daratumumab, as single agent or in combination with lenalidomide (POLLUX) or bortezomib (CASTOR), has been demonstrated in randomized controlled trials (RCT) with impressive hazard ratio. On the other hand, elotuzumab, in combination with lenalidomide/dexamethasone (ELOQUENT-2), has resulted in superior PFS and overall survival (OS) to lenalidomide/dexamethasone. Moreover, next-generation proteasome inhibitors including carfilzomib (ASPIRE) and ixazomib (TOURMALINE-MM1), in combination with lenalidomide and dexamethasone, have also been shown to result in superior PFS to lenalidomide and dexamethasone in RCTs. Moreover, apart from naked monoclonal antibodies, immunotherapy in the form of antibody-drug conjugate targeting B-cell maturation antigen (BCMA) has demonstrated significant efficacy in patients with advanced MM. Furthermore, bispecific T-cell engager (BiTE) targeting BCMA is also under clinical trials in relapsed MM. Finally, chimeric antigen receptor-T cells (CAR-T cells) against BCMA have been shown to be efficacious in advanced MM in the US and China.

IA20 Phase 1 development of new immune-checkpoint inhibitors for solid tumors—lessons learnt and future perspective. Brigette B.Y. Ma. Chinese University of Hong Kong, Hong Kong.

Over 900 immuno-oncology (IO) drugs are currently in clinical development, either as monotherapy or in combination in over 2,000 clinical trials. Many of these trials target overlapping clinical indications, and some of these trials have failed phase III confirmation despite the promising results observed in earlier trials. Moreover, there are a myriad of companion diagnostic kits for PD-L1 expression and lack of harmonization in the way predictive biomarkers are defined. Therefore, a more rational approach to developing IO drugs is needed. This talk will discuss some of the challenges of IO drug development and identify possible solutions to these problems.

IA21 From uncovering the mechanisms underlying nasopharyngeal carcinoma metastasis to discovering antimetastasis agents. Chao-Nan (Miles) Qian. Sun Yat-sen University Cancer Center, Guangzhou, China.

Distant metastasis is the main reason for treatment failure in nasopharyngeal carcinoma (NPC). The underlying mechanism for NPC metastasis is poorly understood. There is no effective drug for preventing or blocking NPC metastasis. We first established and validated high- and low-metastasis NPC cellular clones from the parental cell line CNE2. We then performed whole-genome expression profiling on the cells collected in vitro and in vivo. Among the top 25 upregulated genes and the bottom 25 downregulated genes in high-metastasis cells, most of them have not been characterized yet in terms of regulating cancer progression. By performing a series of functional studies, we have confirmed that serglycin, SPINK6, IL-8, and WNT5A can promote NPC metastasis via both autocrine and paracrine manners. Moreover, SPINK6 is a new ligand for epidermal growth factor receptor (EGFR). We then established a cell-based screening system for identifying antimetastasis compounds by engineering the high- and low-metastasis cells with fluorescent reporter genes that have been transfected into the cells or knocked-in to the cell genome. The screening system was optimized for sufficient fluorescent signals generated from 5000 cells 24 hr after incubation with the compound. The system was further validated by using known agents that could either promote or inhibit cellular motility. Finally, the system was used to screen over 13,000 compounds from two university compound libraries. We have then identified 33 highly efficient antimetastasis lead compounds; some of them been confirmed by in vitro and in vivo function studies. Interestingly, we have also identified 9 lead compounds that can promote cellular motility, which should have a great potential for application in wound healing and coronary collateral circulation establishment. Further preclinical and clinical studies of these lead compounds are warranted.

IA28 Overcome castration-resistant prostate cancer by targeting cancer stem cells, EMT and immune microenvironment. Wei-Qiang Gao. State Key Laboratory of Oncogenes and Related Genes, School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China.

Androgen deprivation therapy (ADT) is a main treatment for prostate cancer (PCa). However, relapse occurs in nearly all patients, and the disease eventually becomes castration resistant. The mechanism underlying castration resistance remains poorly understood. Here we present evidence that cancer stem cells and specific immune cells in the tumor microenvironment are important players in the development of castration-resistant prostate cancer (CRPC). We demonstrated that castration can lead to epithelial-mesenchymal transition (EMT) of the prostate epithelial cancer cells. The CRPC cells possess EMT traits that are associated with prostate stem cell features. Our coupled time lapse imaging and promoter-report system analyses revealed that $hTERT^{high}$ and $Numb^{low}$ prostate cancer cells represent prostate cancer stem cells (CSCs) that can undergo both symmetric and asymmetric divisions, to show their direct, vivid self-renewal characteristics.

In addition, we recently identified a novel subpopulation of $CD4^{low}$ ($HLA\text{-}G^{+}$) T cells that undergo significant expansion in PCa patients after ADT. In a mouse PCa model, a similar $CD4^{low}$ T population becomes noticeable at the early stage of CRPC formation. Phenotypic characterizations define these cells as IL-4-producing TH17 cells, a cell population associated with poor prognosis in patients, and essential for the development of CRPC in mouse models. Mechanistically, the $CD4^{low}$ ($HLA\text{-}G^{+}$) T cells promote intratumoral accumulation of $CD11b^{low}F4/80^{hi}$ cells, which are capable of driving androgen-independent growth of prostate cancer cells. Furthermore, we found that following androgen deprivation, elevated PGE_2 signaling inhibits the expression of CD4 in thymocytes, and subsequently induces the polarization of $CD4^{low}$ naïve T cells towards IL-4-producing TH17 cells via upregulation of IL23R. Therapeutically, inactivating PGE2 signaling with celecoxib, a selective COX-2 inhibitor, dramatically suppresses the formation of CRPC.

Collectively, our current study has revealed that EMT, CSCs, and specific immune cells in the tumor microenvironment play critical roles in the development of CRPC. A combination of ADT with inhibition of either or all of the 3 targets (EMT, CSCs and the $CD4^{low}$ ($HLA\text{-}G^{+}$) T cells) would enhance the efficacy for the treatment of prostate cancer.

IA35 Astronomy accelerates pathology: Multiplex immunofluorescence imaging of the tumor microenvironment. Janis M. Taube[1], Sneha Berry[1], Benjamin Green[2], Julie E. Stein[1], Nicolas Giraldo[3], Tricia Cottrell[3] Suzanne L. Topalian[4], Seyoun Park[5], Alan Yuille[6], Robert A. Anders[3], Drew M. Pardoll[3], Evan J. Lipson[2], Alexander Szalay[7]. [1]Department of Dermatology, Johns Hopkins University, Baltimore, MD, USA [2]Department of Oncology, Johns Hopkins University, Baltimore, MD, USA, [3]Department of Pathology, Johns Hopkins University, Baltimore, MD, USA, [4]Department of Surgery, Johns Hopkins University, Baltimore, MD, USA, [5]Department of Radiology, Johns Hopkins University, Baltimore, MD, USA, [6]Department of Computer Science, Johns Hopkins University, Baltimore, MD, USA, [7]Department of Astronomy, Johns Hopkins University, Baltimore, MD.

Innovative characterization of the tumor microenvironment with a focus on spatially resolved interactions at the single-cell level will provide critical mechanistic insights into

therapeutic responses and potentially identify biomarkers for patient selection. We have developed a method to study ~35-40 markers simultaneously across an entire slide using multispectral, multiplex immunofluorescence. The extent of the data generated has increased the complexity of image analysis and data management. Our collaborator is Dr. Alex Szalay, whose work on the Deep Sky Survey/Hubble telescope correlates well with the multiparameter coordinate data set our approach generates. We have used the astronomy experience to measurably improve microscopic image analysis and have used this new pipeline to generate a high-quality dataset of coordinately plotted immune cells, tumor cells, and immunoactive protein expression. Each tumor in the database is linked to clinical information such as patient age, lesion location, objective response to anti-PD-1, overall survival, irAEs, etc. This approach will facilitate studies of how features in the pretreatment tumor microenvironment such as marker intensity (e.g., PD-1^{low} vs. PD-1^{high} cell populations in situ), marker coexpression (e.g., allowing for assessment of immune checkpoint expression by cell type, and identification of rare cell populations), and cell proximity measurements relate to clinical outcomes. A web-based interface for viewing and analyzing the images acquired on our system is under development. Our goal is to ultimately make the resultant database open access, so other investigators may also query this resource.

IA38 Bile acids, the microbiome, and antitumor immunity in HCC. Tim F. Greten. NCI-CCR Liver Cancer Program, Thoracic and GI Malignancies Branch, CCR, National Cancer Institute, Bethesda, MD, USA.

Immunotherapy has gained a lot of interest in the context of HCC. The basic idea of currently employed immune-based treatment approaches is to activate tumor specific immune responses using immune checkpoint inhibitors. However, this approach dismisses multiple mechanisms that tumors have developed to escape immune surveillance and to suppress antitumor immunity. We study how local factors in the tumor microenvironment such as stress-dependent mechanisms or diet-induced mechanisms (NASH) lead to immune suppression in the liver. We have recently started looking at the effect of the gut microbiome on antitumor immunity in the liver. It is known that dysbiosis impairs the efficacy of immunotherapy, including the immune checkpoint blockade anti-PD1 treatment in melanoma patients. However, the role of gut bacteria in antitumor surveillance in the liver is still poorly understood. Using a primary liver model, we found that altering commensal gut bacteria induced a liver-selective antitumor effect. A selective increase of hepatic CXCR6+ NKT cells was observed, independent of mouse strain, gender, or presence of liver tumor. In vivo studies using both antibody-mediated cell depletion and NKT-deficient mice confirmed that NKT cells mediated inhibition of tumor growth in the liver. Further investigation showed that NKT cell accumulation was regulated by the expression of CXCL16, the solo ligand for CXCR6, on liver sinusoidal endothelial cells, which form the lining of liver capillaries. Primary bile acids increased CXCL16 expression, while secondary bile acids showed the opposite effect. Removing gram-positive bacteria, which contains the bacteria mediating primary-to-secondary bile acid conversion, by vancomycin was sufficient to induce hepatic NKT cell accumulation and decrease liver tumor growth. Feeding secondary bile acids or colonization of bile acid-metabolizing bacteria reversed both NKT cell accumulation and inhibition of liver tumor growth in mice with altered gut commensal bacteria. In nontumor liver tissue from patients with primary liver cancer, primary bile acid CDCA levels correlated with CXCL16 expression; the opposite was found with secondary bile acid GLCA, indicating the finding also applies to humans.

IA40 Precision prevention of cancer: From target identification to clinical practice. Zigang Dong. The Hormel Institute, University of Minnesota, Minneapolis, MN, USA.

Despite the intensive efforts and substantial advances that have occurred through focusing on improving treatment, cancer is still a leading cause of human death worldwide. Many have believed that this could be avoided by focusing on cancer prevention. Cancer incidence of lung, stomach, and many other types has already decreased dramatically in recent years in the USA due to smoking cessation and other prevention programs. During our study of identifying preventive targets and agents, we have found that signal transduction pathways play a critical role in carcinogenesis and thus can be used as a target for cancer prevention. We have identified many protein kinases, acetylase/decentase, methylglase/demethylglase for histone modification, and transcriptional factors, and the role of such modification in cell neoplastic transformation and tumorigenesis. We have discovered natural agents and chemicals that are inhibitors of these kind of enzymes and thus are powerful agents for chemoprevention agents for cancer. Such knowledge will help the translation to new clinical practice of preventive medicine.

CANCER EPIGENETICS

A01 Putative promoters within gene bodies control exon expression via TET1 mediated H3K36 methylation. Ling Ma[1], Yinghui Huang[1], Hongyang Wang[1], Guangyuan Du[1], Ali Sakhawat[1], Tahir Muhammad[1], Xianling Cong[2]. [1]Beijing University of Technology, Beijing, China, [2]Jilin University, Changchun, China.

Hypermethylation of CpG islands in gene promoters has been implicated in gene silencing, and DNA demethylating drugs, such as 5-aza-2'-deoxycytidine, have been used clinically to reactivate tumor suppressor genes for cancer treatment. However, it is not clear why some genes with hypermethylated promoters exhibit high expression levels, nor why drug-induced demethylation of promoters is less successful clinically than expected. One possibility is that CpG islands (CGIs) located in in gene bodies, could also influence gene expression. In this study we investigated how exon-associated methylation of CGIs, as well as exon-associated histone methylation, could affect exon expression, and explain, at least in part, why DNA demethylation alone may be insufficient for full activation of some genes. By RNA-seq and reduced representation bisulfite sequencing, we found a frequent lack of correlation between the level of promoter methylation and gene expression. By CHIP-qPCR and luciferase reporter assays we identified putative promoters within gene bodies, and we showed that the activities of these putative promoters were affected by the methylation level of the CGI nearby. 5-Aza-2'-deoxycytidine reversed the DNA hypermethylation at promoter CGIs effectively, but not the CGIs within gene bodies. We also found that TET1-methylcytosine dioxygenase could demethylate CGIs both in promoter and gene bodies. Furthermore, we revealed a novel mechanism involving H3K36me3-mediated regulation of putative promoters. That is, 5-Hydroxymethylcytosine (5hmC) produced by TET-1 recruited methyl CpG binding protein 2 (MeCP2) and CREB1 as a co-activator to the Histone-Lysine N-Methyltransferase SETD2 promoter, to enhance its gene expression and resulted in increased levels of the transcription-associated histone, H3K36me3, in gene bodies. Our results support the possibility that putative promoters within gene bodies could contribute to gene expression when the parental promoter is silenced by methylation. Furthermore, we find that TET1 influences transcriptional activity of putative promoters in a mechanism that involves both intragenic DNA demethylation and trimethylation of histone H3K36.

A02 *TURBOR,* a LDHA-interacting lncRNA, promotes cancer cell proliferation via enhancing the Warburg effect. Huili Wang, Kequan Lin, Lin Zhu, Shaojun Zhang, Le Li, Yilie Liao, Baichao Zhang, Ming Yang, Dong Wang. Tsinghua University, Beijing, China.

Aerobic glycolysis, also known as the Warburg effect, is a hallmark of cancer and essential for malignancies, but it still remains not well understood how it is specifically modulated in cancer cells. Here, by large-scale functional screening, we identified a tumor-associated and broadly expressed oncogenic long noncoding RNA *TURBOR*, also called *LINC00973*, which is highly expressed in multiple types of human cancers. Notably, knocking down *TURBOR* significantly inhibits the proliferation of multiple types of cancer cells and reduces tumor growth in vivo. Mechanistically, *TURBOR* directly binds to lactate dehydrogenase A (LDHA), an essential glycolysis-associated enzyme, and promotes its tetramerization, thereby enhancing its enzymatic activity and promoting glycolysis. Clinically, high expression of *TURBOR* is significantly associated with poor prognosis in many types of human cancers. Together, our results identified a new lncRNA player and disclosed a cancer-specific regulation mechanism for the Warburg effect, and suggest a potential target against one of cancer vulnerabilities for developing broad-acting anticancer therapies.

A03 Epigenetic activation of a novel isoform of the lncRNA HOTAIR(-N) in cancer cells, a critical role for G-quadruplex. Bin Shan[1], Janarthanan Jayawickramarajah[2], John S. Alsager[1]. [1]Washington State University, Pullman, WA, USA, [2]Tulane University, New Orleans, LA, USA.

The *trans* acting, long noncoding RNA (lncRNA) HOTAIR is dysregulated in cancer. Several HOTAIR isoforms have been described. However, which isoform accounts for HOTAIR expression and functions in cancer is unknown. Unlike HOTAIR's canonical intergenic isoform NR_003716 (named HOTAIR-C hereafter), the uncharacterized novel isoform NR_047517 (named HOTAIR-N hereafter) starts from the first intron of HOXC11 and forms a head-to-head transcription locus with HOXC11. In the current study, we identified HOTAIR-N as the dominant isoform that regulates the gene expression programs and networks for cell proliferation, survival, and death in lung and breast cancer cells. Moreover, HOTAIR-N expression was elevated in tumors from patients with lung, breast, and kidney cancers. In congruence to the elevated expression of HOTAIR-N, the CpG island within the HOTAIR-N and HOXC11 overlapping locus was marked with epigenetic markers for active transcription, including reduced DNA cytosine methylation, increased histone H3 lysine 4 trimethylation, and increased histone H3 lysine 27 acetylation. In addition, we identified

a G-quadruplex (G4) motif rich region in the HOTAIR-N-HOXC11 CpG island of the HOTAIR-N and HOXC11 overlapping locus. Two of the G4 motifs were able to form G4 in vitro, and HOTAIR-N expression was increased by a G4 stabilizing ligand and decreased by a G4 disruptive ligand. HOTAIR-N expression was reduced by inhibition of Bloom, a DNA helicase that resolves G4. We also for the first time provided transcriptomes regulated by HOTAIR-N and Bloom in cancer cells that are important resources for the exploration of lncRNA, DNA helicases, and G4 in cancer. Taken together, our findings indicate that the HOTAIR-N-HOXC11 CpG island is critical in upregulation of HOTAIR-N expression via epigenetic mechanisms in cancer cells. Disruption of G4 may represent a novel therapeutic approach for cancer and other G4 associated diseases.

A04 N6-Nethyladenosine (m6A) related genetic variation is associated with lung cancer risk. Zhening Pu. Center of Clinical Research, Wuxi People's Hospital of Nanjing Medical University, Jiangsu, China.

Background: Lung cancer is the leading cause of cancer deaths worldwide. Previous studies have identified several lung cancer susceptibility loci. However, how to explain them turned out to be a lot more complex than we had hoped. N6-methyladenosine (m^6A) methylation is the most prevalent internal modification of post-transcription in mammalian RNA and has been shown recently to play essential roles in lung cancer. Our purpose was to uncover the possible role of m^6A modification in lung cancer susceptibility in this study.

Methods and Results: We systematically evaluated the association between m^6A related variants (SNPs in the region of predicted m6A region according to motif sequence) and lung cancer risk by meta-analysis of multiple genome-wide association studies (GWASs) data (12,843 cases vs 12,639 controls). In addition, the rs869638 (C > G: OR = 1.09, 95% CI = 1.05-1.13, meta $P = 2.27 \times 10^{-6}$) in *MPZL3* was located next to an experiment-validated m^6A site from Rmbase and m6Avar database. Notably, rs869638 showed high linkage disequilibrium ($r^2 = 0.91$) with rs1056562 reported as a new and complex susceptibility loci of lung cancer by James et al. recently. Then we conducted bioinformatics analysis and functional experiments in cell line. We further showed that *MPZL*3 could be a novel lung adenocarcinoma (LUAD) susceptible gene, while m^6A methylation might play a role at 11q23.3.

Conclusions: We provide first evidence that susceptibility SNP of lung cancer (rs869638) at m^6A motifs may alter the expression of *MPZL3* by modifying the m^6A methylation status. The precious resources of GWAS and m6A sites are warranted to be systematically reviewed to provide additional clues for underlying mechanism of known susceptibility locus.

A05 Long noncoding RNA CISAL mediates BRCA1-dependent mitochondrial fission and cisplatin chemosensitivity via spatially tethering transcription factor-GABPA. Song Fan[1,2,3],* Tian Tian[4],* Xiaobin Lv[5,6],* Xinyuan Lei[1],* Shunrong Li[1], Zhaoyu Lin[1], Xiaofeng Lin[1], Guokai Pan[1,2], Shule Xie[1], Yin Zhang[1], Xinyu Lin[1], Zhanpeng Ou[1], Weixiong Chen[1], Sheng Sun[7], Hanqing Zhang[1], Sigeng Lin[8], Mo Liu[1], Qunxing Li[1], Binghui Zeng[9], Zhiming Tu[3], Lile He[3], Filippos Kontos[3], Ying Ruan[10], Soldano Ferrone[3], Dechen Lin[1], Bakhos A. Tannous[11], and Jinsong Li[1,2]. [1]Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation of Sun Yat-Sen Memorial Hospital, Guangzhou, China, [2]Department of Oral and Maxillofacial Surgery, Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University, Guangzhou, China, [3]Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [4]Department of Neurobiology, Key Laboratory of Human Functional Genomics of Jiangsu, Nanjing Medical University, Nanjing, Jiangsu, China, [5]Markey Cancer Center, University of Kentucky, College of Medicine, Lexington, KY, USA, [6]Nanchang Key Laboratory of Cancer Pathogenesis and Translational Research, Center Laboratory, the Third Affiliated Hospital, Nanchang University, Nanchang, China, [7]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA, [8]Department of Oral and Maxillofacial Surgery, Hainan General Hospital, Hainan, China, [9]Guanghua School of Stomatology, Hospital of Stomatology, Guangdong Provincial Key Laboratory of Stomatology, Sun Yat-sen University, Guangzhou, China, [10]Department of Oral Medicine, Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University, Guangzhou, China , [11]Experimental Therapeutics and Molecular Imaging Lab, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. *These authors contributed equally to this work.

Long noncoding RNAs (lncRNAs) are increasingly reported to regulate gene expression at thousands of locations across multiple chromosomes, thereby modulating cancer cell biology, but the function of lncRNAs in neoadjuvant chemosensitivity remains elusive. Here, we show that the lncRNA LINC01011, which we termed cisplatin sensitivity-associated lncRNA(CISAL), controls mitochondrial fission and cisplatin sensitivity by inhibiting BRCA1 transcriptional activity and its downstream signaling miR-593-MFF axis in TSCC models. Mechanistically, we found that CISAL directly binds the BRCA1 promoter and forms an RNA-DNA triplex structure, recruiting BRCA1 transcription factor-GABPA away from the downstream regulatory binding region, rather than current functionality of lncRNAs in transcriptional regulatory programs, such as competing the binding sites or playing as the decoy/sponge. Importantly, the clinical relevance of these findings is suggested by the significant association of CISAL and BRCA1 expression levels in TSCC tumors with neoadjuvant chemosensitivity and overall survival. We propose a novel model where lncRNAs are tethered at gene promoter by RNA-DNA

triplex formation, spatially recruiting transcription factors away from DNA binding sites. Our study uncovers a lncRNA-BRCA1-dependent network in mitochondrial fission and chemosensitivity, and the potential of CISAL-BRCA1 signaling as a novel target to predict or improve chemosensitivity.

A06 171, a new BET inhibitor, inhibits tumor growth through cell proliferation inhibition more than apoptosis induction. Mohammadali Soleimani Damaneh. Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.

Epigenetics is a relatively novel, important, exciting area of the cell and it has gigantic implications for new anticancer drug (NACD) therapeutics. Bromodomain and extra-terminal (BET) protein is one of the epigenetic reader domains and has emerged as a promising new target class for small molecule drug discovery. Inhibition of bromodomains, including BRD4, has a very nice epigenetic target for cancer therapy. Major collaborative focus of our project concerns the developments of chemical agents for the treatment of cancer. As a matter of fact, many efforts have been made to find new BRD4 inhibitors; this is why we have to understand and develop new anticancer agents (NACAs) for BRD4 inhibition. We have been working in this area for several years and recently we figured out some results; finally, a new BRD4 inhibitor Hjp-b-171 was derived from one PLK1-BRD4 dual inhibitor BI-2536. The most important is that Hjp-b-171 has potent antitumor activity and it selectively blocks the BET. Actually, the antitumor effect of Hjp-b-171 compound was effective approvingly as much better than positive control (+)-JQ-1 and OTX-015, according to results including both in vitro and in vivo antitumor experiments. In conclusion, this research showed that a new BRD4 inhibitor, Hjp-b-171, is more effective in inhibiting cell proliferation and potently downregulates several common oncogenes, including c-MYC. Metabolism and pharmacokinetics data identified that Hjp-b-171 has the potential to become one candidate drug as a BRD4 inhibitor.

A08 Methylation landscape in Barrett's esophagus and esophageal adenocarcinoma reveals distinct subgroups. Jammula S[1], A Katz-Summercorn[2], X Li[2], D Loureda[2], Subash VV[2], Crawte J[2], Abbas S[2], Ginny Devonshire[1], MacRae S[2], Tavaré S[1], Fitzgerald RC[2], Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium. [1]Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, United Kingdom, [2]Medical Research Council Cancer Unit, Hutchison/Medical Research Council Research Centre, University of Cambridge, Cambridge, United Kingdom.

In recent years, the incidence of esophageal adenocarcinoma (EAC) has increased markedly in Western countries, with a five-year survival rate of under 15%. EAC is associated with chronic acid reflux thart causes changes in differentiation of the cells lining the lower esophagus resulting in intestinal metaplasia, otherwise known as Barrett's esophagus (BE). Despite our understanding of genomic changes in BE/EAC very little is known about epigenetic diversity and molecular mechanisms underlying the metaplastic transformation. To date small cohort studies suggest that the methylation profile of EAC resembles lower GI cancers with several cases being associated with CpG Island Methyaltor Phenotype. However, differences between BE and EAC, along with an understanding of the level of heterogeneity in large populations and the association with specific genomic events, are still not well understood. Here, using array-based methylation data derived from 150 BE and 285 EAC and samples from 100 control samples (39 squamous oesophagus, 38 gastric cardia, and 23 duodenum), we have characterized the epigenetic changes in both BE and EAC and combined this with whole-genome sequencing and RNA seq where available. From our genome-wide analysis we observe that both BE and EAC can be grouped into four distinct subtypes with unique genetic changes underlying them. The first two subtypes (I and II) show a significant gain in methylation mainly in regions enriched with CpG islands along with a distinct pattern of methylation in gene bodies distinguishing two subtypes. Subtype I is dominated by BE cases (82%) with unique expression pattern related to metabolic process—mainly fatty acid oxidation and a high proportion (67%) harboring CDKN2A alterations. Subtype II is predominantly EAC cases (87%) with recurrent mutations in TP53 (78%), CCND1 (21%), and GATA4 amplifications (22%). The third subtype (III) is characterized by hypomethylation with signs of genome instability supported by large-scale rearrangements and high copy number changes both globally and at focal regions specifically affecting ERBB2 (29%) and CCNE1 (21%). The fourth subtype consists of cases that do not undergo many changes at the methylation level but rather have a high level of immune infiltration and a very poor survival rate (median survival Subtype I 3 years, Subtype II 2.14 years, Subtype III 2.8 years, Subtype IV 2.01 years, p-value=0.13). Of note, we could confirm from the RNAseq data that CCND2, MGMT, and CHFR are epigenetically silenced in 35%, 31%, and 18% of cases (across all subgroups), raising the possibility that these are potential therapeutic targets. In summary, this is a comprehensive integrated analysis of methylation across a large cohort of EAC and BE cases and provides clinically relevant information on molecular subgroups with differential prognosis and potential therapy targets.

A09 Valproic acid exhibits antitumor activity selectively against EGFR/ErbB2/ErbB3-coexpressing pancreatic cancer via induction of ErbB members-targeting microRNAs. Qun Ren, Tingting Lin, Weimin Zuo, Ruxue Jia, Linhui Xie, Rong Lin, Hu Zhao, Jin Chen, Yan Lei, Ping Wang, Huiyue Dong, Lianghu Huang, Jinquan Cai, Yonghai Peng, Zongyang Yu, Jianming Tan, Shuiliang Wang. Department of Urology, The 900th Hospital of the Joint Logistics Team (the Former Fuzhou General Hospital), Fujian Medical University, Fuzhou, Fujian, China, Fujian Key Laboratory of Transplant Biology, Affiliated Dongfang Hospital, Xiamen University School of Medicine, Fuzhou, Fujian, China.

Deregulated ErbB signaling plays an important role in tumorigenesis of pancreatic cancer. A number of attempts have been taken to target this pathway for systemic therapy; however, patients with pancreatic cancer benefit little from current existed therapies targeting the ErbB signaling. In the current studies seeking novel strategy to treat pancreatic cancer, we discovered that VPA, a clinically used antiepilepsy drug with reported HDACi activity, exhibited a promising antitumor activity selectively against ErbB members-coexpressing pancreatic cancer. VPA preferentially inhibited cell proliferation/survival of EGFR/ErbB2/ErbB3-coexpressing pancreatic cancer cells in a dose-dependent manner, which was accompanied by significant reduction of cyclin D1 as well as induction of P21. Meanwhile, VPA potently induced caspase-dependent apoptosis in EGFR/ErbB2/ErbB3-coexpressing pancreatic cancer cells within its clinically achievable range [40~100 mg/L (0.24~0.6 mmol/L)]. Mechanistic investigations revealed that VPA treatment resulted in significant inactivation of Akt and MAPK signalings via simultaneous downregulation of EGFR, ErbB2, as well as ErbB3 in pancreatic cancer cells, which was mainly attributed to induction of several miRNAs targeting the ErbB family members both in vitro and in vivo. Moreover, the anti-pancreatic cancer activity of VPA was further validated in tumor xenograft model. In summary, we demonstrate that VPA simultaneously downregulates EGFR, ErbB2, and ErbB3 via induction of ErbB family members targeting miRNAs in pancreatic cancer cells, which subsequently inhibits the downstream Akt and MAPK signalings and induces cell apoptosis and growth arrest. Our data strongly suggest that VPA may be added to the treatment regimens for pancreatic cancer patients with co-overexpression of ErbB family members.
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A10 The role of G9a in multiple myeloma. Xi Yun Zhang. Cancer Science Institute, YLL School of Medicine, National University of Singapore, Singapore.

Multiple myeloma (MM) is a mostly incurable plasma cell malignancy characterized by the overproliferative plasma cells in the bone marrow. A variety of complicated primary and secondary genetic events associated with disease progression have been observed. Among them there are mutations resulting in hyperactivation of NF-Kb pathway, which is present in at least 17% of multiple myeloma (MM) tumors and 40% of MM cell lines (MMCLs). Besides the genetic events, epigenetic deregulation is another prominent factor in MM pathogenesis. The changes of epigenetic landscape in MM are related to the stage and drug resistance of the disease, and thus epigenetic targeting drugs could be an important strategy to treat MM. By conducting epigenetics library screening on several MM cell lines, we found a group of G9a inhibitors have high efficacy and significantly inhibit cell viability of MM cells while the role of G9a in multiple myeloma remains to be investigated. In this study, we showed that G9a, an important epigenetics regulator in cancer, is highly expressed across different MM cell lines. Also, depletion of G9a inhibits cell proliferation and colony formation in MM. To further study the mechanisms, we conducted RNA-seq for G9a depleted MM cells and found NF-Kb pathway was affected by G9a depletion. One of the mostly downregulated factors of NF-Kb pathway is RelB. The level of H3K9me2 at RelB promoter decreases after G9a knockdown. These findings indicated that G9a may regulate the NF-Kb pathway in MM through RelB, which provides us with more inspirations in design G9a-targeting drug combinations improving the therapeutic option for MM patients with hyperactivation of NF-Kb pathway.

A11 Potential application of Septin9 methylation assay in monitoring prognosis and recurrence of colorectal cancer. Zhengbin Wu[1], Zheqiang Zhou[2], Meixiang Li[2], Yanhua Cao[3], Jing He[3], Ji Liu[3], Benjamin Yu[4], De-Hua Yu[3]. [1]Hanchuan Renmin Hospital, Department of Surgery, Hanchuan, China, [2]Department of Oncology, The 2nd People's Hospital of Shenzhen, Shenzhen, China, [3]USK Bioscience CO, LTD, Shenzhen, China, [4]Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

The blood-based Septin9 methylation assay for early detection of colorectal cancer (CRC) has been approved in clinical setting in a number of countries. In the present study, we investigated the dynamic changes of Septin9 methylation in blood from patients with CRC pre- and post-operation and its potential as an auxiliary parameter to monitoring disease prognosis and staging. A total of 64 CRC patients with positive detection of blood Septin9

methylation prior surgery were enrolled in this study. After surgery for one week, the plasma Septin9 methylation was negative in 84.4% (54/64) of the tested patients. However, the plasma Septin9 methylation levels were reduced, but were still detected positive in the remaining 10 patients (15.6%, 10/64). Interestingly, all the 10 CRC patients with positive Septin9 methylation were diagnosed with distant metastasis. In addition, we compared the positivity of Septin9 methylation and conventional tumor biomarkers CEA and CA199 in another cohort of patients with recurrent CRC. Our results showed that Septin9 methylation and CEA/CA199 were detected in 75% (9/12) and 58% (7/12) of the CRC patients, respectively. Together, our results suggest that plasma Septin9 methylation is a potential new molecular biomarker for monitoring the prognosis and recurrence, besides its current application in early screening in CRC.

A12 Roles of tRNA methylation regulated by DNA methyltransferase 2 (Dnmt2) in tumorigenesis of triple-negative breast cancer. Jun Yin, Mei Zhang. University of Pittsburgh Department of Developmental Biology, University of Pittsburgh Cancer Institute, Magee Women's Research Institute, Women's Cancer Research Center, Pittsburgh, PA, USA.

Background: The enzymes that are responsible for methylation of DNA, histones, and RNAs are often deregulated or mutated in cancer, and are important in maintaining cancer hallmarks such as proliferation and invasion. DNA and histone methylation has been widely studied in cancer. In stark contrast, despite observations nearly 40 years ago of RNA methylation, our understanding of the function of RNA methylation and its involvement in tumorigenesis is extremely limited. Studies have shown that distinct tRNA methylation (1-methylguanine (m1G) and 5-methylcytosine (m5C) tRNA-Phe) has been found in tumor cells relative to normal tissue. In addition, several tRNA methyltransferases are related directly to DNA repair and carcinogenesis. Moreover, abnormal tRNA modifications have also been reported in cancers which are caused by rearrangement of chromosomes in cancer cells. It has been reported recently that specific tRNAs are upregulated in human breast cancer cells as they gain metastatic activity. However, the direct correlation between RNA modification and cancer initiation remains limited. DNA methyltransferase 2 (Dnmt2), also named tRNA-aspartate methyltransferase 1 (Trdmt1), methylates tRNA instead of DNA. It is only one of two known m5C tRNA methyltransferases in higher eukaryotes. Database analysis from over 1,000 breast cancer patients shows DNMT2 is overexpressed in basal-like breast cancer and associated with poor prognosis. Using a p53 -/- mouse model of human breast cancer, we identified and comprehensively characterized a tumor-initiating cell (TIC) population. Intriguingly, genome-wide gene expression analysis of this TIC population showed elevated expression of Dnmt2.

Hypothesis: Epigenetic disruptions caused by Dnmt2 abnormalities may lead to tumorigenesis. Our studies will provide valuable insights into drug design and cancer treatment.

Methods: Lentiviral transduction were performed to knockdown Dnmt2 in human breast cancer cell lines. Real time qPCR were administered in Dnmt2 control and KD cells to verify the expression of Dnmt2. By using these cells, tRNA methylation was analyzed by bisulfite method. In vitro proliferation measurement and Matrigel colony-forming assay were performed to determine the roles of Dnmt2 in tumorigenesis. In vivo limiting dilution transformation assay showed that Dnmt2 plays a critical role for tumor initiation.

Results: By bisulfite assay, C38 and C47 were demethylated in the Dnmt2 KD cells. Cell proliferation and tumorigenic potential were suppressed in Dnmt2 KD cells using both in vitro and in vivo assays. Expression of several genes critical for cancer stem cell activities was decreased in Dnmt2 KD cells, supporting the role of Dnmt2 in tumorigenesis.

CANCER METABOLISM

A13 HSD17B4 regulates the progression of prostate cancer and degrades via CMA pathway. Ye Zhang, Yongheng Chen. Key Laboratory of Cancer Proteomics of National Health Commission, XiangYa Hospital, Central South University, Changsha, China.

Prostate cancer (PC) is driven by dysregulation of hormone metabolism. 17β-Hydroxysteroid dehydrogenase type 4 (*HSD17B4*) is a key enzyme in the androgen metabolism and participates in the generation of dihydrotestosterone (DHT), which is a vital androgen in cellular processes and associated with the progression of prostate cancer. In this study, we show that HSD17B4 protein is upregulated in prostate cancer, while its K669 acetylation level is downregulated. In human prostate cancer tissues, HSD17B4 is positively correlated with Ki-67, and the overexpression of HSD17B4 promotes the proliferation and migration of prostate cancer cells. Further results found the increase of HSD17B4 K669 acetylation level promotes its degradation via chaperone-mediated autophagy (CMA). SIRT3 interacts with HSD17B4 to deacetylate the K669 acetylation and stabilize its protein level, while CREBBP acetylates HSD17B4 and slows down the progression of prostate cancer.

Collectively, our study reveals a novel regulation mechanism of HSD17B4 and a direct functional link of this regulation to prostate cancer development.

A15 LncRNAs-directed PTEN enzymatic switch governs epithelial–mesenchymal transition. C. Li[1,2],* Q. Hu[1],* S. Wang[1], Y. Li[1], B. Wen[3], Y. Zhang[1], K. Liang[1], J. Yao[1], Y. Ye[4], H. Hsiao[1], T.K. Nguyen[1], P.K. Park[1], S.D. Egranov[1], D.H. Hawke[5], J.R. Marks[6], L. Han[4], M.C. Hung[1,7], B. Zhang[3], C. Lin[1], L. Yang[1]. [1]Department of Molecular and Cellular Oncology, Baylor College of Medicine, Houston, TX, USA, [2]Department of Experimental Therapeutics, Baylor College of Medicine, Houston, TX, USA, [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA, [4]Department of Biochemistry and Molecular Biology, The University of Texas Health Science Center at Houston McGroven Medical School, Houston, TX, USA, [5]Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, [6]Department of Surgery, Duke University School of Medicine, Durham, NC, USA, [7]China Medical University, Taichung, Taiwan. *These authors contributed equally.

Epithelial-to-mesenchymal transition (EMT) has been demonstrated to be required for embryonic development, tissue repair, and cancer metastasis. Factors such as transcription factors TWIST and SNAI1 are known to contribute to EMT, but the underlying mechanisms that regulate these factors are limited. Our findings show that K27-linked polyubiquitination of phosphatase and tensin homologue (PTEN) at lysines 66 and 80 switches its phosphoinositide/protein tyrosine phosphatase activity to protein serine/threonine phosphatase activity. Mechanistically, high glucose, TGF-β, CTGF, SHH, and IL-6 induce the expression of a long noncoding RNA, *GAEA* (*G*lucose *A*roused for *E*MT *A*ctivation), which associates with an RNA-binding E3 ligase, MEX3C, and enhances its enzymatic activity, leading to the K27-linked polyubiquitination of PTEN. PTENK27-polyUb promotes dephosphorylation and stabilization of TWIST and SNAI1, resulting in enhanced EMT. In mice, genetic inhibition of PTENK27-polyUb attenuated EMT markers during mammary gland morphogenesis in pregnancy/lactation and during cutaneous wound healing processes. Our findings illustrate an unexpected paradigm in which the lncRNA-dependent switch in PTEN protein serine/threonine phosphatase activity is important for physiologic homeostasis and disease development.

A16 Targeting IL6 abrogates cancer-promoting activity of SASP from therapy-induced senescent cells malignant glioma cells. Jiayu Gu, Wenbo Zhu. Sun Yat-sen University, Guangzhou, China.

Activation of cyclic adenosine monophosphate (cAMP) pathway induces glial differentiation of glioblastoma (GBM) cells, but the ultimate fate of differentiated cells remains poorly known. Transcriptomic analyses reveal significant changes of cell cycle and senescence-related pathway in differentiated GBM cells induced by dbcAMP, evidenced by cell cycle arrest and β-galactosidase positive staining. We further elucidate that ROS derived from enhanced mitochondria function accounts for senescence induction. Interestingly, the accumulation of H3K9me3 tags in PI3K signal molecules mediates mitochondria function enhancement and subsequent senescence. Moreover, we find that IL-6 released by dbcAMP induced senescent cells potentiate the glycolysis phenotype of GBM cells, which is reversed by IL-6 signal blockage. Temozolomide, the first-line drug for GBM treatment, also induces the occurrence of senescence and IL-6 release. In vivo, the combo of IL6-receptor antibody plus temozolomide significantly prolongs the life of experimental mouse with GBM. Taken together, these results indicate that differentiated GBM cells undergo senescence and release IL-6 to induce the glycolysis of other GBM cells, which could be a novel synergic target for differentiation-inducing therapy.

CANCER PREVENTION

A17 Hemodynamic shear stress regulates the survival of circulating tumor cells in hematogenous dissemination through Piezo1-mediated YAP/TAZ nuclear translocation. Keming Li[1,2], Ying Xin[1,2], Youhua Tan[1,2]. [1]Hong Kong Polytechnic University Shenzhen Research Institute, Shenzhen, China, [2]Department of Biomedical Engineering, the Hong Kong Polytechnic University, Hong Kong, China.

Background: Metastasis accounts for over 90% of cancer-related deaths. Tumor cells metastasize to distant organs mainly through hematogenous dissemination, where they experience considerable levels of shear stress, and less than 0.01% of them can generate metastatic tumors. However, the influence of hemodynamic shear stress on the survival of circulating tumor cells (CTCs) remains elusive. The mechanisms of how CTCs in suspension sense fluid shear stress are unclear.

Methods: A circulatory microfluidic system was developed to mimic blood circulation in vitro. The survival of suspended breast tumor cells in fluid shear flow was tested by MTS assay. The expressions of the related genes and proteins were measured by qPCR and Western blotting. To explore the mechanotransduction pathway, siRNA/shRNAs, various inhibitors or active forms of plasmids were used to modulate the expression of the related genes.

Results: In this study, we found that the majority of breast CTCs could be eliminated by fluid shear stress and tumor cells after shear stress treatment exhibited high level of Piezo1. Silencing Piezo1 or blocking calcium entry through Piezo1 increased the survival of CTCs in shear flow, while activating Piezo1 suppressed tumor cell survival. These data suggest that tumor cells in suspension sense fluid shear stress via Piezo1 mediated calcium influx. Shear-activated Piezo1 enhanced actomyosin activity, the inhibition/activation of which significantly increased/decreased the survival of suspended tumor cells in shear flow. Importantly, fluid shear stress induced nuclear translocation of YAP/TAZ, which was regulated by Piezo1-actomyosin signaling. Inhibiting YAP/TAZ enhanced the viability of suspended CTCs in shear flow, while activating their activity suppressed tumor cell survival. The effects of Piezo1 and actomyosin on tumor cell survival in shear stress could be rescued by YAP/TAZ activity, suggesting that fluid shear stress regulates the survival of suspended tumor cells through Piezo1-mediated activation of actomyosin and YAP/TAZ. Blocking the nuclear import of YAP/TAZ enhanced the survival of CTCs in shear flow and attenuated the dependence of cell survival on shear stress. In addition, shear stress enhanced the expression of MST1/2 and LATS1/2 in Hippo signaling, which further suppressed YAP/TAZ activity. Inhibiting MST1/2 or LATS1/2 significantly decreased tumor cell survival in shear flow, which could be rescued by silencing YAP/TAZ. These findings suggest that shear-induced apoptosis partially depends on Hippo signaling. Further, our data show that p73 expression was significantly increased in shear stress and bound with nuclear translocated YAP/TAZ. This binding facilitated the transcription of proapoptosis gene BAX and PUMA, which further mediated tumor cell apoptosis.

Conclusion: In summary, our findings suggest that fluid shear stress considerably decreases tumor cell survival. CTCs in suspension sense shear stress through Piezo1-actomyosin-YAP/TAZ-p73-BAX signaling. This study unveils the key roles of Piezo1 and YAP/TAZ in the survival of suspended CTCs in hemodynamic shear flow, which may have important implications in targeting CTCs in blood circulation during metastasis.

A18 TCF4N, a new tumor-suppressing isoform of T-cell-specific transcription factor 4 in colorectal cancer. Jian Zou, Yaling Hu, Zhaohui Huang, Bo Zhang, Ying Yin, Jingjing Wang, Yingdi Jiang, Li Ji, Lingli Gong. Wuxi People's Hospital of Nanjing Medical University, Jiangsu, China.

The crucial roles of Wnt/β-catenin pathway in various cancers have made the β-catenin-Tcf4 complex an ideal therapeutic target. The N-terminal of TCF4 is necessary for high-affinity interaction with β-catenin. Due to the responsive site of TCF4 interacting with β-catenin span at least 53 amino acids, it is challenging to disrupt the interaction with small molecules. A further concern is the overlap of the binding sites of β-catenin with TCF4 and E-cadherin, which implies a risk of interrupting the normal physiologic function of β-catenin in cell-cell adhesive junctions. Recently, several alternatively spliced isoforms of TCF4 have been found in various cancers. One of these isoforms, TCF4N, containing the N-terminal interaction domain for β-catenin and lacking the DNA binding domain, functions as a dominant negative factor of Tcf4 and inhibits interactions between β-catenin and TCF/LEF transcription factors. Thus, targeting this isoform might represent a novel approach to inhibit tumorigenesis and progression in cancers with maladjusted Wnt signaling. Here we report that TCF4N mRNA is significantly downregulated in colorectal cancer (CRC) tissues as compared to it in paired adjacent noncancerous tissues. Kaplan-Meier analyses revealed that lower TCF4N expression is associated with poor survival. In vitro and in vivo functional assays indicated that TCF4N is a tumor suppressor in CRC. It inhibits cell proliferation, invasion, and metastasis, and promotes doxorubicin-induced apoptosis. Double immunofluorescent and cellular component Western blot assay showed that the ectopic TCF4N is mainly expressed in cell membrane of CRC cells, and especially localizes in cell-cell contacts. It resulted in an increase of cell aggregation, a rearrangement of F-actin stress fibers, and downregulation of EMT markers. Transcriptome sequence showed that TCF4N is negatively associated with canonical Wnt signaling pathway, frizzled binding, and epithelial-to-mesenchymal transition (EMT), and positively correlated with actin filament organization, bicellular tight junction and apoptotic process, indicating modulating cell junction may be a major mechanism of the tumor suppression of TCF4N. Mechanically, TCF4N binds β-catenin and increases the stability of the β-catenin-E-cadherin complex in cell membrane of CRC cells, even in the presence of Wnt-1. Moreover, TCF4N impairs Wnt-1 induced β-catenin nuclear translocation and E-cadherin downregulation in CRC cells. The membrane localization of β-catenin shows phosphorylation at Thr41/Ser45, and this phosphorylation is decreased in the presence of Wnt-1. Using constitutive phosphorylated or nonphosphorylated mutations, we revealed that phosphorylation at Thr41/Ser45 determines the membrane localization and nuclear translocation of β-catenin and the following transcriptional

activity. It also determined cell-cell junctions, aggregation, and EMT phenotype, followed with cell proliferation, invasion, and apoptosis. The interaction of TCF4N and β-catenin is Thr41/Ser45 phosphorylation dependent. TCF4N maintains β-catenin in the phosphorylation state at Thr41/Ser45 whether Wnt signaling is activated. To explore the implication of TCF4N in tumorigenesis, we developed a eukaryotic protein targeting TCF4N (TAT-TCF4N), which fused to a cell-penetrating peptide, and confirmed that this recombinant protein efficiently penetrates into cells and binds β-catenin in cell membrane. This recombinant protein shows tumor inhibitory potentials in antitumor cell growth, metastasis and enhancing chemosensitivity in vivo. In conclusion, the present study uncovers that TCF4N is a novel antitumorigenic isoform of TCF4 in CRC via modulating cell contacts and β-catenin stability in cell membrane. Its loss or low expression in CRC is associated with tumor growth, metastasis, and chemosensitivity. Remarkably, the present study demonstrates that the cell-penetrating recombinant TCF4N is a novel anticancer strategy in CRC.

A19 The second somatic mutation hit on top of genetic APC mutations causes skin tumor. Ting Niu, Jiangchao Li. Guangdong Pharmaceutical University, Guangzhou, China.

Inactivation of adenomatous polyposis coli (APC) gene is the initiating event in about 80% of human colon cancer cases; *Apc*Min/+ mice also develop benign polyps in the intestinal tract. Few *Apc*Min/+ mouse would develop into breast carcinoma. Here, we also find about 1.5% (6/400) *Apc*Min/+ mice would suffer skin neoplasm in *Apc*Min/+ mice. Our investigation shows that it is skin tumor not derived from adenoma of intestine of mice. To understand why only few *Apc*Min/+ mice obtained the sebaceous adenoma while other most *Apc*Min/+mice did not, sequencing data showed that mRNA expression and somatic mutation skin tumor happen on the background of the genetic APC mutation. In clinical data, APC mutation presents about 27% in cutaneous carcinoma from cancer database, and previous reports show that up to 90% of FAP patient accompany nongastrointestinal tumor including hepatoblastomas, desmoid tumors, and brain cancer. Also, *Apc*Min/+ mice on background of TP53 knockout increase tumor number. All this suggests that the second somatic mutation hit on the germline mutation would increase the tumor rate in skin of *Apc*Min/+mice, suggesting that the second somatic mutation should be avoided if the inherited mutation existing in our body, it would decrease tumor incidence.

A20 A new role for FOXO3a in HCC metastasis. Manqing Cao, Ti Zhang. Tianjin Medical University Cancer Institute & Hospital, Tianjin, China.

Metastasis and recurrence seriously affect the long-term prognosis of patients with hepatocellular carcinoma (HCC), and a better understanding of the molecular mechanisms underlying HCC metastasis may potentially improve clinical treatment. FOXO3a is generally identified as a tumor suppressor. However, recent studies have suggested that FOXO3a promoted tumor metastasis. Whether FOXO3a promotes HCC prognosis has not yet been elucidated. Here, we found that FOXO3a was overexpressed in HCC tissues and mainly located in nuclear, which was associated with poor prognosis. Overexpression of FOXO3a in MHCC-97H cells promoted cell migration and motility. Considering the higher ROS level in MHCC-97H cells, we believe that FOXO3a might promote HCC metastasis in specific condition. Furthermore, RNA-sequencing analysis revealed that FOXO3a was closely related to cell adhesion pathway and the expression of THBS1 was significantly influenced. We proved that THBS1 could be regulated by FOXO3a at both mRNA and protein levels. Therefore, we hypothesize that FOXO3a may regulate THBS1 and thus activating cell adhesion pathway to promote HCC metastasis. Our study will help to further understand the function of FOXO3a in modulating HCC metastasis, and provide new strategy for HCC treatment.

A21 NOL7, a possible facilitator of melanoma survival and metastasis. Yumei Li[1,2],* Fan Chen[1,2],* Weiyu Shen[1,2],* Bifei Li[1,2,3], Ning Zheng[1,2], Lee Jia[1,2,3]. [1]Cancer Metastasis Alert and Prevention Center, and Pharmaceutical Photocatalysis of State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou, Fujian, China, [2]Fujian Provincial Key Laboratory of Cancer Metastasis Chemoprevention and Chemotherapy, Fuzhou University, Fuzhou, Fujian, China, [3]Institute of Oceanography, Minjiang University, Fuzhou, Fujian, China. *These authors contributed equally to this work and should be considered co-first authors.

Background: Metastasis is the principal cause of melanoma-associated death and a major challenge in today's melanoma management. It is crucial to make certain of relevant survival factors in the process of melanoma cell metastasis. Nucleolar protein 7 (NOL7) is a RNA binding protein and controls RNA fate from synthesis to degradation.

Purpose: To explore whether NOL7 is associated with the process of cancer metastasis survival and uncover its mechanisms.

Methods: A mouse model of melanoma metastasis was established to obtain the postmetastatic melanoma cells. Specifically, B16F10 cells were defined as premetastatic melanoma cells. The cells, B16F10M, derived from pulmonary metastatic nodules of B16F10 cells via trypsinization were defined as postmetastatic melanoma cells. Western blotting and quantitative real-time PCR were used to investigate the expression differences between these two cells from the quantitative and qualitative, respectively. NOL7 gene knockdown in B16F10 cells was displayed to determine the functions in metastasis survival ability of melanoma cells.

Results: The postmetastatic melanoma cells, B16F10M, were achieved successfully using the C57BL/6 mouse mode. B16F10M expressed higher NOL7 level than B16F10. In addition, NOL7 gene depletion in B16F10 cells suppresses melanoma cells proliferation, survival, and metastasis, respectively.

Conclusion: NOL7 functions as tumor facilitator in melanoma cells and may be a novel biomarker and therapeutic target of melanoma.

FEMALE-SPECIFIC CANCERS

A22 HPV integration detection of cervical cancer clinical samples by next-generation sequencing technology for cancer prevention, evaluation, and monitoring. Bo Meng[1], Lili Quan[2], Jidong Lang[1], Wenjuan Yang[1], Yu Song[2], Lanyou Chen[1], Jianyu Zhao[1], Ruyi Dong[1], Geng Tian[1]. [1]Gynaecology and Obstetrics Department, Sanmenxia Central Hospital, Sanmenxia, China, [2]Geneis Technology Ltd., Beijing, China.

Introductions: Human papillomavirus (HPV) infection has been reported as an important cause of cervical cancer for many years (1,2), and HPV testing has been applied for prevention of cervical cancer broadly. In addition to HPV infection, integration of HPV DNA is a crucial genetic event in cervical carcinogenesis (3). Therefore, it is important to know the HPV integration hot spots in human genome to predict the cervical cancer development status of patients. Although many studies have addressed the HPV integration sites in human genome (4-8), due to the significant personal difference of integration distribution of human genome, to fully understand the characteristic of HPV integration will still need a lot more effort.

Material and Methods: We designed HPV probes for 18 high-risk HPV types and compared the capture efficiency of three different combinations and chose the best one for further study. By using next-generation sequencing (NGS) method, we enriched HPV integration sites of 40 TCT, 3 tissue and 4 cf DNA samples from patients and sequenced them. We used our in-house developed method for genome structure variation analysis.

Results: The results showed that: 1) we detected positive HPV integration sites from all three type of samples and the positive integration rate are 85% in TCT, 66.7% in tissue, and 50% in cfDNA samples; 2) the identified integration site of each sample is significantly different including number and location, which indicated the personal diagnostic value of it; 3) based on the CIN stage of TCT samples, we calculated the positive integrated samples and found almost 75% samples that were earlier than ASC-US stage (CIN1) were integrated by HPV, which is a bit higher than previous study (42.8%). It could be caused by two reasons. One is for the limited number of the tested samples and the other is that our method is more sensitive than others and has lower detection limitation. The integration sites could locate anywhere in human chromosomes; however, in some specific area, more integration seems to be accumulated. We compared our integrated gene list with previous studies and they had very high concordance. Also, the genes mostly came from pathways with function of alternative splicing, sequence variant and *ect*. We further verified some of the integration sites by PCR and Sanger sequencing. Two of them were verified successfully. Further study is being carried out.

Conclusions: In conclusion, we developed a HPV panel that could successfully detect 18 hrHPV in different types of patient samples with high sensitivity and accuracy. It could be used as an early diagnosis product to predict the risk of carcinogenesis and as a diagnostic method for cancer treatment effect evaluation and recurrence monitoring.

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A23 Long noncoding RNA FLJ33360 participates in ovarian cancer progression by sponging miR-30b-3p. Meiqin Yang[1], Zhensheng Zhai[2], Shuang Guo[1], Xiaoxi Li[1], Yongxia Zhu[1], Yue Wang[1]. [1]Department of Gynecology and Obstetrics, [2]Department of Hepato-Biliary-Pancreatic Surgery, People's Hospital of Zhengzhou University, Henan Provincial People's Hospital, Zhengzhou, Henan, China.

Background: Long noncoding RNAs (lncRNAs) have been reported to play a key role in the development and progression of human malignancies. FLJ33360 is a lncRNA with unknown functions. This study was designed to determine the clinical significance and mechanism of FLJ33360 in ovarian cancer.

Materials and Methods: The clinical significance of FLJ33360 in ovarian cancer was determined using the Gene Expression Profiling Interactive Analysis (GEPIA) database, Kaplan-Meier Plotter database, quantitative reverse transcription polymerase chain reaction (qRT-PCR), and statistical analysis. The regulatory relationships between FLJ33360 and miR-30b-3p were explored through bioinformatics, Gene Expression Omnibus (GEO) database, ArrayExpress database, and meta-analysis. The possible pathways were predicted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. In addition, the key target genes were identified using protein-protein interaction (PPI) network, The Cancer Genome Atlas (TCGA) database, and correlation analysis.

Results: The FLJ33360 expression was significantly downregulated in ovarian cancer tissue (P=0.0011) and was closely associated with disease FIGO stage (P=0.027) and recurrence (P=0.002). FLJ33360 has value in the diagnosis of ovarian cancer (area under the curve =0.793). Function analysis demonstrated that FLJ33360 can act as a molecular sponge of miR-30b-3p to regulate the expression of target genes, which were mainly involved in positive regulation of smooth muscle cell migration, the unsaturated fatty acid metabolic process, and positive regulation of epithelial to mesenchymal transition. Among these target genes, BCL2 is the hub gene.

Conclusion: FLJ33360 is a potential biomarker for early diagnosis and assessment of prognosis in ovarian cancer, which can regulate the expression of genes by sponging miR-30b-3p and thus participate in the development of ovarian cancer.

A24 Tumor immunologic phenotype signature-based drug screening reveals aurora kinase inhibitors enhancing immunotherapy efficacy. Haiyan Wang, Dong Wang. Tsinghua University, Beijing, China.

Checkpoint blockade immunotherapy only benefits a fraction of cancer patients, partially due to the poor infiltration of effective T cells into the tumor microenvironment. Here, using triple-negative breast cancer (TNBC) as an experimental model, we identified that aurora kinase inhibitors ENMD-2076 and TAK-901 promote effective T-cell infiltration into the tumor microenvironment and thus significantly enhance the efficacy of immunotherapy. We firstly defined an immune gene signature with 15 tumor immunologic phenotype (TIP) associated genes. Then, by combining this gene signature and high-throughput sequencing-based high-throughput screening (HTS2), we found that compound ENMD-2076 and TAK-901 could reprogram the expression of TIP signature genes and particularly upregulate expression of the Th1 type chemokine genes *CXCL10* and *CXCL11* in cancer cells, which consequently promotes effective T-cell infiltrating into tumor microenvironment and thus significantly improves anti-PD-1 efficacy in inhibiting the tumor growth of TNBC in preclinical models. Moreover, we demonstrate this inhibitor-induced transcriptional activation of *CXCL10* and *CXCL11* is mediated through aurora kinase A and STAT3. Our discoveries suggest the strong therapeutic potential of combining ENMD-2076 or TAK-901 with checkpoint blockade immunotherapy for the treatment of triple-negative breast cancer.

A26 A novel biophysical-based marker with multilevel, multiparameter expression for cervical cancer detection.
Hongmei Tao, Xing Tang, Yue Lin, Chris Chang Yu, Xuedong Du. Anpac Bio-Medical Science Co., Ltd., Shanghai, China.

Background: Cervical cancer is the most common malignant tumor of female reproductive tract, with the second highest incidence in female malignant tumors and even the highest in some developing countries. Cervical cancer accounts for about 500,000 new cases worldwide each year, accounting for 5% of all new cases of cancer, more than 80% of which are in developing countries. The number of deaths of cervical cancer in China is about 53,000 per year, accounting for about 18.4% of all female malignant tumor deaths. Cervical cancer is a leading disease that endangers the health and life of women in China. An emerging novel liquid biopsy technology called Cancer Differentiation Analysis (CDA) has been evaluated as a viable cervical cancer detection tool. As a biophysical-based marker with multilevel and multiparameter expression features, CDA has shown the capability of detecting cervical cancer with a high degree of sensitivity and specificity.

Methods: In this study, 376 cervical cancer samples with pathologic information and 922 samples from healthy female individuals were measured in a single blind test. Peripheral blood of each individual was drawn in EDTA tubes. One class of biophysical property in blood samples was utilized for CDA tests. The CDA data were first processed using an algorithm built from data base and subsequently analyzed using SPSS. The results are shown in **Table 1**.

Results: The average CDA values of cervical cancer and control groups were 50.99 and 36.69 (rel. units), respectively. The results indicated that cervical cancer could be significantly distinguished from the control ($p < 0.001$). The results indicated that CDA technology has a very good sensitivity and specificity (83.5% and 90.0%, respectively).

Conclusions: Initial results showed that CDA technology could effectively screen cervical cancer patients from healthy individuals. As a novel biophysical-based cancer detection approach with multilevel and multiparameter expressions, CDA technology could be a potential candidate for cervical cancer detection.

A27 Cancer Differentiation Analysis technology as a novel technology for ovarian cancer detection.
Yue Lin, Hongmei Tao, Xing Tang, Xuedong Du, Chris Chang Yu. Anpac Bio-Medical Science Co., Ltd., Shanghai, China.

Background: With increasing incidence and mortality, cancer is the leading cause of death in China and is a major public health problem. From 2003 to 2010, the incidence and mortality of ovarian cancer maintained a stable trend in China, but due to the large population, the new cases and deaths of ovarian cancer remain high. In 2015, approximately 52,100 women in China were diagnosed with ovarian cancer, and about 22,500 women died of ovarian cancer.

Cancer Differentiation Analysis (CDA) technology is a novel biophysical-based cancer detection approach with multilevel and multiparameter diagnostic method that detects signals from protein, cellular, and molecular levels, in which multiple aspects of information can be collected to improve diagnostic accuracy. Initial results showed that CDA technology is capable of detecting ovarian cancer with a high degree of sensitivity and specificity.

Methods: In this study, samples from 449 ovarian cancer patients and 922 healthy female individuals were measured. Peripheral blood of each individual was drawn in EDTA tubes. One class of biophysical property in blood samples was utilized for CDA tests. CDA data were conducted using SPSS, and the results are shown in **Table 1**.

Table 1 (A26) Results from Statistical Analysis of CDA

Group	Sample Size	Gender (Female %)	Age Range (year)	Average Age (year)	Median Age (year)	Average CDA (rel. units)	Median CDA (rel. units)	SD of CDA (rel. units)	AUC (rel. units)	Sensitivity	Specificity
Control	922	100	21-91	59	62	36.69	36.50	5.17	/	/	/
Cervical cancer	376	100	31-80	52	52	50.99	51.08	7.50	0.939	83.5%	90.0%

Table 1 (A27) Results from Statistical Analysis of CDA

Group	Sample Size	Gender (Female %)	Age Range (year)	Average Age (year)	Median Age (year)	Average CDA (rel. units)	Median CDA (rel. units)	SD of CDA (rel. units)	AUC (rel. units)	Sensitivity	Specificity
Control	922	100	21-91	59	62	36.69	36.50	5.17	/	/	/
Ovarian cancer	449	100	27-82	60	62	51.09	50.90	7.00	0.950	87.1%	90.0%

Results: The average CDA values of ovarian cancer and control groups were 51.09 and 36.69 (rel. units), respectively. The results indicated that ovarian cancer could be significantly distinguished from the control ($p < 0.001$). Area under ROC curve (AUC) was 0.950, and sensitivity and specificity was 87.1% and 90.0%, respectively.

Conclusions: Initial results showed that CDA technology could effectively distinguish ovarian cancer from healthy individuals. As a novel biophysical-based cancer detection approach with multilevel and multiparameter expressions, CDA could be a potential candidate for ovarian cancer detection.

A28 β1,4-Galactosyltransferase V modulates breast cancer stem cells through Wnt/β-catenin signaling pathway. Wei Tang, Jing Li. Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao, China.

Breast cancer is one of the most common malignant cancers among woman worldwide. In 2017, over 252,710 invasive breast cancer cases were diagnosed among women, and approximately 40,610 women were expected to die from breast cancer in the United States. Emerging evidences suggest that the existence of breast cancer stem cells (BCSCs) contributes to initiation, development, and recurrence of breast carcinomas. β1, 4-galactosyltransferase V (B4GalT5) catalyzes the addition of galactose to GlcNAcβ1-4Man of N-glycans, which is involved in embryogenesis as a growth regulator, but its impact on BCSC maintenance remains unclear. In this study, we found that B4GalT5 was upregulated in breast carcinoma and correlated with decreased patient survival by online database analysis. Notably, B4GalT5 expression was significantly upregulated in CD44+CD24$^{-/low}$ cells compared to other population by FACS sorting, and FACS analysis showed that B4GalT5 could significantly regulate the proportion of side population (SP) cells and CD44+CD24$^{-/low}$ cells. The abilities of wound healing and tumorsphere formation were positively associated with expression level of B4GalT5, which were affected by siRNA and a pharmacologic inhibitor, triptolide, indicating that B4GalT5 could regulate self-renewal ability of BCSCs. Mechanistically, we performed Western blotting and immunofluorescence to verify that Wnt signaling pathway was activated in the CD44+CD24$^{-/low}$ cells sorted from MCF-7ADR cells. The activation of Wnt signaling pathway was positively correlated with B4GalT5 by evaluation expression levels of Wnt signaling related proteins, such as β-catenin and Frizzled-1. In addition, B4GalT5 knockdown promoted degradation of β-catenin and Wnt receptor Frizzled-1 by ubiquitin proteasome pathway and lysosome pathway, respectively. Furthermore, RCA-I pulldown assay demonstrated regulation of N-glycosylation of Frizzled-1 by B4GalT5. Interestingly, we also found that B4GalT5 could locate on cell surface of MCF-7ADR, but not in MCF-7 that has lower proportion of BCSCs. However, cell surface B4GalT5 could not enrich ESA+CD44+CD24$^{-/low}$ cells by FACS analysis, so its role on cell surface needed further investigation. Taken together, these results suggest that B4GalT5 is involved in maintaining the stemness of BCSCs through regulating activation of Wnt/β-catenin signaling. Therefore, our study highlighted a previously unknown role of B4GalT5 in regulating stemness of breast cancer, and targeting this glycosyltransferase might be a promising therapeutic strategy.

A29 Targeting the unfolded protein response to overcome chemotherapy resistance. Xi Chen. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.

Limited sensitivity to chemotherapy and an immunosuppressive tumor microenvironment drives breast cancer mortality in HER2-negative breast cancer. We have identified the IRE1/XBP1 branch of the unfolded protein response (UPR) as a previously unexplored therapeutic vulnerability in MYC-driven breast cancer that enhances chemotherapy sensitivity and reverses the immunosuppressive tumor microenvironment. IRE1 is amplified in ~10% of human breast cancer, and frequently co-amplified with the MYC oncogene. Activation of MYC is synthetic lethal with IRE1/XBP1 pathway inhibition. We found that inhibition of the IRE1/XBP1 pathway with a highly selective IRE1 RNase inhibitor suppresses MYC-high-expressing, but not MYC-low-expressing, tumor growth in patient-derived xenograft (PDX) models. IRE1 inhibitor substantially enhances docetaxel efficacy, resulting in rapid tumor regression and complete eradication of the MYC^{high} PDX tumors. Furthermore, the IRE1 inhibitor plus docetaxel therapy triggers massive cytotoxic T-cell infiltration and depletion of immunosuppressive myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. Collectively, these data establish the synthetic lethal interaction of the IRE1/XBP1 pathway with MYC hyperactivation and reveal IRE1/XBP1 pathway as a critical resistance mechanism to chemotherapy.

A30 Aberrant expression of ROS1 is uncommon in ovarian cancer. Li Chen[1], Oscar Gee-Wan Wong[1], Ivy Tsz-Lo Wong[1], Jun Wang[2], Annie Nga-Yin Cheung[1,2]. [1]Department of Pathology, The University of Hong Kong, Hong Kong, [2]Department of Pathology, HKU-Shenzhen Hospital, Shenzhen, China.

Background: The receptor tyrosine kinase ROS1 is overexpressed in multiple cancer types and is demonstrated to drive tumorigenesis by fusing with a number of genes including FIG, CD74, and SLC34A2. However, in the context of ovarian cancer, the frequency and the clinicopathologic significance of ROS1 expression and gene fusion are largely undetermined. Herein, we aim to examine the expression profile of ROS1 and to detect the presence of known reported ROS1 fusion genes in ovarian cancer.

Methods: Immunohistochemistry for ROS1 was carried out on 94 formalin-fixed, paraffin-embedded ovarian carcinoma tissues (41 clear-cell, 24 serous, 19 endometrioid, and 10 mucinous); 10 benign and 9 borderline tumor cases were included for comparison. RT-qPCR was also performed to examine the expression profile of ROS1 at the mRNA level in a panel of 13 ovarian cancer cell lines and 2 normal ovarian epithelial cell lines. Given that ROS1 is often fused with other genes in cancer, fusion-specific qPCR was further conducted by designing primers targeting three most common ROS1 fusion genes, FIG-ROS1, CD74-ROS1, and SLC34A2-ROS1. To evaluate ROS1 expression at the protein level, immunoblotting of cell lysates was also performed in the above cell lines. Subcellular localization of ROS1 was determined by immunofluorescence.

Results: In our cohort of ovarian tumor clinical samples, ROS1 exhibited negative staining in all the cancer tissues. Interestingly, in a serous carcinoma cell line, SKOV3, we observed a higher expression of ROS1 transcripts compared to the other ovarian cancer cell lines. However, no expression of the three known ROS1 fusion transcripts was detected by RT-qPCR, suggesting that SKOV3 cells might not display genomic rearrangement leading to ROS1 gene fusion. Our immunoblotting analysis revealed that ROS1 protein products were barely detectable in all the cancer cell lines including SKOV3. In concordance with the immunoblotting results, fluorescent signals could not be detected in SKOV3 cells probed with the ROS1 antibody. Although the undetectable protein expression is contradictory to the expectation from the elevated ROS1 mRNA level in SKOV3, we believe this discordance may be caused by underlying post-transcriptional mechanisms.

Conclusion: This study shows that our cohort of ovarian tumor tissue and panel of ovarian cancer cell lines do not express ROS1 proteins and any common ROS1 fusion products. The role of ROS1 in ovarian carcinogenesis still remains to be elucidated.

A31 Nuclear PAK4 impairs homologous recombination via RAD51C to promote genome instability in high-grade serous ovarian cancer. Ivy Tsz-Lo Wong, Oscar Gee-Wan Wong, Annie Nga-Yin Cheung. Department of Pathology, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong SAR, China.

p21-activating kinase 4 (PAK4) is a nucleo-cytoplasmic shuttling protein frequently overexpressed in cancer. In particular, nuclear PAK4 (nPAK4) expression correlates with clinical aggressiveness and survival in ovarian cancer, although the mechanism still remains unclear. By analyzing the nPAK4 transcriptome and nPAK4-bound genomic landscape, we previously found that nPAK4 affects transcription of genes involved in DNA damage repair in high-grade serous ovarian cancer (HGSC) cells, and identified RAD51C as a potential direct transcriptional repression target of nPAK4. In this study, we further validated the mode of regulation of RAD51C imposed by nPAK4, and interrogated whether nPAK4 functionally affects DNA repair through RAD51C to disrupt genome stability in HGSC. We demonstrated by ChIP-PCR that nPAK4 occupies the RAD51C promoter region at its consensus DNA binding site in HGSC cells. Overexpression and knockdown studies confirmed nPAK4 negatively regulates RAD51C expression in HGSC cells, whereas nPAK4 expression but not that of cytoplasmic PAK4 or total PAK4, correlates with RAD51C expression in a panel of serous ovarian cancer cell lines. Analysis of 316 ovarian serous cystadenocarcinoma samples from TCGA showed that gene alterations in *PAK4* and *RAD51C* were mutually exclusive, suggesting a potential coregulatory role of PAK4/RAD51C in HGSC. Further investigation on nPAK4-mediated DNA repair activity revealed nPAK4 significantly reduced homologous recombination competence in a DR-GFP reporter assay, he effect of which is in part contributed by RAD51C. Concurrently, we found a significant elevation in the number of micronuclei formed in PAK4 overexpressing SKOV3 compared to control SKOV3 cells. In addition, PAK4 overexpression exacerbates the formation of aberrant chromosomal structures upon exposure to the chemotherapy drug cisplatin. Correlation studies of TCGA data from ovarian serous cystadenocarcinoma patients revealed that genome-wide copy number aberrations are positively correlated with PAK4 expression. Taken together, we found that HGSC nPAK4 is a novel negative regulator of RAD51C. The impact of nPAK4-mediated RAD51C downregulation contributes to genome instability in HGSC by functionally disrupting homologous recombination.

A32 Multiple alterations on genes encoding components of the SWItch/Sucrose Non-Fermentable complex could be found in human papillomavirus-negative cervical cancer. Oscar G.W. Wong[1], Claire L.Y. Cheung[1], Stephanie S. Liu[2], Hextan Y.S. Ngan[2], Annie N.Y. Cheung[1]. [1]Department of Pathology, The University of Hong Kong, Hong Kong, [2]Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong.

Cervical cancer without apparent association with human papillomavirus (HPV) infection is a rare type of the malignancy with poor prognosis but little is known about it. With HPV vaccine becoming widely accepted, it is expected that the proportion of HPV-negative cancer will rise. Understanding the molecular etiology of HPV-negative cancer is therefore important for the management of cervical cancer in the future. To compare the genetics of HPV-negative and -positive tumors, we characterized the coding genomes of 24 cervical cancers with known high-risk HPV (hrHPV) statues verified by INNO-LiPA HPV Genotyping Extra II assay using whole-exome sequencing (WES). In total we analyzed 4 HPV-negative and 20 HPV-positive cervical cancer samples by WES. There was no significant difference between the number of nonsynonymous SNPs and INDELs between HPV-negative and HPV-positive cancers. Here a preliminary analysis of the WES data focusing on 27 genes encoding components of the (SWItch/Sucrose Non-Fermentable) SWI/SNF complex is presented. In contrast to previous genomic data form TCGA, no protein changing SNP or INDEL was identified on *ARID1A*. Instead a small insertion adding one amino acid to the ARID1B protein was found in one of the HPV-negative cancers but not in the HPV-positive cases. Several SNPs were also found on some other genes encoding subunits of SWI/SNF complex in at least one of the HPV-negative cancers. Our data suggested that while mutations in SWI/SNF complex are common among HPV-negative cervical cancers, the genetic makeup of HPV-negative cervical cancer may be more diversified than previously identified.

A33 S-Nitrosoglutathione inhibits triple-negative breast cancer metastasis by promoting CYR61 protein S-nitrosylation. Yusheng Lu[1,2], Sudan He[2], Xingtian Yang[2], Suhong Yu[2], Lee Jia[1,2]. [1]Institute of Oceanography, Minjiang University, Fuzhou, Fujian, China, [2]Cancer Metastasis Alert and Prevention Center, and Fujian Provincial Key Laboratory of Cancer Metastasis Chemoprevention and Chemotherapy, Fuzhou University, Fuzhou, China.

The human cysteine-rich protein 61 (CYR61) plays a key role in cancer metastasis, including cancer cell proliferation, migration, adhesion, invasion, differentiation, and survival. Nitro oxide (NO) can covalently bind to the SH group of cysteines (termed S-nitrosylation) to regulate protein functions. CYR61 is highly expressed in breast cancer in particular, in the triple-negative breast cancer (TNBC) cell line MDA-MB-231. Inspired by our previous successes in S-nitrosylation of various molecules, we here present the first evidence that S-nitrosylation of CYR61 can significantly inhibit metastatic aggressiveness of MDA-MB-231 cell line. S-nitrosylation by S-nitrosoglutathione (GSNO) of CYR61 reaches a plateau quickly and is confirmed by spectroscopic analysis and biotin-switch assay. S-nitrosylation of CYR61 protein in MDA-MB-231 cells significantly attenuates the metastatic ability of these cells, including inhibiting hetero-adhesion of cancer cells to endothelial cells, reducing cancer cell migration and invasion ability, interrupting cancer cells/platelets interplay, and making the adhered cells unattached. The attenuation increases proportionally with the degree of S-nitrosylation to CYR61 naturally expressed or genetically manipulated, and can be demonstrated in mice, where S-nitrosylation of these cell lines inhibits their acute seeding in lungs after tail vein injection, and late development into the metastatic nodes. The present novel findings demonstrate that both endogenous and exogenous S-nitrosylation of CYR61 can operate in concert to attenuate the metastatic aggressiveness of the cysteine-rich TNBC.

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A34 Association of nutritional status with platinum-containing chemotherapy-induced nausea and vomit in patients with advanced breast cancer. Wei Li, Jin Lu, Yuechuan Zhang, Xiangliang Liu, Wei Ji, Lingyu Li, Jiuwei Cui. Cancer center, The First Affiliated Hospital of Jilin University, Changchun, Jilin, China.

Objective: By analyzing different nutritional indicators of patients with advanced breast cancer, to determine the relationship between the indicators and the occurrence of chemotherapy-induced nausea and vomiting.

Methods: Chemotherapy-induced nausea and vomiting (CINV) refers to nausea (a state characterized by the regurgitation caused by stomach upset and/or the urge of vomiting) and vomiting (the reflection of vomiting the stomach content) that are caused by or related to chemotherapy drugs. It is the adverse event of Grade 2 or above in Common Terminology Criteria for Adverse Events (CTCAE) grades. 173 patients with advanced breast cancer who had complete nutritional status data and received chemotherapy were divided into two groups according to whether CINV occurred during their treatment. The relationship between CINV and different nutritional indicators was retrospectively analyzed.

Results: All patients enrolled were women. Among them, in the CINV group, there were 90 cases with an average age of 55.09±10.76 years. There were 83 cases without CINV. The average age was 56.53±9.96 years. The age differences between patients with/without CINV showed no statistical significance. The mean albumin (Alb) of patients with/without CINV was 38.57±4.70 g/L and 40.86±4.10g g/L, and the difference was statistically significant (P<0.05). The mean prealbumin (PA) of patients with/without CINV was 0.22±0.09 g/L and 0.23±0.05 g/L, and showed no statistical significance. The mean NLR was 2.67±2.43 and 2.15±1.08, respectively, and showed no statistical significance. The mean PLR was 199.58±230.46 and 180.58±147.41) respectively, and showed no statistical significance. The mean hemoglobin (Hb) in patients with CINV was 116.92±20.63 g/L and was 124.72±12.70 g/L in the other group, and the difference was statistically significant. The C-reactive protein value in each group was 10.14±24.32 mg/L and 5.68±15.02 mg/L, and showed no statistical significance. The PG-SGA scores of patients with/without CINV were 7.73±4.08 points and 2.76±1.79 points, and the difference was statistically significant. Binary logistic analysis included Hb, Alb, and PG-SGA as analysis factors, indicating that high PG-SGA score was a risk factor for CINV (B=0.652, S.E.=0.097, Waldχ2=40.63, Exp(B)=1.919, 95.0% CI=1.607~2.450, P<0.001).

Conclusions: In the univariate analysis, the differences of albumin, hemoglobin, and PG-SGA scores were statistically significant in patients with advanced breast cancer who had CINV or not. The PG-SGA score was significantly associated with the occurrence of CINV. A high PG-SGA score is an independent risk factor for CINV.

A35 Chinese medicine formulae WD-3 induces breast cancer cell death and suppresses aerobic glycolysis.

Xiaodan Zhu[1], Qing Xue[1], Chongyi Lu[2], Meng Yang[2], Jianliang You[1], Chunhui Jin[1]. [1]Nanjing University of Chinese Medicine Wuxi Hospital, Wuxi, Jiangsu, China, [2]Anti Cancer Biotech (Beijing) CO, LTD, Beijing, China.

Background: Breast cancer cells heavily relied on the glycolytic pathway regardless of oxygen tension. According to traditional Chinese medicine (TCM), spleen deficiency was one of the manifestations of breast cancer. Our previously clinical studies had found that spleen-regulating prescription WD-3 could inhibit breast cancer growth. However, the mechanism involved in WD-3 mediated antitumor activity is not entirely clear. The macroscopic spleen of TCM and the microscopic mitochondria of modern medicine had similar functions. The hypothesis about "Glycolytic-Mitochondria-Spleen-Tumor" became a possibility. The present study was designed to investigate the antitumor activity of WD-3 against breast cancer in vitro and explore the mechanisms by which WD-3 acts on breast cancer cells with focus on glycolytic pathway.

Methods: 4 different human breast cancer cell lines (MDA-MB-231, BT-549, MCF-7, MCF-7/ADR-RES) were used to investigate the inhibitory effect of WD-3 on proliferation of breast cancer cells by MTT assay. Morphologic changes of cells under the action of WD-3 were observed by Hoechst 33258 nuclear staining confocal scanning microscope and fluorescence microscope. By transfection and filtering, 4 green and red fluorescent protein transferred human breast cancer models were established, including MDA-MB-231 DUAL, BT-549 DUAL, MCF-7 DUAL, and MCF-7/ADR-RES DUAL (the cytoplasm express RFP, and the nucleus express GFP). The morphologic changes and apoptosis of the cell models on the effect of WD-3 were observed with inverted real-time fluorescence microscopy imaging. Flow cytometry was used to detect breast cancer cell death rate. The levels of ATP, ADP, and AMP in breast cancer cells after WD-3 intervention were measured to evaluate the energy supply of breast cancer cells. The expression of hexokinase II protein and mRNA was detected by Western blotting and RT-PCR, and the effect of WD-3 on hexokinase II was further investigated.

Results: The experimental results showed that WD-3 had an obvious inhibitory effect on the proliferation of 4 human breast cancer cell line MDA-MB-231, BT-549, MCF-7, and MCF-7/ADR-RES in a concentration- and time-dependent manner. The apoptosis ratio was increased as evidenced by flow cytometric analysis (p<0.05). Besides apoptosis, pyroptosis was observed by inverted real-time fluorescence microscopy imaging. However, WD-3 can reduce the levels of ATP and EC in BT-549 cells, and inhibit the expression of hexokinase II protein and mRNA. There was no significant difference in the levels of ATP and EC or the expression of hexokinase II protein and mRNA in the other cell lines.

Conclusion: WD-3 can significantly inhibit proliferation of breast cancer cells. Inhibition of breast cancer cell proliferation by interfering with the glycolytic pathway may be one of the antitumor mechanisms of WD-3. It may be considered for further studies in vivo and in clinical trials.

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A36 SMAD4 somatic mutations in head and neck carcinoma (HNSCC) are associated with tumor progression. Chung-Ji Liu, Li-Han Lin. Department of Oral and Maxillofacial Surgery, Taipei MacKay Memorial Hospital, Taipei, Taiwan.

In recent years, the incidence and mortality rate of head and neck squamous cell carcinoma (HNSCC) have been increasing worldwide. Therefore, to understand the genomic alterations involved in HNSCC carcinogenesis is a crucial issue for diagnosis or therapy. The tumor suppressor gene Smad4 (DPC4) at chromosome 18q21.1 belongs to the Smad family, which mediates the TGFbeta signaling pathway suppressing epithelial cell growth. In this study, a multiplex PCR-based NGS analysis was performed to identify that 4% of tumors harbor damaging SMAD4 mutation. Experiments by knocking down of endogenous SMAD4 expression and exogenous expression of SMAD4 domains unequivocally designated that SMAD4 was suppressive to the migration and invasion of HNSCC cells. Functional analysis identified that the nonsense mutation in SMAD4 may render the loss of suppression against tumor progression. This is the first study that used the multiplex PCR-based NGS analysis to signify the eminent occurrence of nonsynonymous SMAD4 mutations in HNSCC, which are prognostic determinants of patients. The gene analysis proposed in this study may facilitate the identification of SMAD4 mutation as a diagnostic marker or therapeutic target in head and neck cancer. The analysis strategy proposed for SMAD4 mutation detection in this study may be validated as a platform to assist mutation screening.

A38 Regulation of T-ALL associated gene expression in the 3D genome. Lu Yang[1], Fengling Chen[2], Haichuan Zhu[1], Xiaojun Huang[3], Yang Chen2, Hong Wu[1]. [1]The MOE Key Laboratory of Cell Proliferation and Differentiation, School of Life Sciences, Peking-Tsinghua Center for Life Sciences, Beijing Advanced Innovation Center for Genomics, Peking University, Beijing, China, [2]MOE Key Laboratory of Bioinformatics; Bioinformatics Division and Center for Synthetic & Systems Biology, BNRist, Department of Automation, Tsinghua University, Beijing, China, [3]The People's Hospital, Peking University Institute of Hematology, Peking University, Beijing, China.

Acute T lymphoblastic leukemia (T-ALL) is a common hematologic malignancy characterized by the development and infiltration of large numbers of immature T cells. Recent multiplexed genomic analyses, including RNA sequencing and whole-exome sequencing (WES) of large T-ALL cohorts, have identified many novel driver genes and pathways involved in T-ALL leukemogenesis. However, these studies are limited in the coding sequences and cannot identify those alternations that alter the expressions of the driver genes. As genome-wide association studies uncovered that more than 90% of disease-associated variants lie in noncoding DNA and recent understanding that chromosome 3D structure plays important roles in gene regulation, studying T-ALL-associated chromosomal structure variations in the context of the 3D genome will enable the identification of novel regulatory components and provide insights into the molecular mechanisms underlying T-ALL. To comprehensively map 3D chromatin organization in T-ALL, we conducted chromatin interaction analysis by Bridge Linker-Hi-C (BL-Hi-C) to map neighborhoods and other cis-regulatory interactions in patients with T-ALL and healthy controls. We observed global 3D genome alterations and increased number of contact domain boundaries in T-ALL. Unsupervised hierarchical clustering can classify T-ALL as three subtypes, named early T-cell progenitor (ETP), ETP-like, and non-ETP. We also identified 30 novel translocations. By integrating RNA-seq and ChIP-seq analysis, we found that the Sub-topologically associating domain (SubTAD) formation between URE and *SPI1* promoter is correlated to a higher expression of *SPI1* in ETP-ALL. We also observed that TADs containing the oncogenic transcription factor loci exhibit increased intra-TAD connectivity and gene expression. Overall, our work provides a comprehensive view of chromatin organization in T-ALL and a resource to discover molecular mechanisms of dysregulated genes in T-ALL.

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A39 Chronic inflammation does not increase somatic mutation in colon mucosa cells. Jimin Han[1,2],* Yu Chen[1,2],* Xusheng Wang[1,2], Yaojiong Wu[1,2]. [1]School of Life Sciences, Tsinghua University, Beijing, China, [2]The Shenzhen Key Laboratory of Health Sciences and Technology, Graduate School at Shenzhen, Tsinghua University, Beijing, China. *Co-first authors.

Cancers are caused by the accumulation of genomic alterations, and it is also well established that chronic inflammation increases cancer risk. In this study, we attempted to evaluate whether chronic inflammation contributed to the accumulation of genomic alterations. To this end, we utilized the repeated dextran sodium sulfate (DSS) treatment-induced chronic colitis model in mice. Whole-exome sequencing (WES) (200x coverage) was performed to detect somatic variations in colon mucosa cells. The exome of colon muscularis mucosae cells in

the same mice was also sequenced to eliminate germline variations. We found that there was no significant difference in SNP/InDel mutation between mice with colitis and age-matched healthy control mice. Similarly, the number of CNV in the colitis mice was also not significantly different from that of the control mice. Notably, we found that the SNP/InDel number was obviously elevated in elder mice (56 week, SNP/InDel: 860) compared with young mice (8 week, SNP/InDel: 629). Hence, our data indicate that chronic inflammation does not increase the number of gene mutations in colon epithelial cells in mice, but aging does.

GI CANCERS

A40 Does detection of LN metastasis mean worse prognosis in stage M0 colon cancer? A modified stage based on LNR. Yang Lv[1], Qing-Yang Feng[1], Song-Bin Lin[2], Yi-Hao Mao[1], Yu-Qiu Xu[1], Peng Zheng[1], Liang-Liang Yang[1], Guo-dong He[1], Jian-Min Xu[1]. [1]Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China, [2]Department of General Surgery, Zhongshan Hospital Xiamen Branch, Fudan University, Xiamen, China.

Lymph node ratio (LNR) seems to be more precise than N stage in classifying cancer stage. Thus we aim to construct a modified classification system based on LNR for colon cancer without distant metastasis. This study enrolled two independent cohorts of patients. Primary cohort enrolled 2,152 patients from 2008 to 2013 in Zhongshan Hospital, Fudan University. Validation cohort consisted of 77,406 patients from SEER registry from 2004 to 2014. The inclusion criteria were pathologically confirmed colon cancer, AJCC stage I/II/III. The exclusion criteria included incomplete follow-up information, rectal cancer, and multiple primary sites. The prognostic value of LNR for overall survival was evaluated. Cut-off value of LNR was determined by the X-tile. Predictive performance of modified classification was determined by concordance index. After analysis, 0.05 and 0.50 were determined as the best threshold value of LNR. A value of <0.05, 0.05-0.50, and >0.50 was reclassified as the mN0, mN1, and mN2 stage. A modified classification based on mN0, mN1, and mN2 was further constructed for stage I\II\III colon cancer. C-index of modified classification was statistically more precise than AJCC classification (0.687 versus 0.605, P<0.001). Same results can also be determined in the validation cohort (0.715 versus 0.640, P<0.001). Furthermore, a prognostic nomogram including independent factors was constructed. Constructed nomogram showed good performance according to calibration curve. Clinical value of LNR level was preferable than classic N stage in colon cancer patients. Our proposed classification based on LNR and AJCC T category can effectively differentiate the patients with varied survival outcomes.

Author contributions statement: Yang Lv, Qing-Yang Feng, Song-Bin Lin and Yi-Hao Mao contributed equally to this work.

A41 PET-CT standard uptake value predict bevacizumab-containing chemotherapy response and prognosis for unresectable colorectal cancer liver metastasis. Yang Lv[1], Qing-Yang Feng[1], Wen-tao Tang[1], Yu-Qiu Xu[1], Song-Bin Lin[2], Yi-Hao Mao[1], Peng Zheng[1], Liang-Liang Yang[1], Guo-dong He[1], Jian-Min Xu[1]. [1]Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China, [2]Department of General Surgery, Zhongshan Hospital Xiamen Branch, Fudan University, Xiamen, China.

Background: Standard uptake value (SUV) of PET-CT is an indicator of tumor metabolic response. In this paper, we aim to explore the clinical value of SUV on the unresectable colorectal cancer liver metastasis (CRLM) patients receiving bevacizumab-containing chemotherapy.

Method: This study was performed retrospectively. A total of 185 CRLM patients between April 2011 to December 2015 with complete clinical data were included in this study. All the enrolled patients were assigned into two treatment cohorts (bevacizumab plus first-line chemotherapy cohort and chemotherapy only cohort). A blind, independent radiologist evaluated images for RECIST and morphologic response. All clinical variables and various PET/CT parameters were statistically compared with progression-free survival (PFS) and overall survival (OS). Primary and metastatic tumor SUV were selected for analysis.

Results: Among the 185 patients, 101 patients received first-line chemotherapy plus bevacizumab (beva cohort); 84 patients only received first-line chemotherapy (CMT cohort). Baseline characteristics of two cohorts showed no statistical difference (P>0.05). Primary SUV level was correlated with primary tumor size, while metastatic SUV was statistically correlated with metastatic tumor number and tumor size (P=0.000). Primary lesion, metastatic lesion SUV, and elevation of SUV demonstrated prognostic role for OS (P<0.05). SUV gap was statistically associated with optimal response in bevacizumab cohort (P=0.03) and no-PD status in chemotherapy cohort (P=0.019), respectively. After multivariate analysis, elevated SUV is an independent risk factor for OS (P=0.000). Besides, elevation of SUV between metastatic and primary lesion can be a predictive factor for bevacizumab survival benefit.

Conclusion: PET-CT scan is important for CRLM patients. Our study demonstrated that an elevation of SUV was a better prognostic and predictive marker for CRLM patients.

Author contributions statement: Yang Lv, Qing-Yang Feng, Wen-Tao Tang and Yu-Qiu Xu contributed equally to this work.

A42 CD-associated microRNAs in four types of gastrointestinal cancers using RNA-seq samples, clinical data analysis, and in vitro study. Hanyu Zhang[1,2], Parham Jabbarzadeh Kaboli[1,2], Mingxing Li[1,2], Yueshui Zhao[1,2], Xu Wu[1,2], Jing Li[1,2,3], Zhangang Xiao[1,2]. [1]Laboratory of Molecular Pharmacology, Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou, Sichuan, China, [2]South Sichuan Institution for Translational Medicine, Luzhou, Sichuan, China, [3]Department of Oncology and Hematology, Hospital (T.C.M) Affiliated to Southwest Medical University, Luzhou, Sichuan, China.

Introduction: MicroRNAs (miRNAs) are small noncoding RNAs that act as tumor suppressors or oncogenes. Their importance in prognosis, diagnosis, and treatment of gastric and colorectal cancer has been widely reported; however, the associations of miRNAs to cluster of differentiation (CD) molecules in gastrointestinal (GI) cancers was not greatly studied. In the current study, our goals were to analyze the relationship between miRNAs and CD molecules in GI cancer, to identify miRNAs that may have an effect on particular CD molecules, and to validate this hypothesis in the GI cancer cell lines. The objective of the present study is to measure the mRNA levels of CD molecules and confirm biologically functional miRNAs in GI cancer. Accordingly, we have taken full advantage of the database for the analysis of miRNA, as well as hundreds of RNA-Seq from the TCGA consortium. Negatively correlated CD molecules and miRNAs in clinical data of patients with GI cancer have also been studied.

Methods: TMK1 and BGC823 gastric cancer cell lines, SW480 colorectal cancer cell line, and Eca109 esophageal carcinoma cell line were obtained from The Chinese University of Hong Kong. We purchased miR-559, miR-200a, and miR-1236 mimics from GenePharma (China). To understand the relationship between miRNAs and CD molecules in GI cancer, we used plenty of websites that analyzed CD molecules or miRNAs. Moreover, to verify the association between overall survival and miRNAs expression and the relationship between miRNA and CD molecules, we analyzed hundreds of patient RNA-Seq samples with gastric, colon, rectum, pancreatic, and esophageal cancers. Finally, these miRNAs identified as potentially important regulators of CD molecules specifically were robustly selected and validated by qPCR in four types of GI cancer cell line.

Results: Our in vitro study demonstrates that miR-200a and miR1236 are potential therapeutic markers in GI cancer. In the present study, miR-559 as a CD-associated miRNA in GI cancer is reported for the first time and its further study is suggested. We concluded that miR-200, miR-1236, and miR-559 are closely associated with CD86, CD160, and CD81, respectively. In addition to the miRNAs negatively correlated with the expression level of CD molecules, we were curious to find their associations with the prognosis of cancer patients as well. Thus we performed survival analysis in GI cancers from TCGA datasets. The resulting Kaplan–Meier survival curves show that the miRNA panel is able to separate patients into higher and lower risk groups by median PI. Based on this data, the high expression of miR-148a was significantly associated with poor survival in colon cancer. This situation is the same as miR-6808 in rectum cancer. In stomach cancer, patients with low levels of miR-4458 and miR-200a expression showed reduced survival time compared with patients with high levels of these two miRNAs expression. For patients with high expression of miR-4458, miR-148A, miR-429, miR-135A1 and miR-547 had a significantly good prognosis in pancreatic cancer ($P < 0.05$, log-rank test). In summary, from the analysis of TCGA data, some miRNAs are purposely discovered as prognosis markers in types of cancers.

Conclusion: We pinpoint all miRNAs expressed in immune cells that might negatively regulate CD molecules. Although a few studies have continuously suggested that GI cancers have been inhibited by CD-associated miRNAs, direct involvement of CD molecules regulated by miRNAs in GI cancers has not been revealed yet. In summary, our finding revealed miR-200, miR-1236, and miR-559 are closely associated with CD86, CD160, and CD81, respectively. Collectively, these results highlight novel immunologically miRNA-based strategies in patients with GI cancers.

A43 Hormonal suppression of symmetric stem cell division leads to reduced gastric tumorigenesis. Wenju Chang[1,2,3], Hongshan Wang[1,3,4], Woosook Kim[3], Antonia Sepulveda[5], Adam J. Bass[6], Timothy C. Wang[3]. [1]Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China, [2]Shanghai Engineering Research Center of Colorectal Cancer Minimally Invasive (17DZ2252600), Shanghai, China, [3]Division of Digestive and Liver Diseases, Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, NY, USA, [4]Gastric Cancer Center of Zhongshan Hospital, Fudan University, Shanghai, China, [5]Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, NY, USA, [6]Division of Molecular and Cellular Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.

Background: Epithelial cancers are thought to originate most commonly from stem cells, but in most tissue the exact origin and mechanism for cancer initiation has remained obscure. To date, the cell of origin for gastric cancer and the mechanisms that have evolved by stem cells to limit the acquisition of mutations and tumor development have not been well defined. In the mammalian gastrointestinal tract, few stem cells have been described that undergo predominant asymmetric cell division, and the role of deregulated cell division of stem cell in tumorigenesis remains unclear.

Method: Transgene mice including *Cck2r*- CreERT2-BAC mice, *Eef1a1*-LSL-*Notch1(IC)* mice, LSL-*Trp53*^{R172H} mice, *Apc*^{flox} mice, *Lgr5*-DTR-GFP mice, *Lgr5*-GFP-*IRES*-CreERT2 mice, GAS-KO mice and *Cck2r*$^{-/-}$ mice were used for this study. Lineage tracing analysis and assessment of immunofluorescence was applied to assess the stem cell properties of *Cck2r* and *Lgr5* expressing cell. Crypt and single-cell isolation and cultures were performed to assess pair-cell analysis. Mouse gastric cancer model were established by gene knockout of *Apc* plus *P53* mutant, and *h.felis*/MNU induction model. Whole-exome sequencing was analyzed based on gastrin-deficient (GAS-KO) mice and WT mice on the C57BL/6J background treated with gastrin pump under *h.felis*/MNU induction model of gastric antrum cancer.

Result: We report 4 major discoveries regarding gastric antral stem cells and cancer biology: 1) While we previously identified the Cck2r^{+} cell as the +4 stem cell in the gastric antrum, we show here that it marks a DLL1^{high}/NICD^{low}/Numb^{high} stem cell population, which is a label-retaining cell that normally undergoes predominant asymmetric cell division. 2) Notch signaling and the antral niche cell-derived hormone, gastrin, play a key role in maintaining Cck2r^{+} stem cell quiescence and asymmetric cell division. This suggests a potential role for gut endocrine cells in providing such niche signals. 3) Cck2r^{+} stem cells serve as a cell of origin for gastric antrum cancer. With both conditional knockouts of tumor suppressor genes, and in unbiased carcinogenesis studies, Cck2r^{+} cells appear especially capable of giving rise to neoplastic lesions. Gastrin deficiency caused deregulation of symmetric division of Cck2r^{+} cell, resulting in accumulation of gene mutation during carcinogenesis and more rapid tumor progression. 4) The suppression of symmetric division and antral tumor development by gastrin was associated with a marked reduction in gastric cancer mutational load as revealed by exomic sequencing.

Conclusion: Gastric tumorigenesis is associated with increased symmetric cell division of Cck2r^{+} stem cell in gastric antrum that facilitates mutation accumulation, and the hormone gastrin from adjacent niche cells provides an important niche signal involved regulation of asymmetric/symmetric division by the Cck2r^{+} stem cell.

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A44 Association of PI3 kinase with KIT is crucial for secondary mutation of KIT-mediated resistance to imatinib in the relapsed GISTs. Guangrong Zhu, Jun Shi, Shaoting Zhang, Ling Huang, Jianmin Sun. School of Basic Medical Sciences, Ningxia Medical University, Yinchuan, China.

Secondary mutation of KIT-mediated resistance to imatinib leads to relapse and treatment failure of GISTs. In this study, we found that combination of primary and secondary mutation but not either mutation alone of KIT dramatically enhanced the ligand-independent activation of the receptor and reduced its sensitivity to Imatinib which is used in the targeted therapy of gastrointestinal stromal tumors (GISTs). It has been reported that PI3 kinase is essential for the ligand-independent activation of primary mutation of KIT in GISTs. By blocking the association with PI3 kinase, we found that the ligand-independent activation of the combined primary and secondary mutation of KIT, the receptor-mediated cell survival and proliferation were inhibited. Unlike that, PI3 kinase inhibitor could not inhibit the ligand-independent activation of the combined primary and secondary mutation of KIT although the inhibitor can inhibit the cell survival and proliferation. Furthermore, loss of the association with PI3 kinase, but not PI3 kinase inhibitor, improved the sensitivity of the combined primary and secondary mutation of KIT to Imatinib, meaning that PI3 kinase plays a crucial role in secondary mutation of KIT-mediated resistance to the targeted therapy in the recurrence of GISTs.

A45 Using deep learning system in endoscopy for screening of early esophageal squamous cell carcinoma based on white light imaging. Bing Li, Yun-Shi Zhong, Ping-Hong Zhou. Endoscopy Center, Zhongshan Hospital of Fudan University, Shanghai, China.

Background and Aims: Artificial intelligence (AI)-based technologies in medicine, including a triage or screening tool for gastrointestinal disease, is advancing rapidly. Whereas most computer-aided systems have focused on colonoscopy examination, however, less effort has been made on improving the efficiency of screening for esophageal squamous cell carcinoma (ESCC). Here, we developed and validated a novel system of computer-aided detection with a deep neural network (DNN-CAD) to localize and identify early ESCC under conventional endoscopic white light imaging (WLI).

Methods: We collected 2,428 (1,332 abnormal, 1,096 normal) esophagoscope images from 746 patients during January 2016 to April 2018 to set up a novel DNN-CAD system. To evaluate the accuracy of the DNN-CAD system, we prepared a validation dataset contains 187 images from 52 patients. Eight endoscopists, who were divided into three groups including senior, mid-level, and junior, were asked to review the images of the validation set. The diagnostic results, including accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were compared between DNN-CAD and endoscopists.

Results: DNN-CAD receiver-operating characteristic curve showed the area under the curve was 0.9637. For 187 images in validation dataset, DNN-CAD correctly identified 89 of the 91 early ESCC lesions, with a sensitivity of 98.8%, a specificity of 85.4%, and an accuracy of 91.4%. The positive predictive and negative predictive values of the DNN-CAD were 86.4% and 97.6%, respectively. The senior group achieved a result with an average diagnostic accuracy of 90.6%, whereas the average diagnostic accuracy of the junior group was lower at 77.7%. The accuracy for early ESCC of the DNN-CAD was similar to that of the endoscopists in the senior group, and higher than that in other groups with significant difference (P<0.05). In addition, DNN-CAD achieved the highest sensitivity, while the specificity was lower than the average rate of endoscopists (89.6%). After referring to the results of DNN-CAD, the average diagnostic abilities of endoscopists were all improved, in terms of sensitivity (74.4% vs. 84.4%, P = 0.033), specificity (89.6% vs. 92.3%, P = 0.419), accuracy (82.2% vs. 89.9%, P = 0.011), PPV (88.3% vs. 91.6%, P = 0.327), and NPV (79.6% vs. 87.7%, P = 0.040).

Conclusions: This novel CNN-CAD system for screening of early ESCC has high accuracy and sensitivity, which can help endoscopists based on conventional endoscopy with standard white light to detect lesions ignored before.

A46 Inhibitor of DNA binding 1 (Id1) mediates stemness of colorectal cancer cells through the Id1-c-Myc-PLAC8 axis via the Wnt/β-catenin and Shh signaling pathways.

Yanxia Sun[1,2],* Xiaolan Lai[3],* Yue Yu[1,2], Jieyu Li[2,4], Lei Cao[3], Wansong Lin[2,4], Chuanzhong Huang[2,4], Jinrong Liao[2,4], Wei Chen[1,2], Chao Li[5], Chunkang Yang[6], Mingan Ying[6], Qiang Chen[3], Yunbin Ye[2,4]. [1]School of Basic Medical Sciences, Fujian Medical University, Fuzhou, Fujian Province, China, [2]Laboratory of Immuno-Oncology, Fujian Provincial Cancer Hospital, Fuzhou, Fujian Province, China, [3]Department of Medical Oncology, Union Hospital of Fujian Medical University, Fuzhou, Fujian Province, China, [4]Fujian Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian Province, China, [5]Department of Pathology, Fujian Provincial Cancer Hospital, Fuzhou, Fujian Province, China, [6]Department of Abdominal Surgery, Fujian Provincial Cancer Hospital, Fuzhou, Fujian Province, China. *These authors contributed equally to this work.

Background/Aims: Inhibitor of DNA binding 1 (Id1) is essential for the maintenance of self-renewal of neural and embryonic stem cells; however, its roles in human colorectal stem-like cells (CSCs) and epithelial-mesenchymal transition (EMT) are still elusive. This study aimed to examine the associations of Id1 with the stemness of colorectal cancer (CRC) and explore the underlying mechanisms.

Methods: The mRNA and protein levels of Id1 and CD133 were detected by qPCR and immunohistochemistry analysis in normal mucosa and primary CRC specimens. Id1 was stably knockdown (KD) in human colon cancer cell lines. Spheres forming assay and tumorigenic assay were performed to evaluate self-renewal capacity in vitro and tumor initiation in vivo, respectively. CSC and EMT relative markers and TCF/LEF activity were assessed after Id1 KD in HCT116 cells.

Results: qPCR assay showed higher Id1 and CD133 mRNA expression in the CRC specimens than in normal mucosa specimens ($P < 0.05$). Immunohistochemistry detected high cytoplasmic expression of Id1 in 35 CRC specimens (46.7%), and high CD133 expression in 22 CRC specimens (29.3%) and negative expression in 18 normal mucosal specimens. High Id1 expression was found to positively correlate with poor differentiation ($P = 0.034$), and CD133 expression was found to correlate with T category ($P = 0.002$). In addition, Spearman correlation analysis revealed a positive correlation between Id1 and CD133 expression in CRC patients ($P < 0.05$). Id1 KD resulted in suppression of proliferation, cell-colony formation, self-renewal capability, and CSC-like features in HCT116 cells, and Id1 KD impaired the tumor-initiating capability in CRC cells. In addition, Id1 maintained the stemness of CRC cells via the Id1-c-Myc-PLAC8 axis through activating the Wnt/β-catenin and Shh signaling pathways.

Conclusions: Id1 expression is significantly associated with CD133 expression in CRC patients, and Id1 KD impairs CSC-like capacity and reverses EMT traits, partially via the Wnt/β-catenin signaling. Id1 may be a promising therapeutic target against colon CSCs.

A47 Circular RNA hsa_circ_0006232 acts as the sponge of microRNA-138 to promote colorectal cancer progression. Wentao Tang, Pengyang Zhou, Zhengchuan Niu, Meiling Ji, Wenju Chang, Li Ren, Jianmin Xu. Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China.

Background: As a new class of noncoding RNAs, circular RNAs (circRNAs) play an important role in regulating gene expression through acting as microRNA (miRNA) sponge in the development and various types of disease, especially in cancer. However, the expression profile and function of circRNAs in human colorectal cancer (CRC) remain largely unknown.

Methods: CircRNA expression were profiled by Arraystar human circRNA chip in 8 paired CRC and normal colorectal tissues. RNA in situ hybridization (ISH) of circTRERF1(hsa_circ_0006232) was performed in another 15 paired tissues. Real- time quantitative PCR (qRT-PCR) was used to detect circTRERF1 expression in 133 consecutive CRC tissues. Cell viability, colony formation, and cell cycle assays in vitro and subcutaneous tumor formation assay in vivo in HCT-116 cells were carried out to investigate the function of circTRERF1. RNA pulldown assay through circTRERF1 probe was performed to identify the potential binding miRNA of circTRERF1. The hybridization of circTRERF1 and miR-138 probes was performed to testify their co-localization. Western blot was carried out to test CDK6 expression.

Results: circTRERF1 was the highest-expressed circRNA in CRC tissues. CRC patients with high circTRERF1 expression had shortened survival. Silencing circTRERF1 significantly suppressed CRC cell viability, cloning formation and induced cell cycle G1 arrest, and inhibited subcutaneous tumor growth in nude mice. miR-138 was significantly enriched in the RNA pulldown complex. Furthermore, the tumor-inhibiting effect of circTRERF1 silencing was blocked by miR-138 inhibitor. In addition, both silencing of circTRERF1 and overexpression of miR-138 in CRC cells could downregulate CDK6 expression.

Conclusion: High circTRERF1 expression in CRC tissues may serve as a poor-prognosis predictor for CRC patients. CircTRERF1 promotes CRC progression by acting as the sponge of miR-138 to promote CDK6 expression, suggesting that circTRERF1 is a potential target in CRC treatment.

A48 Tissue residency of innate lymphoid cells altered in microenvironment of colon cancer. Jingtao Chen[1], Jing Wu[1], Hang Cheng[1,2], Tete Li[1], Xinping Lv[1], Guoxia Zang[1], Shan Zhu[1]. [1]Institute of Translational Medicine, The First Hospital, Jilin University, Changchun, China, [2]Department of Pediatrics, The First Hospital, Jilin University, Changchun, China.

Innate lymphoid cells (ILCs) are important tissue-resident innate immune cells that play important roles in the early stages of the immune response during inflammation, tissue repair, and maintenance of epithelial integrity. Various ILC subsets in human tissues are associated with the pathogenesis and progression of chronic infections and inflammatory diseases. However, the role of ILCs in colon cancer, especially in the human colon tumor microenvironment, remains unclear. Therefore, we collected tissue samples from 58 patients with colon cancer and analyzed the role of ILCs in tumor microenvironment. We found the number of ILC3s, especially NKP44+ILC3s, was altered, and negatively correlated with the pathologic grade of colon cancer. RNA-Seq analysis for ILC3s showed thousands of differentially expressed genes, mostly involved in the development of tumor. Hence, ILC3s in tumor tissue may play a certain role in the development of tumors. Understanding the role of ILCs in colon cancer microenvironment may help to develop new strategies for metastatic and recurrent colon cancer.

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A49 Functional characterization of mixed lineage leukemia 4 (MLL4) in PDAC tumorigenesis. Frederic K. C. Fung[1], Yinxin Zhu[1], Chi Hin Wong[1], Chi Han Li[1], Yangchao Chen[1,2]. [1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, [2]Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China.

Pancreatic ductal adenocarcinoma (PDAC) accounted for 90% of pancreatic cancer, is the fourth leading cause of death and characterized by its poor prognosis; therefore, it is emerged to identify a novel gene that can be utilized as a therapeutic target against PDAC. Our previous work identified MLL4, which is a histone H3 lysine 4 (H3K4) methyltransferase of MLL family, as one of the tumorigenesis-associated gene to PDAC tumorigenesis. Our preliminary findings showed that MLL4 and its putative activated targets were frequently upregulated in PDAC cell lines and tissues. Previous study reported that SET domain of MLL4 is the major functional domain for H3H4 methylation and we hypothesize that the SET domain contributes to PDAC development and progression. Here, we aim to characterize the in vitro functional role of SET domain of MLL4 and its activating targets regarding to PDAC tumorigenesis. Results illustrate that ectopic SET expression in PDAC cells promotes cell proliferation, anchorage-dependent colony formation as well as cell

migration. Moreover, depletion of MLL4 activated targets by siRNAs impairs cell growth and alleviates the migratory ability in PDAC cells, suggesting that these activating targets contribute to the PDAC proliferation and metastasis. Taken together, our findings demonstrated that SET domain of MLL4 and its activating targets exhibit the crucial role in PDAC tumorigenesis and development, suggesting MLL4 might be a novel therapeutic target against PDAC.

A50 Variant of SNP rs1317082 at CCSlnc362 (RP11-362K14.5) creates a binding site for miR-4658 and diminishes the susceptibility to CRC. Chaoqin Shen[1],* Tingting Yan[1],* Jing-Yuan Fang[1], Haoyan Chen[1], Jie Hong[1]. [1]State Key Laboratory for Oncogenes and Related Genes, Key Laboratory of Gastroenterology & Hepatology, Ministry of Health, Division of Gastroenterology and Hepatology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Cancer Institute, Shanghai Institute of Digestive Disease, Shanghai, China, [2]Department of Urology, Fudan University Shanghai Cancer Center, Shanghai, China, [3]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China. *These authors contributed equally to this article.

Genome-wide association studies (GWAS) have identified several loci harboring variants that affect the risk of colorectal cancer; however, the specific mechanisms by which germline variation influences the tumorigenesis of colorectal cancer (CRC) remains unrevealed. We found the T>C variant of rs1317082, locating at the exon 1 of lncRNA RP11-362K14.5 (CCSlnc362), was predicted to be a protective locus for cancer. However, the specific role of CCSlnc362 and the interaction between CCSlnc362 and rs1317082 variation in colorectal cancer and its mechanisms remain unclear. Here, we explored the expression and function of CCSlnc362 in CRC cells and tissues. We found lncRNA CCSlnc362 expression was significantly increased in CRC samples. Follow-up functional experiments elucidated that downregulation of CCSlnc362 inhibited cell proliferation, arrested cell cycle, and promoted apoptosis in CRC cells. The T>C variant of rs1317082 at CCSlnc362 exon 1 created a binding site for miR-4658 to reduce the expression of CCSlnc362 and thus decreased the susceptibility to CRC. Our findings have provided supporting evidence for the protective role of rs1317082 variation and the potential oncogenic role of lncRNA CCSlnc362 in CRC. The data shed new light on the relationship between germline variation, miRNAs, and lncRNAs and open a new avenue for targeted therapy in CRC.

A51 Senescent fibroblasts promote tumorigenicity of colorectal cancer via regulating GALC gene expression. Mengdi Yang, Jing Sun, ZhiYu Wang, Guangyu Yao, Hui Zhao. Department of Internal Oncology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China.

Introduction: The mechanism of colorectal cancer (CRC)-associated senescent cells that may play a crucial role in tumor progression remains unclear.

Methods: 16 random patients with CRC who have been treated with XELOX were collected, then we analyzed the tumor stroma ratio (TSR) by hematoxylin-eosin (HE) staining. Immunohistochemistry (IHC) and β-galactosidase (SaβG) staining were used to verify a stable model of senescent fibroblasts. The scanning-electron microscopy (SEM) was used to look at the morphologic and structure difference in coculture system and CRC cells. Last, in vivo experiments and transcriptomics analysis were done to verify the effect and potential mechanisms of senescent fibroblast on tumor formation.

Results: HE staining showed the TSR of CRC was related to the effect of XELOX. IHC showed high expression of galactosylceramidase (GALC) is significant to SAβG staining, which is related to CRC patients' survival. We noted that LV-GALC cells have undergone cell cycle arrest. The morphology and cell cycle characteristics were accompanied by upregulation of p16 and p21 genes and downregulation of hTERT expression. In coculture system, LV-GALC cells significantly increased the proliferation of LoVo cells, which indicated that senescent fibroblasts could promote the viability and metastasis of CRC cells. SEM analysis verified that overexpressed GALC cause cell morphology resembling an aging phenotype. Subcutaneous tumor formation and transcriptomic analysis suggest potential mechanisms for the above results.

Discussion: We demonstrated that senescent fibroblasts critically regulate tumorigenicity of CRC cells through induction of pathways; targeting these processes could improve efficacy of clinical treatment.

A52 SET8 targeted by miR-192/ -215 induces senescence in human gastric carcinoma cells via p21. Xiaojing Zhang, Yin Peng, Yuli Gao, Jian Wang, Zhe Jin. Department of Pathology, School of Basic Medical Sciences, Shenzhen University Health Sciences Center, Shenzhen, Guangdong Province, China.

SET8 (also known as SETD8, PR-SET7, and KMT5A) belongs to the SET domain-containing methyltransferase family and specifically catalyzes H4K20me1. Set8 is involved in diverse biologic processes, including cancer occurrence

and progression. SET8 was also found as a new target as well as a key regulator of the cellular senescence signaling. One aspect of the senescence is "cellular senescence," which means irreversible ceasing of proliferation, a state also called replicative senescence. Given this presumably tumor-suppressive nature, senescence is viewed as a barrier to cancer and therefore a potential target for therapeutic regiment. Herein, we provide evidence that SET8 displays decreased expression in gastric cancer (GC) and is directly regulated by miR-192/-215 in GC. We demonstrate that SET8 is involved in triggering cellular senescence in GC as a tumor suppressor in vivo and in vitro. The 3'UTR of SET8 is targeted by miR-192/-215. Overexpression of miR-192/-215 resulted in promotion of GC cells proliferation, migration, and invasion. Additionally, the effect of SET8 inducing senescence was mediated by regulating the senescence switch gene p21. Taken together, miR-192/-215/SET8/p21 circuit emerges as a key regulator of GC via repressing the expression of senescence proteins that is highly associated with proliferation, cell cycle regulation, migration, and metastasis in GC. The study will provide experimental evidence for investigating the potential therapeutic target in GC.

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A53 Construction of colorectal cancer subtypes based on stroma/immune-specific gene expression and prediction of drug response. Xinyu Zhang, Haoyan Chen, Xiaoqiang Zhu, Dan Ma. Shanghai Institute of Digestive Disease, Shanghai, China.

Objectives: Gene expression profiling has been extensively used to classify molecular subtypes in colorectal cancer (CRC), given that tumor transcriptome signals not only derive from cancer cells but also from the tumor microenvironment, and recent studies have shown that noncancerous components might affect the classification of CRC subtypes. Thus, we hypothesized that using stroma-specific gene signature would be more effective to identify CRC subtypes with clinical relevance.

Subjects and Methods: To this end, we analyzed 1963 gene expression profiles from 13 datasets. We firstly constructed a signature in which genes were both stroma-specifically expressed and associated with drug response. Further, we identified CRC stroma-specific subtypes (CRSS) using K-means clustering based on the signature and verified the classification in another two cohorts. We also used nearest template prediction (NTP) algorithm to predict drug response. We estimated immune and stromal cell populations using microenvironment cell populations-counter (MCP-counter) method.

Results: The CRSS subtypes were associated with distinct clinicopathologic, molecular, and phenotypic characteristics and specific enrichments of gene signatures and signaling pathways: (i) CRSS-A: nonserrated adenomas, colon crypt top derived, glycolytic, epithelial, KRAS-mutant, sensitive to cetuximab, enriched with NK cells; (ii) CRSS-B: nonserrated adenomas, colon crypt base derived, DNA replication activity, epithelial, BRAF wild-type, TP53 mutant, chromosomal stability, distal CRC, sensitive to cetuximab; (iii) CRSS-C: serrated adenomas, colon crypt top derived, interleukin-6 pathway activity, epithelial, microsatellite instability (MSI), BRAF mutant, hypermutation, chromosomal instability, CpG island methylator phenotype, proximal CRC, sensitive to gefitinib, good prognosis, enriched with cytotoxic lymphocytes and monocytic lineage; (iv) CRSS-D: nonserrated adenomas, colon crypt top derived, interleukin-2 pathway activity, epithelial–mesenchymal transition (EMT), chromosomal stability, sensitive to FOLFIRI and FOLFOX chemotherapy regimens; (v) CRSS-E: serrated adenomas, colon crypt base derived, EMT, immune pathways activation, poor prognosis, sensitive to FOLFIRI and FOLFOX, enriched with endothelial cells and fibroblasts; (vi) CRSS-F: serrated adenomas, colon crypt base derived, EMT, IGF1 pathway activity, poor prognosis, sensitive to FOLFIRI and FOLFOX, enriched with endothelial cells, fibroblasts, and monocytic lineage.

Conclusions: We classified CRC into six molecular subtypes (CRSS). The identification of CRSS subtypes is critical, as it provides possibilities to identify robust prognostic models and provide more precise therapeutic options for each subtype after surgical resection.

A54 TMT-based isobaric labeling quantitative proteomics reveals distinctive protein expression profile and potential biomarker in pancreatic cancer. Xiu Dong, Lanping Zhou, Fang Liu, Yulin Sun, Jianqiang Cai, Xiaohang Zhao. State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignant tumors with a 5-year survival rate less than 8%. Currently the diagnosis of PDAC relies on serum CA19-9, whose sensitivity and specificity is limited. The aim of this study was to explore the differential protein expression profile that fosters the aggressive PDAC and identify potential biomarker.

Methods: A total of 5 pairs of PDAC tumor tissues and adjacent normal tissues were first analyzed using TMT-based isobaric labeling approaches. Bioinformatics analyzed differential protein expression profile and identified candidate protein for PDAC diagnosis. Immunohistochemistry in PDAC tissues (n=152) and enzyme-linked immunosorbent assay (ELISA) in PDAC plasmas (n=51) were used to validate its expression. Circulating tumor cells (CTCs) were isolated from the same patients and counted. The receiver operator characteristics (ROC) curve analyzed the value of the multiple markers for PDAC.

Results: A total of 3,771 differentially expressed proteins were identified. When the cutoff line was set at 1.3-fold with paired *P* value less than 0.05, 244 proteins were recognized as differentially expressed proteins, including 173 upregulated proteins and 71 downregulated proteins. GO analysis revealed that upregulated proteins were mainly located in extracellular space or matrix and were associated with cellular signal transduction, cell binding, and adhesion. One of the upregulated proteins, thrombospondin-2 (THBS-2), can be secreted into extracellular matrix, which may play an important role in PDAC development and serve as a potential biomarker. Immunohistochemistry validated that the expression level of THBS-2 was extremely higher in tumor tissue ($P<0.0001$), and appeared in the extracellular matrix. ELISA revealed that the concentration of THBS-2 in PDAC plasma was significantly higher than healthy control ($P=0.0265$). The respective area under ROC curve (AUC) was 0.699 for THBS-2, 0.843 for CTC amount, and 0.857 for the combination of THBS-2 and CTC amount in PDAC vs. noncancer individuals.

Conclusion: We have demonstrated the distinctive protein expression profile of PDAC, which may provide better understanding of the aggressive PDAC signature. In addition, THBS-2 is likely to be important for PDAC development and has shown great potential to be a novel biomarker for PDAC diagnosis, especially when combined with CTCs. Further studies will investigate the function and mechanism of THBS-2 in PDAC progression, and estimate THBS-2 –CTC diagnostic panel for clinical use in large cohort.

A55 PURα mediates epithelial–mesenchymal transition to promote esophageal squamous cell carcinoma metastasis via modulation of Wnt/β-catenin signaling. Jiajia Gao, Lusong Tian, Lanping Zhou, Fang Liu, Yulin Sun, Xiaohang Zhao. State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Esophageal squamous cell carcinoma (ESCC) is one of the most common malignancies worldwide. The lack of effective early detection markers often results in the late diagnosis of the disease. Purine-rich element binding protein alpha (PURα) is a key player in the regulation of the cell cycle and oncogenic transformation. Previous study shows that the expression of PURα increased significantly in esophageal squamous carcinoma tissues; however, the molecular mechanism induced by PURα is unknown. In this study, we investigated the function of PURα in ESCC. In vitro and in vivo studies using several ESCC cell lines revealed that PURα promoted cell proliferation, migration, and invasion. Moreover, exogenous PURα expression caused epithelial-mesenchymal transition (EMT) in ESCC cells, which was confirmed by the downregulation of the epithelial markers and the upregulation of the mesenchymal markers. Mechanistically, PURα potently enhanced the Wnt/β-catenin signaling pathway by upregulating key Wnt component β-catenin. Importantly, the knockdown of β-catenin could reverse the PURα-induced EMT and decrease the migration and invasion ability of ESCC cells. In conclusion, PURα promoted ESCC progression and EMT were regulated by the Wnt/β-catenin signaling pathway, suggesting PURα as a potential molecular target to prevent ESCC progression.

A56 Serum autoantibody as biomarkers for early lymph node metastasis in patients with esophageal squamous cell carcinoma. Yan Zhao, Shuyang Dai, Yan Sun, Lanping Zhou, Yulin Sun, Xiaohang Zhao. State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Esophageal squamous cell carcinoma (ESCC) is the fourth most common malignant cancer in China with the overall 5-year survival rate less than 30%. It was generally accepted that lymph node metastasis (LNM) is a predominant unfavorable prognostic factor in ESCC. However, as many as 10-30% of early-stage (T1) cases that are comprised of mucosal and submucosal cancer can also metastasize to regional lymph nodes and behave like advanced tumor. Therefore, it is needed to find some effective biomarkers for distinguishing LNM cases from early-stage patients. In the present study, we adopted the Human Proteome Microarrays containing 19,394 recombinant proteins to comprehensively discover the differentially expressed serum autoantibodies between healthy controls as well as patients with T1N0M0 (group A) and T1N1M0 (group B) stages. A total of 185 and 195 tumor-associated antigens (TAAs) were identified in group A and B, respectively. Also, 42 TAAs showed significant difference between group A and B. Furthermore, Western blot and indirect ELISA were used to validate these differently expressed TAAs in the validation

cohort. Some candidates showed good discrimination between LNM and nonmetastatic cases. The in-depth validation is still ongoing in our lab. In conclusion, the novel serum autoantibodies may serve as potential biomarkers for early-stage ESCC with LN metastasis.

A57 Characterization of Epstein-Barr virus transcripts in gastric adenocarcinoma. Yarong Tian, Guojiang Xie, Sanna Abrahamsson, Sri Harsha Meghadri, Jonas Carlsten, Ka-Wei Tang. Institute of Biomedicine, Sahlgrenska Academy, Gothenburg, Sweden.

Approximately every tenth gastric tumor is caused by the Epstein-Barr virus (EBV). EBV sequences detected in gastric cancer RNA-Seq data generated by The Cancer Genome Atlas have generated conflicting results. The goal of this study is i) to characterize the EBV transcripts in gastric gancer samples and ii) to investigate the role of EBV in gastric cancer. Transcriptome datasets of 25 EBV-positive gastric adenocarcinoma were downloaded from publicly available database. Then prinseq-lite tool was used for quality control of the reads. The filtered reads were mapped against the EBV reference genome and the alignment results were analyzed. Split reads, transcription start site, and poly-A containing reads were further analyzed in order to determine strand specificity. The majority of viral RNA originates from the BamHI-A region. By identifying the transcription start site and poly-A site, we conclude that RPMS1, a long noncoding RNA, is the major EBV-transcript in gastric cancer. Poly-A containing reads were only detected at the predicted site downstream of the polyA-signal in RPMS1 exon 7. Furthermore, a splice-reads analysis suggests the existence of different splice-variants of the RPMS1 transcript. In this study, we have characterized the expression pattern of EBV RNA in gastric cancer, which may provide insight into EBV oncogenesis. EBV as the main associated infectious agent in gastric cancer mainly transcribes RPMS1 in the tumor. However, we know nothing about the function of this long transcript. Better characterization of this gene will be helpful for understanding EBV-associated gastric cancer.

OTHER

A58 Smad1 inhibits p53 acetylation and functions in glioblastoma. Ying Yin, Lingli Gong, Jian Zou, Bo Zhang, Zhening Pu, Yaling Hu, Jingjing Wang, Li Ji. Center of Clinical Research, Wuxi People's Hospital of Nanjing Medical University, Nanjing, China.

Numerous studies indicated that loss-of-function mutations of *TP53/*p53 regulate malignant phenotypes by gain-of-function (GOF) in virous cancers. However, p53 is frequently increased and associated with pathologic grade of glioma and overall survival, suggesting that GOF cannot fully explain the roles of p53 protein in gliomas. Post-translational modification (PTM) aberrant is a key molecular event that causes p53 tumor suppressor function loss or transformation in tumors except mutations. Recent studies have reported that acetylation is critically required for p53 activation, while whether or how p53 acetylation contributes to malignant of gliomas is still unknown. Here, we analyzed the databases from The Cancer Genome Atlas (TCGA) and REMBRANT (Repository for Molecular BRA in Neoplasia DaTa) and determined that *TP53* mRNA is higher in lower-grade glioma (LGG) and glioblastoma (GBM) than it in normal brains. To explore the pathologic association of acetylated p53 (Ac-p53), we detected p53 and acetylated p53 level in GBM tissue microarray using immunohistochemistry (IHC) with p53 and acetyl-p53 (Lys382) antibody. IHC results confirmed the frequency increase of p53 protein in GBM tissues. Kaplan-Meier analyses indicated that p53 or Ac-p53 positive patients have better survival predicted than those with negative p53 or Ac-p53. All the Ac-p53 positive cases were both p53 positive, while nearly half of p53 positive cases showed acetylation negative. Importantly, in p53 positive cases, patients with acetylated p53 have better survival outcomes than those with negative Ac-p53. Here, we reported that the acetylation of p53 in GBM is negatively associated with Smad1. Functional studies showed that Smad1 was an oncoprotein in GBM by promoting tumorigenesis and chemoresistance in vitro and in vivo. cDNA microarray, luciferase reporter, and ChIP assays revealed that the tumor promotion of Smad1 is partially through negatively regulating p53 pathway. The negative correlation between Smad1 and Ac-p53 was further confirmed in GBM tissues by IHC, double immunofluorescence (IF) and Western blot assay. It suggests that Smad1 is an important regulator of p53 acetylation in GBM. p300 is essential for p53 acetylation in GBM cells. A novel finding is that Smad1 forms a ternary complex with p53 (including p53 mutants) and p300. Our study demonstrated that Smad1 binds p53 and blocks p53-p300 interaction via inhibiting p53 phosphorylation at S46/S392, and the regulation of Smad1 on p53 phosphorylation is p53-binding dependently. We revealed

that the MH2 domain of Smad1 is responsible for the interaction with p53, and the region of L3 loop (418–428aa) localizing in MH2 domain was identified as a determinant of Smad1-p53 interaction. Smad1 also negatively regulates the acetylation of mutated p53. For the first time, the present study demonstrated that acetylation at C-terminal domain (CTD) reverses the GOF of the p53 mutates (R273C, R248Q, R175H) in GBM, explaining why p53 acetylation predicts better outcomes, no matter mutated or wide type. It also suggests that loss of p53 acetylation derived from high Smad1 expression is a risk factor for GBM patients. Interestingly, Smad1 is also acetylated by p300 and p300 mediated acetylation is required for Smad1 activity. p300 depletion almost abolishes promoter-binding of Smad1 as detected by ChIP-qPCR. Collectively, this study demonstrated that acetylation is a decisive factor for p53 in tumor suppressor or oncogene and Smad1 acts as an oncoprotein partially through impairing p53 phosphorylation and following acetylation in GBM.

A59 Inhibition of STAT3 signaling pathway by niclosamide improves antitumor activity and impairs melanoma metastasis. Yongxia Zhu, Meiqin Yang, Yue Wang. Department of Obstetrics and Gynecology, Henan Provincial People's Hospital, Zhengzhou, China.

The incidence of melanoma, one of the most malignant cancer, is increasing worldwide. The aggressiveness of melanoma is dependent on the high metastatic potential of melanoma cells, which can explain the need for new candidates that inhibit melanoma growth and metastasis. Drug repurposing, using drugs already approved for other indication, may allow progressing through the drug development process more quickly as well as reducing project costs compared to traditional de novo drug discovery. Niclosamide, an FDA-approved anthelmintic drug, has been used to treat tapeworm infection for approximately 50 years. Niclosamide has been identified as a potent STAT3 inhibitor that can suppress STAT3 phosphorylation at Tyr705 and transcript activity. Notably, STAT3 activity increased melanoma invasiveness and is required for active melanogenesis by regulating tyrosinase gene expression and transcript activity. In addition, STAT3 is widely expressed in melanoma, suggesting that STAT3 is a promising anticancer target for melanoma therapy. We hypothesized that niclosamide, a STAT3 inhibitor, might be useful in the treatment of patient with melanoma. To verify this hypothesis, this study evaluated the biologic activities of niclosamide in melanoma in vitro and in vivo. Firstly, we found that niclosamide showed potent growth inhibition against melanoma cell lines and induced apoptosis of cancer cells in a concentration-dependent manner. Further, Western blot analysis indicated that the occurrence of cell apoptosis was correlated with the activation of Bax and cleaved caspase-3 and decreased the expression of Bcl-2. Moreover, niclosamide markedly impaired melanoma cells migration and invasion, with the reduction of phosphorylated $STAT3^{Tyr705}$ and inhibition of matrix metalloproteinase (MMP) -2 and MMP-9. Furthermore, in the xenograft model of A375, intraperitoneal administration of niclosamide could inhibit tumor growth and tumor weight in a dose-dependent manner without obvious side effects. Meanwhile, histologic and immunohistochemical analyses related a decrease in Ki-67-positive cells, p-$STAT^{Try705}$-positive cells and an increase in cleaved caspase-3-positive cells. Importantly, niclosamide significantly inhibited the pulmonary metastasis in B16-F10 melanoma lung metastasis model, including the number of lung metastatic nodules and the lung/body coefficient. Taken together, these results demonstrated that niclosamide may be a promising candidate for inhibiting melanoma growth and metastasis.

A60 Trigger-activated autonomous stepwise assembly of net-like DNA nanostructure and its dendritic amplification for the fluorescence imaging of biomolecules within living cells. Huo Xu[1], Lisha Wang[2], Yifan Jiang[1], Zhifa Shen[2], Zai-Sheng Wu[1]. [1]Cancer Metastasis Alert and Prevention Center, Pharmaceutical Photocatalysis of State Key Laboratory of Photocatalysis on Energy and Environment, and Fujian Provincial Key Laboratory of Cancer Metastasis Chemoprevention and Chemotherapy, College of Chemistry, Fuzhou University, Fuzhou, China, [2]Key Laboratory of Laboratory Medicine, Ministry of Education of China, Zhejiang Provincial Key Laboratory of Medicine Genetics, School of Laboratory Medicine and Life Sciences, Institute of Functional Nucleic Acids and Personalized Cancer Theranostics, Wenzhou Medical University, Wenzhou, China.

Visualization, sensitive, specific, and stable detection of tumor suppressor- or oncogene- microRNAs (miRNAs) remains challenging and is highly significant for clinical diagnostics. To solve this problem, we have developed a target-triggered assembly of net-like DNA nanostructure for intracellular miRNA imaging based on branched hybridization chain reaction (HCR). In this approach, only two hairpin-shaped metastable probes (HP1 and HP2) were involved. Intracellular miRNAs trigger the self-assembly of net-like DNA nanostructure in the cytoplasm with high efficiency and stability, achieving the goal of improved signal amplification capacity (1:N) for the low expression of miRNA in the complex intracellular environment. The process of target-triggered net-like nanostructure assembly was confirmed by nondenaturing polyacrylamide gel electrophoresis (n-PAGE) analysis. Moreover, its morphologies were validated by AFM images, and the specificity and sensitivity of intracellular miRNA were also demonstrated by confocal laser scanning microscopy and flow cytometry. By taking advantage of intelligent design,

the proposed method was also successfully applied to image other miRNA inside living cells, which provides an effective, universal, and stable platform for miRNA detection in living cells.

A61 SUMOylation is required for ZFHX3 to promote cell proliferation and tumor growth in breast cancer cells. Rui Wu[1,2], Jiali Fang[1], Wenxuan Chen[2], Jinming Liu[1], Mingcheng Liu[1,2], Gui Ma[1], Zhiqian Zhang[2], Baotong Zhang[3], Jin-Tang Dong[1,2,3]. [1]Department of Genetics and Cell Biology, College of Life Sciences, Nankai University, Tianjin, China, [2]Southern University of Science and Technology, School of Medicine, Shenzhen, Guangdong, China, [3]Emory Winship Cancer Institute, Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, GA, USA.

ZFHX3 is a large transcription factor that plays important roles in multiple physiologic and pathologic processes including development and tumorigenesis. ZFHX3 clearly has a tumor-suppressor activity in prostate cancer according to genetic studies, but ZFHX3 also promotes cell proliferation in mammary gland development. Our previous study demonstrated that ZFHX3 can be SUMOylated, and SUMOylated ZFHX3 forms nuclear body-like structures in epithelial cells. However, it is currently unknown whether SUMOylation alters the function of ZFHX3 in cell proliferation and tumorigenesis. Here we report the effect of SUMOylation on the function of ZFHX3 in cell proliferation and tumorigenesis along with how ZFHX3 SUMOylation is regulated in breast cancer cells. We found that ZFHX3 was SUMOylated by SUMO1, SUMO2, and SUMO3 at multiple lysine (K) residues, and K2806 was the major SUMO acceptor site. The E2 SUMO-conjugating enzyme UBC9 was essential for ZFHX3's SUMOylation, and PIASXα was identified as the major E3 ligase for the SUMOylation of ZFHX3. Among all the 6 deSUMOylation enzymes, SENP1 was identified as the major one for the deSUMOylation of ZFHX3. Interestingly, SUMOylation stabilized ZFHX3, and the stabilization was mediated by reduced protein degradation via the ubiquitin proteasome pathway, as SUMOylation compromised the binding of the ubiquitin E3 ligase EFP to ZFHX3 and subsequent ZFHX3 degradation. Functionally, overexpression of ZFHX3 in triple-negative breast cancer cell line MDA-MB-231 promoted, but loss of SUMOylation dramatically reduced, cell proliferation, cell migration, and tumor growth in nude mice. These results suggest that different from a tumor-suppressor activity in prostate cancer, ZFHX3 plays an oncogenic role in breast cancer, and SUMOylation is a novel mechanism for maintaining the oncogenic activity of ZFHX3 in breast cancer.

A62 Improved distribution of liposomal doxorubicin in tumors by bacterial proteolytic activity. Hiroaki Shirai, Kosuke Tsukada. Graduate School of Fundamental Science and Technology, Keio University, Yokohama, Kanagawa, Japan.

Poor penetration of anticancer drugs in tumors is one of the limitations of chemotherapy (1). To overcome this limitation, anaerobic bacteria have been used for cancer therapy because bacterial motility wins high interstitial fluid pressure of tumors and thus is able to penetrate in tumors (2). Notably, the efficacy of bacterial cancer therapy is particularly high in combination with conventional chemotherapy; however, the mechanisms of this synergy remain unclear. Thus, we aim to understand the mechanisms of the synergy of the combination therapy. This work sets focus on the role of bacterial proteolytic activity on ECM of collagen in drug delivery in tumors. The objective of this work is to mathematically model interstitial fluid flow, transport of liposomal doxorubicin (Doxil), and bacterial transport in tumors. For the geometry of tumors, a cylindroid with a radius of 4 mm is used for the modeling. Interstitial fluid flow in tumors is described as Darcy's law. Penetration of liposomal doxorubicin in tumors included infiltration from blood vessels, diffusion, and interstitial fluid flow. Bacterial penetration in tumors included motility and growth terms. Bacterial proteolysis rate on ECM of collagen is expressed in Michaelis-Menten kinetics. The governing equations above are solved by finite element method using COMSOL Multiphysics with appropriate initial and boundary conditions. Simulated interstitial fluid pressure in tumor is high in tumors and drops at the periphery, which agreed with previous experimental work (3). Simulated distribution of liposomal doxorubicin in osteocarcinoma agreed reasonably well with previous experimental literature (4,5). Penetration of liposomal doxorubicin in tumors is limited, in part due to high interstitial fluid pressure. *Clostridium* penetrated in tumors due to motility. Maximum reaction rate, V_{max}, was changed at 0 (without reaction), 0.002, 0.003 mg ml^{-1} min^{-1} as V_{max} value of *Clostridium* on ECM of collagen is unknown from literature. With higher reaction rate, liposomal doxorubicin distributed in tumors faster. Collage content of tumors decreased over time due to bacterial proteolytic activity. Decrease in collagen content increased hydraulic conductivity in tumor tissues, which decreased interstitial fluid pressure. Decreased interstitial fluid pressure in turn increased infiltration of liposomal doxorubicin from blood vessels to tissues, and thus, increased distribution of doxorubicin in tumors. Moreover, decrease in collagen content allowed liposomal doxorubicin to diffusion in the tissues faster. Finally, the simulated doxorubicin concentrations in tumor tissues at 4 h are 2.6, 5.9 ug ml^{-1} at V_{max} of 0, 0.003 mg ml^{-1} min^{-1}, respectively, while those of previous work at 4 h were 2.5 and 13.0 ug ml^{-1} for Doxil alone and Doxil and C. noyvi-NT, respectively (6). This work concludes that bacterial proteolytic activity

improved distribution of liposomal doxorubicin in tumors by decreasing interstitial fluid pressure and increasing diffusion of liposomal doxoroubicin in the tissues. This work at least in part explains the previous experimental work that coadministration of Doxil and *C. noyvi-NT* enhanced doxorubicin concentration 5–6 times compared with Doxil alone (6).

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A63 IGF1R overexpression and HPV16 variants induce resistance to radiotherapy in uterine cervical cancer.

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Several causes of the variable radiotherapy (RT) efficacy have been studied without convincing results. If the HPV-16 infection has been hypothesized to be a predictor of poor response to RT, the real clinical impact of HPV-16 and its prognosis significance is still to be demonstrated. In recent studies it was reported that factor receptor insulin-like growth1 (IGF1R) is overexpressed by effect of E6 AA-a, and E-G350 HPV-16 variants. Previous studies have shown a role for IGF1R in cellular radioresistance in cervical carcinoma; Moreno-Acosta et al. (2012, 2017) found that the overexpression of IGF1R is a predictive marker for patients (HPV16 (+)) undergoing radiotherapy because overexpression of this receptor confers 28.6 times greater risk of treatment failure, and that the frequency of protein expression of IGF1R alpha and beta was very similar; however, it was observed that only IGFIR beta significantly affected OS. The aim of the present study was to prospectively report the detection of HPV-16 variants, gene expression IGF1R and assess the relationship with treatment response. Detection of HPV 16 variants of 19 patients by PCR-SSCP and direct sequencing and analysis of IGF1R gene expression by real-time PCR were performed. Of these patients, 15 underwent exclusive radiotherapy and four underwent radiochemotherapy. Three months after treatment completion, out of the 15 patients receiving exclusive RT, 8 experienced complete responses: 3 with the European T350 variant (E-T350 and IGF1R low expression, 2 with the European G350 variant (E-G350) and IGF1R negative expression), 2 with an undetermined European variant (E-Nd) and IGF1R negative expression), and 1 with an Asian-American variant (AAa) and IGF1R negative expression. The other 7 experienced no complete response: Three patients were diagnosed with a partial response (2 E-T350, 1 E- G350, and IGF1R overexpression), 3 had a stable tumor (2 E-G350, 1 E-Nd and IGF1R overexpression), and 1 experienced tumor progression (AAa and IGF1R overexpression). We concluded that presence of E-G350 and non-European (e.g., AA) variants and overexpression of IGF1R in the no complete response group could be related with radioresistance. Larger prospective trials are needed to validate the presence of HPV-16 variants and IGF1R expression as biomarkers of radioresistance. Studies about the identification of prognostic and predictive biomarkers in the path towards an adequate therapeutic management in the area of radiation oncology of patients with uterine cervical cancer have been developed. These indicate the importance of future utility of growth factors such as IGF1R and the presence of variants of HPV16 as potential biomarkers. For these already considered therapeutic targets has been working on its modulation through the search, design, and development of inhibitors or radio sensitizers both synthetic and natural.

A64 Astaxanthin improves breast cancer via mitochondria.

Xiao-ding Lin, Xie Xi. Key Laboratory of Tropical Biological Resources of the Ministry of Education, Hainan University, Haikou, China.

Purpose: To investigate the anticancer effect of astaxanthin (CAS:472-61-7) on MDA-MB-231 cells and underlying mechanism.

Methods: The anticancer effect of astaxanthin was tested on MDA-MB-231 cells in vitro by CCK8 and live/dead cell viability assay. JC-1 probes used to observe the effect of astaxanthin on mitochondrial membrane potential of MDA-MB-231 cells under a fluorescence microscope. MitoSox Red was used to determine mitochondrial ROS (mt ROS) production under a fluorescence microscope. Immunofluorescence was used to detect Nrf2 translocation. Western blot was used to evaluate the expression of Bax/Bcl-2/Nrf2/Keap1/HO-1.

Results: Astaxanthin decreased the viability of MDA-MB-231 cells. Astaxanthin acted on mitochondria of MDA-MB-231 cells, reduced mitochondrial membrane potential and mt ROS production. Astaxanthin reduced the MDA-MB-231 cell viability through decreased intracellular generation of ROS. Astaxanthin promoted the expression of Bax, reduced the expression of Bcl-2, and activated the Nrf2-ARE signaling pathway.

Conclusion: The results suggest that astaxanthin could be a potential mitochondrial targeted drug that can inhibit breast cancer cells.

A65 RNA-binding protein CELF6 is cell cycle-regulated and controls cancer cell proliferation by stabilizing p21.

Gang Liu, Qianwen Zhang, Li Xia, Mengjuan Shi, Haowei Zhang, Naihan Xu. State Key Laboratory of Chemical Oncogenomics, The Graduate School at Shenzhen, Tsinghua University, Shenzhen, China.

RNA-binding proteins (RBPs) play crucial role in post-transcriptional regulation in eukaryotes and control multiple aspects of cell behavior. RBPs control the expression of numerous oncogenes or tumor suppressors through post-transcriptional gene regulation. Many RBPs are deregulated in cancer and play critical roles in tumorigenesis and cancer development. CUGBP Elav-like family (CELF) proteins are highly conserved based on their sequence-specific binding to RNA containing CUG repeats. CELF6, a member of the CELF family of RNA-binding proteins, regulates muscle-specific alternative splicing and contributes to the pathogenesis of myotonic dystrophy (DM); however, the role of CELF6 in cancer is less appreciated. Here, we show that the expression of CELF6 is cell cycle-regulated. The cell cycle-dependent expression of CELF6 is mediated through the ubiquitin-proteasome pathway; SCF-β-TrCP recognizes a non-phospho motif in CELF6 and regulates its degradation. Gene expression profiling and KEGG pathway enrichment analysis reveal that the p53 signaling is enriched in *CELF6* knockout cells. Overexpression or depletion of CELF6 modulates p21 gene expression; CELF6 binds to the 3'UTR of p21 transcript and regulates its stability. CELF6 is downregulated in human colorectal cancer tissues; depletion of *CELF6* promotes cell cycle progression, cell proliferation, and colony formation whereas overexpression of CELF6 induces G1 phase arrest. Collectively, these data demonstrate that CELF6 is a potential tumor suppressor and that CELF6 regulates cell proliferation and cell cycle progression via modulating p21 stability.

IMMUNOTHERAPY

B01 Interaction between tumor-infiltrating T cells and B cells controls the progression of colorectal cancer. Ping Tao[1], Li-jie Ma[2], Liang Hong[2]. [1]Department of Laboratory Medicine, Shanghai TCM-integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [2]Department of General Surgery, Shanghai Fifth People's Hospital, Fudan University, Shanghai, China.

Backgrounds: Colorectal cancer (CRC) is one of the common malignant tumors worldwide. With the rapid development of immunotherapy in recent years, it has been successfully applied to solid tumors such as prostate cancer, renal cell carcinoma, and breast cancer. As an immunogenic tumor and immune-targeted disease, CRC has gradually attracted widespread attention. The immune system has specific and nonspecific responses to tumors, and its mechanism is very complex, involving a variety of immune cells and their secretory products. Therefore, in-depth analysis of the distribution, subgroups, and phenotypes of immune cells in the CRC tissue microenvironment will help to understand the pathogenesis and metastasis mechanism of CRC.

Methods: Multichannel flow cytometry analysis was applied in 10 fresh specimens of colorectal tumor tissues. Multicolor immunofluorescence staining was performed on a TMA including 90 CRC cases. RT-PCR analysis was performed on 16 frozen CRC tissues. B lymphocytes knockout model in vivo and subcutaneous transplantation colorectal tumor model were performed on 6- to 7-week-old male C57BL/6 mice.

Results: Multicolor immunofluorescence staining found that tumor-infiltrating T cells and B cells were in close contact with each other in tumor tissues, and the density was significantly positively correlated with the prognosis of CRC patients. The density of TIB was not only significantly positively correlated with the enhanced expression of killer cytokines such as granzyme B and IFN-γ, but also significantly positively correlated with the expression of tumor proliferating molecule Ki-67 and tumor cell activation molecule, Caspase-3. The expression of costimulatory molecules CD27, CD40, and activated molecule CD38 was significantly positively correlated with the prognosis of CRC patients. A mouse model of depleted mature B lymphocytes was constructed by injecting anti-CD20 monoclonal antibody through tail vein injection, and a human colon cancer cell line, HCT116, was transplanted to construct a subcutaneous/orthovascular tumor model. Furthermore, B lymphocytes depletion group had significant growth of transplanted tumors, and activated T lymphocytes are significantly reduced.

Conclusions: It is helpful to reveal that tumor-infiltrating T cells and B cells are closely related to their functional interactions in CRC, revealing the correlation between the crosstalk and local enhanced immune activation. Thus, to carry out targeted immunologic intervention studies and to develop a basis for tumor immunotherapy research will ultimately improve the prognosis of patients with CRC.

B02 A highly selective small-molecule CSF-1R inhibitor potentiates Bcl-2 inhibitor in AML cell lines. Shuqun Yang, Baowei Zhao, Mingming Zhang, Yuan Zhao, Xin Chen, Lei Zhang, Hongping Yu, Zhui Chen, Yaochang Xu. Abbisko Therapeutics, Shanghai, China.

CSF-1R is the receptor for colony-stimulating factor 1 (CSF-1). Its signaling is critical for macrophage/myeloid-derived suppressing cells (MDSC) recruitment, proliferation, and survival. We have discovered a novel, highly selective, and potent small-molecule CSF-1R inhibitor, ABSK021. To explore its utilization in hematologic malignancy, we screened it as a single agent or in combination with other therapeutic agents in various leukemia cells. As a result, we found that ABSK021 demonstrates strong synergy with Bcl-2 inhibitor venetoclax in acute myeloid leukemia (AML) setting. ABSK021 has potent biochemical and cellular activities against CSF-1R (IC50<30 nM), resulting in strong inhibition of CSF-1R phosphorylation and proliferation of myeloid cell line. Kinomescan and cellular proliferation assay demonstrated the superior selectivity of ABSK021 against other kinases. Efficacy studies showed that ABSK021 strongly enhanced antitumor efficacy of anti-PD-1 antibody in syngeneic models. When combined with Bcl-2 inhibitor venetoclax, ABSK021 demonstrated enhanced antiproliferation inhibition in AML cell lines. The synergy of these two inhibitors may shed light on future clinical management of AML.

B03 A novel Bayesian phase I/II design for immunotherapy. Suyu Liu. The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Immunotherapy is an innovative treatment approach that stimulates a patient's immune system to fight cancer. It demonstrates characteristics distinct from conventional chemotherapy and stands to revolutionize cancer treatment. We propose a Bayesian phase I/II dose-finding design that incorporates the unique features of immunotherapy by simultaneously considering three outcomes: immune response, toxicity, and efficacy. The objective is to identify the biologically optimal dose, defined as the dose with the highest desirability in the risk-benefit tradeoff. An Emax model is utilized to describe the marginal distribution of the immune response. Conditional on the immune response, we

jointly model toxicity and efficacy using a latent variable approach. Using the accumulating data, we adaptively randomize patients to experimental doses based on the continuously updated model estimates. A simulation study shows that our proposed design has good operating characteristics in terms of selecting the target dose and allocating patients to the target dose.

B04 An optogenetic controllable T cell system for hepatocellular carcinoma immunotherapy. Yingchao Wang, Bixing Zhao, Xiaolong Liu, Jingfeng Liu. Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou, China.

Rationale: T cell-based immunotherapy increasingly shows broad application prospects in cancer treatment, but its performance in solid tumors is far from our expectation, partly due to the reinhibition of infiltrated T cells by immunosuppressive tumor microenvironment. Here we presented an artificial synthetic optogenetic circuit to control the immune responses of engineered T cells on demand for promoting and enhancing the therapeutic efficiency of cancer immunotherapy.

Methods: We designed and synthesized blue-light inducible artificial immune signaling circuit and transgene expression system. The blue light triggered transgene expression was investigated by luciferase activity assay, qPCR, and ELISA. The in vitro cytotoxicity and proliferation assays were carried out on engineered T cells. The in vivo antitumor activity of engineered T cells was investigated on xenograft model of human hepatocellular carcinoma.

Results: Blue light stimulation could spatiotemporally control gene expression of specific cytokines (IL2, IL15, and TNF-α) in both engineered 293T cells and human primary T cells. This optogenetic engineering strategy significantly enhanced the expansion ability and cytolytic activity of primary T cells upon light irradiation, and the light-activated T cells showed high efficiency of elimination against xenograft of hepatocellular carcinoma cells.

Conclusions: The current study represented an engineered remotely controlled T-cell system for solid tumor treatment, and provided a potential strategy to partially overcome the intrinsic shortages of current immune cell therapy.

B05 Novel plate-based detection method for T cell activation/proliferation, migration, and cytotoxicity assay using image cytometry. Alyssa Johnston, Bo Zhu, Liang Bai, Ruwei Li, Liang Bai and Leo Li-Ying Chan. Nexcelom Bioscience LLC, Lawrence, MA, USA.

The field of cancer immunotherapy has grown significantly due to numerous successful trials in antibody-based treatments, chimeric antigen receptor (CAR) T cell therapy, and cancer vaccines. For cancer vaccines, tumor cell antigens are used to induce immune responses to target specific cancer disease; subsequently, numerous assays have been developed and established for immune monitoring of cancer vaccines in both academic and industry settings. Traditionally, ELISA or ELISpot are standard assays used to detect specific cytokines concentration for measuring T-cell response. In addition to the standard assays, other methods such as T-cell activation/proliferation, migration, and cytotoxicity assays are performed to understand the functionality of immune responses. In general, these functional assays are performed using flow cytometry or qualitative analysis by microscopy. In recent years, image cytometry has been employed to develop robust assays to monitor immune response; the ability to perform high-throughput screening assays for monitoring T-cell immune response is highly critical for cancer vaccine research. The Celigo Image Cytometer can perform whole well bright field (BF) and fluorescence (FL) image acquisition and analysis for a 96- or 384-well plate in less than 5 min for BF and 9 min for FL. Furthermore, directly counting cells within each well allows for more accurate representation of T cell function without perturbation. In this work, we demonstrate the use of Celigo Image Cytometer to perform in vitro cell-based assays to monitor immune response for cancer vaccine research. Three novel methods are developed: label-free T-cell activation and proliferation measurement, T-cell migration assay, and a T cell-mediated cytotoxicity assay using direct cell counting. For T-cell activation and proliferation, primary T cells are seeded into a 96-well plate and stimulated with antibodies or coculture with cancer cells. The cells are directly counted in the well over 48 hours using the image cytometer in bright field images. To measure T-cell migration, T cells are seeded in a transwell plate with chemokines in the bottom well. As the T cells migrate through the transwell and drop to the bottom of the wells, the cells can be directly counted using bright field or fluorescence imaging with Hoechst staining. Finally, T cell-mediated cytotoxicity assay is performed by coculturing CD8 T cells with calcein AM- or fluorescent protein-labeled cancer cells. As target cancer cells are killed, the fluorescence dissipates, and by counting the changes in the number of “live” target cells, the cytotoxicity percentages are measured.

B06 Combination immunotherapy targeting CD47 and PD-L1 enhances antitumor responses in pancreatic cancer. Yu Pan[1], Fengchun Lu[1], Xingxing Yu[1], Qinglin Fei[1], Ping Xiong[2], Zhijiang Chen[1], Xianchao Lin[1], Zelin Hou[1], Minggui Pan[3], Heguang Huang[1]. [1]Department of General Surgery, Fujian Medical University Union Hospital, Fuzhou, China, [2]Department of Obstetrics and Gynecology, 900 Hospital of the Joint Logistics Team, Fuzhou, China, [3]Department of Oncology and Hematology and Division of Research, Kaiser Permanente, Santa Clara, CA, USA.

Background: Human pancreatic ductal adenocarcinoma (PDAC) exhibits marginal responses to anti-PD-1/PD-L1 immunotherapy and the most important factor related to the response of immune checkpoint inhibitors is tumor microenvironment (TME). A deeper understanding of the TME may be crucial to developing effective immunotherapeutic strategies.

Methods: Using immunohistochemistry, we examined tumor-infiltrating $CD68^+$ pan-macrophages ($CD68^+$ M), $CD163^+$ M2 macrophages ($CD163^+$ M2), tumor expression of CD47 and PD-L1 in 106 cases of PDAC, and conducted correlation and survival analyses using the Kaplan–Meier method and Cox regression. Moreover, the efficacy of anti-CD47 therapy was confirmed in patient-derived PDAC xenografts. Also, the combinational efficacy of targeting CD47 and PD-L1 in pancreatic cancer was examined in mouse xenograft models.

Results: We found that $CD68^+$ M was positively correlated with the expression of CD47, but no significant correlation was found in $CD163^+$ M2 and CD47 expression. Higher levels of tumor-infiltrating $CD68^+$ M, $CD163^+$ M2, and tumor expression of CD47 were significantly associated with worse survival. Survival analysis of combinations of these variables revealed that $CD47^{high}/CD68^+$ M^{high} and $CD47^{high}/CD163^+$ $M2^{high}$ were significantly correlated with shorter survival and higher hazard ratio, whereas $CD47^{low}/CD68^+$ M^{low} and $CD47^{low}/CD163^+$ $M2^{low}$ were correlated with longer survival. Using patient-derived xenografts, we comfirmed the inhibiting effect of anti-CD47 therapy in PDAC tumor growth. Futhermore, we found that the combination of anti-CD47 and anti-PD-L1 therapy exerted synergistic inhibition of PDAC growth in mouse xenograft models.

Conclusion: Our results indicated that tumor expression of CD47 and proportion of tumor-associated macrophages can be the useful prognostic biomarkers. Combination immunotherapy targeting CD47 and PD-L1 may improve antitumor responses in PDAC.

B07 STAT3 inhibition enhances CDN-induced STING signaling and antitumor immunity. Jianwen Pei,* Yibo Zhang,* Qinhong Luo, Wenlv Zheng, Wanxuan Li, Xin Zeng, Qinkai Li, Junmin Quan. State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen, China. *These authors contributed equally to this work.

Cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway is a key regulator in innate immunity and has emerged as a promising drug target in cancer treatment, but the utility of this pathway in therapeutic development is complicated by its dichotomous roles in tumor development and immunity. The activation of the STING pathway and the induced antitumor immunity could be attenuated by the feedback activation of IL-6/STAT3 pathway. Here we report that STAT3 inhibition significantly enhanced the intensity and duration of STING signaling induced by the STING agonist c-diAM$(PS)_2$. Such sensitization effect of STAT3 inhibition on STING signaling depended on STING rather than cGAS, which was mediated by simultaneously upregulating the positive modulators and downregulating the negative modulators of the STING pathway. Furthermore, the combination treatment with the STAT3 inhibitor and STING agonist markedly regressed tumor growth in syngeneic mice by increasing CD8+ T cells and reducing regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. Our work provides a rationale for the combination of STAT3 inhibitors and STING agonists in cancer immunotherapy.

B08 Humanized mouse model: A preclinical platform feasible for CAR-T therapy. Chang Song[1], Yirong Kong[1], Nan Mou[2], Yan Wang[1], Hongwu Li[1], Siying Peng[1], Jijun Yuan[2]. [1]Beijing IDMO Co., Ltd., Beijing, China, [2]Shanghai Genechem Co., Ltd., Shanghai, China.

Introduction: Chimeric antigen receptor (CAR) T cells form part of a broad wave of immunotherapies that are showing promise in clinical trials. This clinical delivery has been based upon preclinical efficacy testing that confirmed the proof of principle of the therapy. However, with recent reports of adverse events associated with CAR T-cell therapy, there is now concern that current preclinical models may not be fit for purpose with respect to CAR T-cell toxicity profiling. CAR T-cell therapy does not exist alone as T cells are generally given in combination with patient preconditioning, and may also include systemic cytokine support, both of which are associated with toxicity. Here, we explore the preclinical humanized mouse models used to validate CAR T-cell function and examine their potential to predict CAR T-cell driven toxicities for the future.

Experimental Design: Study design using patient-derived xenograft (PDX) model: NPI (NOD. Prkdc-/- Il2rg-/-) mice were engrafted with patient tumor tissues. Meanwhile, T cells from the same patient were activated and transfected with CAR genes by either lentivirus (LV) or electroporation. In vivo efficacy study was carried out using engineered CAR T-cells infused into PDX mice. Study design using Hu-CD34 models: NPI (NOD. Prkdc-/- Il2rg-/-) mice were preconditioned and engrafted with human CD34 positive hematopoietic stem cells (HSCs) from cord blood. Human T cells were isolated from spleens of 16~20 weeks reconstituted NPI mice and activated in a certain environment in which they can actively proliferate. The T cells were then transfected with CAR genes by either an integrating gammaretrovirus (RV) or by lentivirus (LV) vectors. Engineered CAR T-cells were infused into the NPI mice or tumor-bearing NPI mice reconstituted with HSCs from the same donor (Hu-CD34 NPI).

Conclusions: (1) Patient-derived xenograft (PDX) model, more clinically relevant, is a better preclinical in vivo efficacy model for CAR T-cell therapy. (2) Xeno-tolerant T cells from Hu-CD34 NPI mice are functional in vitro assay and in vivo efficacy study. (3) Long-term cytokine release and toxicity of on target/off tumor can be further investigated using Hu-CD34 NPI mice. (4) Hu-CD34 NPI models can be used to study selective depletion mediated by the clinically approved therapeutic antibody when the transduced cells are engineered to express the antibody targeted cell surface antigen.

B09 Humanized mouse model: A preclinical platform feasible for a bispecific T cell-engaging antibody (BiTE). Yirong Kong, Jinxia Shi, Yan Wang, Hongwu Li, Siying Peng, Chang Song. Beijing IDMO Co., Ltd., Beijing, China.

Introduction: The selection of appropriate preclinical models based on similarity to human biology and disease genotype and phenotype carries considerable potential to ensure higher predictability of preclinical trials. The design and interpretation of first-in-man trials remains a major challenge in the development of novel anticancer agents. Key study design elements such as schedule, escalation strategy, targeted patient population, etc., rely heavily on preclinical (usually in vivo) data. It is especially difficult to model for preclinical assessment of cancer immunotherapy, the most actively developing area in oncology. Bispecific T-cell engager molecules (BiTEs) constitute a class of bispecific single-chain antibodies for the polyclonal activation and redirection of cytotoxic T cells against pathogenic target cells. Here we proposed preclinical studies in NPI (NOD. Prkdc$^{-/-}$ Il2rg$^{-/-}$) mice engrafted with human CD34 positive hematopoietic stem cells (HSCs) from cord blood, which indicate the importance of the desired balance between potency, PK, and safety for optimizing the performance of a T cell–recruiting bispecific antibody.

Experimental Design: BiTES are engineered from the binding domains of two different antibodies. One end binds to CD3 found on T cells and the other end binds to a tumor-specific protein epitope presented on the surface of the tumor. When both of its binding domains are engaged, the BiTE creates a cytolytic synapse, leading to T cell-mediated killing of the target tumor cell. NPI (NOD. Prkdc$^{-/-}$ Il2rg$^{-/-}$) mice were preconditioned and engrafted with human CD34 positive hematopoietic stem cells from cord blood. Reconstituted mice with B and T cell differentiation were treated with rituximab (CD20) and blinatumumab (CD19 X CD3). By using FACS analysis to check T-cell and B-cell populations in peripheral blood after rituximab or blinatumumab treatment, the efficacy and safety effect of antibody treatment were carefully monitored.

Conclusion: Hu-CD34 NPI mice can be used to evaluate efficacy and safety of BiTEs in vivo. These preclinical studies in Hu-CD34 NPI mouse models indicate the importance of selecting a CD3 affinity resulting in the desired balance between potency, PK, and safety for optimizing the performance of a T cell–recruiting bispecific antibody.

B10 Humanized mouse model: A preclinical platform feasible for immunotherapy. Yirong Kong, Jinxia Shi, Yan Wang, Hongwu Li, Siying Peng, Chang Song. Beijing IDMO Co., Ltd., Beijing, China.

Introduction: The selection of appropriate preclinical models based on similarity to human biology and disease genotype and phenotype carries considerable potential to ensure higher predictability of preclinical trials. The design and interpretation of first-in-man trials remains a major challenge in the development of novel anticancer agents. Key study design elements such as schedule, escalation strategy, targeted patient population, etc., rely heavily on preclinical (usually in vivo) data. It is especially difficult to model for preclinical assessment of cancer immunotherapy, the most actively developing area in oncology. To build a preclinical mouse platform to evaluate immunotherapies for human cancer, we have established a tumor and immune system double-humanized mouse model, "Ideal Immune," by implanting tumor tissue from patients into Hu-CD34 NPI mice (NPI, NOD-Prkdc^{scid}-Il2rg^{em1IDMO}). The Ideal Immune model can mimic the interaction between the human immune system and primary tumor, which allows scientists to evaluate cancer immunotherapies together with better understanding of tumor microenvironment.

Experiment design: CD34^{+} hematopoietic stem cells (HSCs) purified from umbilical cord blood were implanted into NPI mice to establish the Hu-CD34 NPI mouse model.

After HSC transplantation, reconstitution was determined by FACS analysis of peripheral blood from 4 to 16 weeks post-implantation, which showed a steady increase of reconstituted human lymphocytes in Hu-NPI mice. After 16 weeks reconstitution, human CD45+ cells remained stable at 60-90%. All the compartments can be detected in peripheral blood. All the data shown was collected from Hu-CD34 NPI mice reconstituted from the same donor. Various subsets of immune cells were detected in spleen, bone marrow and peripheral blood in Hu-NPI after 24 weeks reconstitution. Female Hu-CD34 NPI mice, reconstituted for 16 weeks, were implanted with NSCLC PDX tumor tissues subcutaneously in the right flank. Dosing began on Day0 in mice with established tumors (group mean 120mm^3). The study endpoint was a tumor volume of 1500mm^3 in the control group. (A) Pembrolizumab was administrated i.p. at 10mg/kg, BIW. The function of CD8 cells in tumor were determined by intracellular cytokine staining. (B) Ipilimumab was administrated i.p. at 10mg/kg, BIW. Depletion of Treg cells was analyzed by FACS.

Conclusions: (1) Various subsets of immune cells were detected in spleen, bone marrow, and peripheral blood in Hu-NPI after 24 weeks reconstitution. (2) Hu-CD34 PDX mouse model can be used to determine the immuno-profiling of tumor infiltrated lymphocytes, such as expression of immune checkpoints (PD1, Ox40, Tim3, Lag3 et al) on CD8 T cells. (3) CD8 cells in tumor are not activated, while turned into killing mode by IFNΥ and TNFα release after anti-PD1 treatment. (4) Treg cells in tumor can be depleted after anti-CTLA4 treatment through ADCC (antibody-dependent cell-mediated cytotoxicity). (5) Hu-CD34 PDX mouse model is a suitable preclinical in vivo model for immunotherapy.

B11 Humanized mouse model: A preclinical platform feasible for antibody-based therapy. Yirong Kong, Jinxia Shi, Yan Wang, Hongwu Li, Siying Peng, Chang Song. Beijing IDMO Co., Ltd., Beijing, China.

Introduction: The selection of appropriate preclinical models based on similarity to human biology and disease genotype and phenotype carries considerable potential to ensure higher predictability of preclinical trials. The design and interpretation of first-in-man trials remains a major challenge in the development of novel anticancer agents. Key study design elements such as schedule, escalation strategy, targeted patient population, etc., rely heavily on preclinical (usually in vivo) data. It is especially difficult to model for preclinical assessment of cancer immunotherapy, the most actively developing area in oncology. To build a preclinical mouse platform to evaluate immunotherapies for human cancer, we have established a tumor and immune system double-humanized mouse model, "Ideal Immune," by implanting tumor tissue from patients into Hu-CD34 NPI mice (NPI, NOD-Prkdc^{em1IDMO}-Il2rg^{em2IDMO}). The Ideal Immune model can mimic the interaction between the human immune system and primary tumor, which allows scientists to evaluate cancer immunotherapies together with better understanding of tumor microenvironment.

Experiment design: Female Hu-CD34 NPI mice, reconstituted for 16~20 weeks, were implanted with MDA-MB-231 or PDX tumor tissues subcutaneously in the right flank. PD-1 antibody dosing began on Day0 in mice with established tumors (group mean 120mm^3). The study endpoint was a tumor volume of 1500mm^3 in the control group. Mean tumor growth and HLA typing results are shown using MDA-MB-231 tumor model, CRC PDX model and NSCLC PDX model. Significant tumor growth inhibition can be seen in these humanized models. Female Hu-CD34 NPI mice, implanted with NSCLC PDX tumor tissues subcutaneously in the right flank were also treated with different combinations of chemotherapy along with immune check point antibodies. Dosing began on Day0 in mice with established tumors (group mean 120mm^3). The study endpoint was a tumor volume of 1500~3000mm^3 in the control group. Mean tumor growth and body weight change are checked. The significant tumor growth inhibition in a PD-1 and CTLA-4 combo-antibodies treatment group, together with dramatic toxicity. However, combo with chemotherapy prolonged tumor-free phase.

Conclusions: Hu-CD34 NPI mice can be used to evaluate efficacy and safety of immune checkpoint antibodies in vivo. These preclinical studies in Hu-CD34 NPI mouse models indicate the importance of selecting a proper combination strategy for cancer immunotherapy to obtain ideal pharmacodynamic results and reducing drug toxicity at the same time.

LEUKEMIA AND LYMPHOMA

B12 Fabrication and evaluation of drug codelivery micellar formulation for the treatment of acute myeloid leukemia. Doudou Yan[1],* Hui Wei[2],* Yangyang Ge[1], Jie Meng[1], Tao Wen[1], Jian Liu[1], Jianxiang Wang[2], Haiyan Xu[1].* [1]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China. *These authors contributed equally to this work.

The treatment of acute myeloid leukemia (AML) has been facing the big challenge of drug resistance and relapse. Although the combination chemotherapy regimen of AML is beneficial to the long-term survival of patients, the administration of separate drugs has several obstacles, including uncertain doses and poor patient compliance as well as increased cost. In this wok, doxorubicin (DOX) and homoharringtonine (HHT) were encapsulated into polymeric micelles, and the therapeutic effect of this codelivery drug system (DHM) was evaluated in vitro and in vivo. The dynamic particle size distribution of DHM was around 78 nm, with encapsulation efficacy of 75% and 64% for DOX and HHT, respectively. The micellar formulation could increase cellular uptake of the drugs in the doxorubicin-resistant AML cell line HL60/A, resulting in greater cytotoxicity than either single drug or the combination of both free drugs (DH) by inhibiting cell division and enhancing apoptosis. DHM also exhibited significantly improved therapeutic effect than DH in AML1-ETO$^+$-c-kit$^+$ mouse model representing the refractory and relapsed status of AML, markedly prolonging the survival of mice without exerting more severe toxicity in vivo. In conclusion, the codelivery drug system could improve antitumor effects both in vitro and in vivo, and holds promising potential in the treatment of AML.

B13 Antitumor activity of CXCR4 peptide antagonist E5 on AML1-ETO mouse model. Yangyang Ge[1],* Jie Meng[1],* Haiyan Xing[2],* Hui Wei[2], Shilin Xu[1], Jian Liu[1], Chen Wang[3], Min Wang[2], Jianxiang Wang[2], Haiyan Xu[1]. [1]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, [2]State Key Laboratory of Experimental Hematology, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China, [3]CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology, Beijing, China. * These authors contributed equally to this work.

The interaction between CXCR4 and CXCL12 plays critical roles in the development, progression. and metastasis of acute myeloid leukemia (AML), because CXCL12 is constrictively secreted from BM stroma and facilitates AML cells migration and retention in BM microenvironment, which contribute to AML growth and chemoresistance. E5 is a chemically synthesized peptide antagonist of CXCR4 and has been demonstrated effective anti-AML effect in the HL60 xenograft animal model as monotherapy and in combination with chemotherapy in our previous work. In this work E5 was fabricated into micellar formulation (M-E5) with 23 nm in diameter and applied to an AML animal model with t(8;21)(q22;q22) translocation representing a refractory leukemia resistant to TK inhibitor and multiple chemotherapy drugs, as well as to multiple kinds of AML cell lines. Experimental results showed that M-E5 was able to bind to U937, KG1, and GFP positive AML1-ETO cells from mouse spleen and bone marrow (BM); meanwhile, it inhibited CXCL12 mediated AML1-ETO cells migration. A single injection of M-E5 could significantly inhibit GFP positive cells homing to spleen and BM, as well as mobilized AML1-ETO cells into peripheral blood (PB) from BM and spleen. The survival of AML1-ETO mice was prolonged significantly. In addition, GFP positive rate in PB, spleen, and BM was decreased significantly after 4 days injection of 2.5, 5, 10 mg/kg M-E5. In conclusion, monotherapy of M-E5 holds promising translational potential in the treatment of AML.

B14 Epstein-Barr virus DNA quantification in blood is insufficient for the detection of development of post-transplant lymphoproliferative disease. Diana Vracar, Isak Holmqvist, Jonas Carlsten, Ka-Wei Tang. Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, Gothenburg, Sweden.

Almost every single adult in the world is infected with Epstein-Barr virus (EBV). Following a primary infection of this herpesvirus during childhood or adolescence, EBV remains latent in our B-lymphocytes for the rest of our lives. Proliferation of the latently infected B-lymphocytes is kept in control by circulating CD8+ T lymphocytes. Patients with pharmaceutically induced inhibition of T-lymphocytes following transplantation have an increased risk of development of post-transplantation lymphoproliferative disease (PTLD) that originates from proliferation of malignant latently EBV-infected B-lymphocytes. PTLD is one of the most common malignancies developed after transplantation. Despite intensive monitoring of patients post-transplantation, many cases are not discovered until symptoms arise from local lymphoma development. Prior to transplantation patient blood is analyzed to determine their sero-status for many viral agents, including EBV. As expected, the patients who developed PTLD were mostly EBV sero-negative prior to transplantation. After transplantation, blood samples were regularly collected for analysis of EBV-DNA quantification. PTLD lymphoma was, in the majority of cases, not discovered until the development of local or systemic lymphoma symptoms including stomach or skeletal pain as well as weight loss, night sweats, and fever. Novel approaches for early detection of PTLD are therefore urgently needed.

B15 PTEN regulates hematopoietic lineage choice through chromatin accessibility. Yilin Wu[1,2,3], Zihan Xu[2], Hong Wu[1,2,3]. [1]The MOE Key Laboratory of Cell Proliferation and Differentiation, School of Life Sciences, Beijing, China, [2]Peking-Tsinghua Center for Life Sciences, Beijing, China, [3]Beijing Advanced Innovation Center for Genomics, Peking University, Beijing, China.

Pten deletion in hematopoietic stem cells (HSCs) leads to increased myeloid and T lineages, accompanied by decreased common lymphoid progenitors and arrested B cell development, proving PTEN's function in regulating hematopoietic lineage choice. However, the underlying molecular mechanism is still unclear. To explore the molecular mechanism of PTEN regulating hematopoietic fate decision, we conducted single-cell RNA-seq (scRNA-seq) analysis. We found that CD38 could divide Prepro-B into CD38- and CD38+ subsets, of which cells in the CD38- subset is more proliferative than the CD38+ subset. PTEN deletion resulted in increased the CD38- Prepro-B subset, which could transit to the CD38+ subset. Together with suppression of B lineage transcription program, PTEN loss leads to the defect of *Igh* rearrangement in CD38+ Prepro-B cells, resulting in the decreased Prepro-B cell differentiation.

We also carried out an integrated analysis of differentiation potentials, gene expression, and epigenetic program of early progenitors to determine how PTEN controls alternative lineage choices. We found that *Pten*^{null} Prepro-B cells had increased myeloid and T lineage potentials. The underlying mechanism is that PTEN deletion changed chromatin accessibility in the CD38- Prepro-B cells. Comparing to the wild-type controls, the distal regulatory elements specific to myeloid and T lineage are more open in the *Pten*^{null} cells and very sensitive to transcription factor activation, whereas those related to B lineage are more closed in *Pten*^{null} CD38- Prepro-B cells. The balance between myeloid and T lineage potential depended on the relative concentration of PU.1 and CEBPA. Heterozygous deletion of PU.1 led to the enhanced impact of CEBPA, resulting in a decrease of T lineage potential but an increase of myeloid lineage potential. Our study revealed the molecular mechanism underlying PTEN-regulated hematopoietic fate decision and proposed a model in which PTEN loss in immature hematopoietic progenitor tilts the lineage balances among myeloid, T, and B cells by altering chromatin accessibilities of the lineage regulatory elements. This model explained the emergence of mixed-phenotype acute leukemia (MPAL) and lineage switch after treatment, providing a new strategy for relevant clinical treatment.

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B16 JQ1, a selective bromodomain inhibitor, augments the immunogenicity of mantle cell lymphoma by influencing the expression of PD-L1. Fengdong Cheng[1], Jie Chen[1], Yuan Ren[2], Xiaohong Zhao[2], Mitchell Smith[1], James Bradner[3], Jianguo Tao[2], Eduardo M. Sotomayor[1]. [1]GW Cancer Center, George Washington University, Washington DC, USA, [2]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA, [3]Harvard Medical School, Boston, MA, USA.

Mantle cell lymphoma (MCL) is an aggressive form of non-Hodgkin's lymphoma, comprising about 6%-8% of NHL cases. Clinical outcome remains poor in patients with MCL due to the emergence of drug resistance and lymphoma progression. New therapeutic strategies are under development to improve the clinical outcome in patients with this disease. Recent studies have highlighted the importance of BRD/acetyl-lysine binding in orchestrating molecular interactions in chromatin biology and regulating gene transcription. The bromodomain (BRD) is a diverse family of evolutionary conserved protein-interaction modules, which recognizes acetylated lysine residues such as those on the N-terminal tails of histones.

Recently we have determined the functional consequences of inhibiting bromodomains in MCL. First, we evaluated the effects of JQ1, a selective small-molecule bromodomain inhibitor, upon MCL's immunogenicity and antigen-specific $CD4^+$ T-cell responses. In vitro treatment of MCL cells with increasing concentrations of JQ1 resulted in a decreased expression of the tolerogenic molecule PD-L1 as compared to control cells. Second, we evaluated the capabilities of JQ1-treated MCL cells to present cognate antigen to naïve or anergic antigen-specific $CD4^+$ T-cells. We found that treatment of MCL cells with JQ1 enhances their antigen-presenting capabilities, leading to effective priming of naïve $CD4^+$ T-cells and restoring the responsiveness of tolerant T cells as determined by their increased production of IL-2 and IFN-gamma in response to cognate antigen. More importantly, in vivo studies clearly demonstrated a strong antitumor effect in JQ1 or Brd4 inhibitor-treated MCL tumor-bearing animals. Mechanistically, we found a decreased STAT3 phosphorylation and c-Myc expression in JQ1-treated MCL cells, which in turn might be responsible for the diminished expression of PD-L1 and augmentation of MCL's immunogenicity. Taken together, we have found that MCL cells treated with the bromodomain-specific inhibitor JQ1 are more inflammatory, display lower expression of the immunosuppressive molecule PD-L1, and are capable of restoring the responsiveness of tolerant T cells. More importantly, JQ1 treatment in vivo inhibits tumor growth in MCL tumor-bearing animals. Our studies therefore have unveiled a previously unknown immunologic effect of BRD inhibitors and have identified a novel immunotherapeutic approach in MCL.

B17 Investigating the molecular mechanism underlying the gender bias in acute T lymphoblastic leukemia.
Liuzhen Zhang, Hong Wu. The MOE Key Laboratory of Cell Proliferation and Differentiation, School of Life Sciences, Peking-Tsinghua Center for Life Sciences, Beijing Advanced Innovation Center for Genomics, Peking University, Beijing, China.

Background: Gender bias in cancer susceptibility is often observed in human cancers. Acute T lymphoblastic leukemia (T-ALL) is an aggressive form of T-cell leukemia that is mainly diagnosed in children and shows a male bias with unknown mechanism. By generating and analyzing the *Pten* null T-ALL models, we and others have demonstrated the important role of WNT-β-catenin pathway in the formation and maintenance of T-ALL leukemia stem cell (LSC) and T-ALL progression. A recent study reports that the androgen receptor (AR) can regulate β-catenin expression, indicating AR signaling pathway may contribute to the male bias observed in T-ALL.

Methods: We first tested the in vivo androgen level in the *Pten*-null T-ALL mice, using Cre^- wild-type (WT) mice as controls. We also tested the therapeutic effects of MDV3100, an androgen receptor antagonist, on the T-ALL mice alone or in combination with an anti-PI3K inhibitor Bay1082439. Finally, we genetically deleted Ar in the *Pten*-null T-ALL model and directly compared the survival and leukemogenesis potential of $Pten^{loxp/loxp}$;*Vec-Cre*$^+$ T-ALL mice and *Pten* $^{loxp/loxp}$;*Vec-Cre*$^+$;$Ar^{loxp/y}$ mice.

Results: We found that the androgen level in *Pten*-null T-ALL mice is significantly higher than that of WT mice. MDV3100 can significantly prolong the lifespan of T-ALL mice. Consistently, genetic deletion of Ar also prolonged the survival of *Pten* $^{loxp/loxp}$;*Vec-Cre*$^+$;*Ar* $^{loxp/+}$ mice, supporting the notion that AR signaling is involved in T-ALL leukemogenesis. Furthermore, MDV3100 and Bay1082439 combination treatment can significantly reduce the leukemic blast number and restore the morphology of spleen and thymus in T-ALL mice.

Conclusions: Our study suggests that AR signaling plays a critical role in T-ALL leukemogenesis. We will conduct more detailed study to investigate the underlying molecular mechanism.

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B18 The genomic landscape of acute T lymphoblastic leukemia in a Chinese cohort.
Bingjie Dong[1], Mei Wang[1], Haichuan Zhu[1], Xiaojun Huang[2], Yingchi Zhang[3], Xiaofan Zhu[3], Hong Wu.[1 1]The MOE Key Laboratory of Cell Proliferation and Differentiation, School of Life Sciences, Peking-Tsinghua Center for Life Sciences, Beijing Advanced Innovation Center for Genomics, Peking University, Beijing, China, [2]The People's Hospital, Peking University Institute of Hematology, Peking University, Beijing, China, [3]State Key Laboratory of Experimental Hematology, Institute of Hematology and Hospital of Blood Diseases Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China.

Background: T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of immature T cells. Although genetic alterations in T-ALL have been studied in the Western populations, a large-scale multi-omic analysis of Chinese T-ALL population is currently lacking. Here we performed a detailed genomic and transcriptomic analyses on Chinese T-ALL patients to identify the landscape of genetic alterations, expression profiles, and survival-related factors associated with different subtypes of T-ALL.

Methods: In collaboration with Peking University People's Hospital and Institute of Hematology and Blood Diseases Hospital, we obtained samples from 127 patients and conducted RNA sequencing and whole-exome sequencing. Our cohort consists of 25 ETP and 86 non-ETP cases; among them 88 are pediatric patients and 23 are adult patients.

Results: We identified 54 recurrently mutated genes, including known T-ALL driver genes, e.g., *NOTCH1*, *FBXW7*, *PTEN*, and *PHF6*. Importantly, we identified a novel recurrent mutated gene *NUP214* in 4 (3.1%) cases. *NUP214* (Nucleoporin 214) is a member of FG-repeat-containing nucleoporins. *NUP214* fusion events have been discovered in T-ALL, including *SET-NUP214*, *NUP214-ABL1*, and *DEK-NUP214*. The mutations we identified may influence NUP214's subcellular localization and function. We further classified these recurrent mutations into nine functional pathways, including epigenetic factors, NOTCH1 signaling pathway, transcriptional factors, JAK-STAT signaling pathway, Ras signaling pathway, cell cycle, PI3K-AKT signaling pathway, ubiquitination, and DNA repair. We observed a significantly higher mutation frequency of epigenetic factors in ETP group than non-ETP group (78.95% in ETP-ALL, 46.84% in non-ETP ALL; p-value = 0.026, two-sided Fisher's exact test). Furthermore, we identified two epigenetic factors *CTCF* and *KMT2C* mutations were enriched in ETP-ALL (p-value = 0.026, 0.0126, respectively). Gene expression analysis demonstrates increased PI3K and MAPK pathway activation in the ETP samples and the risk of relapse or death in Chinese ETP patients is 2.36 times higher (Confidence interval, CI: 1.19-4.68, p-value = 0.01 by log-rank test) than that of non-ETP patients, consistent

with previous reports. Mutations in the Ras pathway were correlated with poor outcome (p-value = 0.03; log-rank test), particularly in non-ETP group (p-value = 0.01). We assessed all mutations through OncoKB database to identify clinically actionable alterations and found that most patients with the Ras pathway mutations have potential actionable drugs.

Conclusions: In summary, we identified a novel recurrent mutated gene *NUP214* in T-ALL. ETP patients form a unique high-risk group, which is immunologically and transcriptome-wide distinct from non-ETP-ALLs. We observed enriched epigenetic factors alterations in ETP-ALL. We found that Ras pathway alterations were related with poor outcome and provided potential therapeutic strategies for patients with Ras pathway alterations.

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LIVER CANCER

B19 Predicting therapy response of transarterial chemoembolization in hepatocellular carcinoma by CT imaging-based deep learning. Jie Peng[1, 2], Shuai Kang[1], Zhengyuan Ning[3], Hangxia Deng[4], Jingxian Shen[5], Hongbo Zhu[1], Zhijiao Duan[1], Yikai Xu[6], Jing Zhang[6], Wei Zhao[7], Xinling Li[7], Jinhua Huang[4], Yu Zhang[3], Li Liu[1]. [1]Hepatology Unit and Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [2]Department of Oncology, The Second Affiliated Hospital of GuiZhou Medical University, Kaili, China, [3]School of Biomedical Engineering, Southern Medical University, Guangzhou, China, [4]Department of Minimal Invasive Interventional Therapy, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Guangzhou, China, [5]Department of Radiology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Guangzhou, China, [6]Department of Medical Imaging Center, Nanfang Hospital, Southern Medical University, Guangzhou, China, [7]Department of Interventional Radiology, Nanfang Hospital, Southern Medical University, Guangzhou, China.

Purpose: We aimed to develop and validate a model of deep learning for the preoperative prediction of response of patients with intermediate-stage hepatocellular carcinoma undergoing transarterial chemoembolization.

Methods: The training and two independent validation CT images were acquired for 540 patients from the Nan Fang Hospital (NFH), 84 patients from Zhu Hai Hospital Affiliated with Jinan University (ZHHAJU), and 136 patients from the Sun Yat-sen University Cancer Center (SYUCC). We built a predictive model from the outputs using transfer learning techniques of a residual convolutional neural network (ResNet50). Then, we revaluated the prediction accuracy for each patch in two independent validation cohorts.

Results: In the training (NFH) set, the deep learning model had a high accuracy of 84.3% and area under the receiver operating characteristic curves (AUROC) of 0.97, 0.96, 0.95, and 0.96 for complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), respectively. In the validation (ZHHAJU) set, the deep learning model had an accuracy of 85.1% and an AUROC of 0.98, 0.96, 0.95, and 0.94 for CR, PR, SD, and PD, respectively. In other validation (SYUCC) set, the deep learning model had an accuracy of 82.8% and an AUROC of 0.97, 0.96, 0.95, and 0.95 for CR, PR, SD and PD, respectively.

Conclusion: The proposed ResNet model showed excellent performance for the individualized and noninvasive estimation of TACE therapy, which may help clinicians to better identify patients with HCC who can benefit from the interventional treatment.

B20 Deficiency in embryonic stem cell marker REX1 activates MKK6-dependent p38 MAPK signaling to drive hepatocarcinogenesis. Steve T. Luk[1], Man Tong[1], Tin-Lok Wong[1], Kai-Yu Ng[1], Terence K. Lee[4,5], Xin-Yuan Guan[2,3], Stephanie Ma[1,2]. [1]School of Biomedical Sciences and [2]State Key Laboratory for Liver Research, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, [3]Department of Clinical Oncology, The University of Hong Kong, Queen Mary Hospital, Hong Kong, [4]Department of Applied Biology & Chemical Technology and [5]State Key Laboratory of Chemical Biology and Drug Discovery, Hong Kong Polytechnic University, Hong Kong.

Embryonic stem cell-related transcription factors are central to the establishment and maintenance of stemness and pluripotency, and their altered expression plays key roles in tumors including hepatocellular carcinoma (HCC), a malignancy with no effective treatment. Here, we report the embryonic stem cell marker REX1 (also known as zinc finger protein 42, ZFP42) to be selectively downregulated in HCC tumors. Deficiency of REX1 in HCC was attributed to a combination of hypermethylation at its promoter as well as histone modification by methylation and acetylation. Clinically, hypermethylation of REX1 was closely associated with neoplastic transition and advanced tumor stage in humans. Functionally, silencing of REX1 potentiated the tumor-initiating and metastasis potential of HCC cell lines and xenografted tumors. Transcriptome profiling studies

revealed REX1 deficiency in HCC cells to be enriched with genes implicated in focal adhesion and MAPK signaling. From this lead, we subsequently found REX1 to bind to the promoter region of MKK6, thereby obstructing its transcription, resulting in altered p38 MAPK signaling. Our work describes a critical repressive function of REX1 in maintenance of HCC cells by regulating MKK6 binding and p38 MAPK signaling. REX1 deficiency induced enhancement of p38 MAPK signaling, leading to F-actin reorganization and activation of NRF2-mediated oxidative stress response, which collectively contributed to enhanced stemness and metastatic capabilities of HCC cells.

B21 Overriding adaptive resistance to sorafenib via combination therapy with SHP2 blockade in hepatocellular carcinoma. Carmen Oi Ning Leung[1], Man Tong[2], Stephanie Ma[2], Terence K. Lee[1]. [1]Hong Kong Polytechnic University, Hong Kong, [2]University of Hong Kong, Hong Kong.

Background and Aims: The survival benefit of sorafenib for HCC patients is unsatisfactory due to the development of adaptive resistance. We investigated the therapeutic potential of Src homology 2 domain–containing phosphatase 2 (SHP2) blockade to override sorafenib resistance, aiming to establish a novel therapeutic strategy against hepatocellular carcinoma (HCC).

Methods: We first examined the in vitro ability of the SHP2 inhibitor SHP099 to sensitize a panel of HCC cell lines, their sorafenib-resistant counterparts, and ex vivo HCC organoid culture to sorafenib. The molecular mechanisms of SHP2 blockade involved in overriding the adaptive resistance to sorafenib were analyzed by flow cytometry, qRT-PCR analysis, immunoprecipitation, immunoblotting, and immunohistochemistry. The combinatorial effect of SHP099 and sorafenib was tested in vivo using our HCC xenograft models and an immune-competent syngeneic model.

Results: Adaptive resistance to sorafenib in HCC cells developed with the activation of RTKs and of their positive effector SHP2. We found that SHP099 abrogated sorafenib resistance in HCC cell lines and organoid culture in vitro by blocking receptor tyrosine kinase (RTK)-mediated RAS/MEK/ERK and AKT reactivation. In addition, this sensitization effect was mediated by suppression of liver cancer stem cells (CSCs) and induction of cellular senescence. SHP099 in combination with sorafenib was highly efficacious in the treatment of xenografts and genetically engineered models of HCC.

Conclusions: SHP2 blockade in combination with sorafenib attenuated the adaptive resistance to sorafenib by impeding RTK-induced reactivation of the MEK/ERK and AKT signaling pathways. SHP099 in combination with sorafenib may be a novel and safe therapeutic strategy against HCC.

B22 Hsa-circRNA-0046600 promotes hepatocellular carcinoma metastasis by competitively binding to MiR-640-HIF-1α. Zhensheng Zhai, Qiang Fu, Chuanjiang Liu, Xu Zhang, Pengchong Jia, Peng Xia, Pan Liu, Tao Qin, Hongwei Zhang. Department of Hepato-Biliary-Pancreatic Surgery, People's Hospital of Zhengzhou University (Henan Provincial People's Hospital), Zhengzhou, Henan, China.

Background: Circular RNAs (circRNAs) play important roles in the development and progression of various human cancers. However, the functions of a large number of circRNAs in hepatocellular carcinoma (HCC) are not known. The purpose of this study was to investigate the biologic function of hsa-circ-0046600 in HCC and to elucidate the possible molecular mechanisms of this circRNA.

Methods: GSE97332, quantitative polymerase chain reaction (qRT-PCR), and fluorescence in situ hybridization (FISH) were used to detect the expression of hsa-circ-0046600 in HCC tissues and cells. Wound scratch assay and Western blotting were used to determine the effect of hsa-circ-0046600 on HCC cell migration. FISH was used to describe the localization of hsa-circ-0046600 in HCC cells. Luciferase reporter assays were used to determine hsa-circ-0046600 directly regulated miRNAs, and meta-analysis confirmed the expression of miRNAs in HCC. Bioinformatics was used to predict the mRNA targeted by miR-640. Important target genes were screened by GO, KEGG analysis, and PPI networks and verified by Western blotting.

Results: This study showed that the expression level of hsa-circ-0046600 in HCC was significantly higher than that in adjacent normal tissue ($P < 0.05$), and hsa-circ-0046600 expression was also associated with tumour size, TNM stage, and pathologic vascular invasion. The knockdown of hsa-circ-0046600 expression significantly inhibited the migration of HepG2 and SK-HEP-1 cells. In HCC, hsa-circ-0046600 can indirectly promote upregulation of HIF-1α via competitive binding to miR-640.

Conclusion: Hsa-circ-0046600 is highly expressed in HCC and can be used as a competitive endogenous RNA (ceRNA) of miR-640 to promote the expression of proteins such as HIF-1α to contribute to the malignant biologic behaviour of liver cancer cells. Therefore, hsa-circ-0046600 can be used as a new biomarker for HCC diagnosis and disease progression and provides a potential target for targeted therapy.

B23 Genomic analyses of hepatolithiasis-associated cholangiocarcinoma (HL-CCA) revealed a distinct subtype feature. Guoyun Xie[1], Jianlian Deng[1], Yupeng Wang[2], Huijuan Luo[1], Xing Zhao[1], Kui Wu[1], Jian Zhou[2], Xinrong Yang[2], Lunda Gu[1]. [1]BGI-Shenzhen, Shenzhen, China, [2]Liver Surgery Department, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China.

Background: Hepatic stone or lithiasis is a known risk factor for intrahepatic cholangiocarcinoma (ICC) in clinic; however, the process and genomic background of the hepatolithiasis-associated cholangiocarcinoma (HL-CCA) are completely unknown. Here, we established an intrahepatic CCA cohort including the patients combining biliary calculi.

Results: Eighteen stone CCA and 47 non-stone CCA paired frozen tissues from patients who received surgical operations between 2011 to 2013 were retrospectively collected. Prognosis analysis revealed that stone-associated patients had worse OS (18.6 months vs 28.6 months, P=0.024, Kaplan-Meier, HR=2.08), compared to those of non-stone ICC. Whole-exome sequence (WES, average 400x) analysis showed that HL-CCA displayed a distinct mutation pattern, with RTK pathway genes *EPHA2, KRAS* and TNF-beta pathway *SMAD4* significantly enriched in stone ICC (P<0.05, Fisher exact test) and epigenetic regulators *IDH1* and *BAP1* more enriched in non-stone group (P<0.1). Moreover, patients with *EPHA2* and *KRAS* mutations had poor OS than their wild-type counterparts, indicating these mutations play important roles in stone-mediated CCA.

Conclusion: Our results demonstrate that biliary stone might be an independent factor to induce a distinct CCA subtype, which may utilize kinase pathway to initiate carcinogenesis and result in a worse prognosis compared to non-stone CCA patients.

B24 Inflammatory microenvironment contributes to self-renewal features of hepatocellular carcinoma via NF-κB/miR-497/SALL4 axis. Bixing Zhao, Yingchao Wang, Xiaolong Liu. The United Innovation of Mengchao Hepatobiliary Technology Key Laboratory of Fujian Province, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou, China.

Increasing evidences have demonstrated the essential roles of inflammatory microenvironment on tumorigenesis and tumor progression. Cancer stem cells (CSCs) with capacity of self-renewal and multipotent differentiation have been proved to be responsible for tumor metastasis and treatment resistance. Although both inflammatory microenvironment and CSCs played crucial roles in tumor initiation and development, currently it is still unclear whether and how the inflammatory microenvironment promotes the formation of CSCs. Here, we show the first evidence that the inflammatory microenvironment connects with CSCs via NF-κB/miR-497/SALL4 axis to promote the self-renewal and metastasis in hepatocellular carcinoma (HCC). We discover that miR-497 directly targets SALL4 and negatively regulates its expression, and further inhibits the self-renewal and metastasis of HCC; more importantly, inflammatory factor TNFα inhibits the expression of miR-497 via NF-kB mediated negative transcriptional regulation, and simultaneously upregulates the expression of SALL4 and promotes self-renewal and metastasis phenotype of HCC cells. Moreover, lower expression of miR-497 is significantly associated with poor prognosis in HCC patients. Taken together, our findings not only revealed a novel signaling pathway (NF-κB/miR-497/SALL4 axis) to connect inflammation with CSCs and clarified the molecular mechanisms underlying the inflammation-mediated CSC phenotypes, but also provided novel molecular targets for developing new anticancer strategies.

B25 Ectopic expression of hypoxia-inducible factor-2α induces multidrug resistance in the treatment of hepatocellular carcinoma. Shu-cai Yang[1,6], Wei-jie Deng[4,6], Li-ping Liu[3], Jie Zhou[1,6], Hui Liu[5,6], Li Zhang[1,6], Bao-hu Zhang[1,6], Tao Jin[2]. [1]Department of Laboratory Medicine, Pingshan District People's Hospital, Shenzhen, Guangdong, China, [2]Department of Oncology, Liyuan Hospital, Tongji Medical College of HUST, Wuhan, Hubei, China, [3]Department of Hepatobiliary Surgery, Shenzhen People's Hospital, Shenzhen, Guangdong, China, [4]Department of Anesthesiology, Pingshan District People's Hospital, Shenzhen, Guangdong, China, [5]Department of Pharmacy, Pingshan District People's Hospital, Shenzhen, Guangdong, China, [6]The 8th Clinical School of Hubei Medical College, Shenzhen, Guangdong, China.

Objective: To study the multidrug resistance of HIF-2α to hepatocellular carcinoma and its mechanism.

Methods: One case of hepatocellular carcinoma and adjacent tissues were selected and the expression of HIF-2α was detected by immunohistochemistry. The expression of HIF-2α mRNA was also detected in two normal liver cell lines, four early-stage liver cancer cell lines, and three advanced liver cancer cell lines. HepG2 and PLC/PRF/5 were infected by HIF-2α lentiviral particles to prepare cells stably expressing HIF-2α, which was confirmed by Western blot. Six conventional chemotherapy drugs (phosphoramide, epirubicin hydrochloride, mitomycin, methotrexate, and sorafenib) were added to HepG2-HIF2α and control cells. MTT assay was used to detect cell inhibition rate; Western blot was used to detect the expression of drug resistance genes MDR1, LRP, and MRP in HIF-2α overexpressing hepatocellular carcinoma (HCC) cells and the corresponding control cells.

Results: Immunohistochemistry result showed that HIF-2α could be expressed in HCC tissues, but was lower than that in adjacent tissues. PCR results showed that HIF2α could be expressed in normal liver cell lines, early-stage HCC cell lines, and advanced HCC cell lines. The ectopic expression of HIF-2α in lentivirus-infected HepG2 and PLC/PRF/5 cells was validated by Western blot. MTT results showed that the inhibition rates of epirubicin hydrochloride, methotrexate, and sorafenib on HepG2-HIF-2α cells were significantly lower than control cells ($p<0.05$), which suggests the phenomenon of drug resistance, while the inhibition rates of fluorouracil, cyclophosphamide, and mitomycin on HepG2-HIF-2α cells were not different from that of the control group ($p>0.05$). This result was further analyzed by the method of Western blot; the results showed that, the expression levels of drug resistance-related genes (MDR1, LRP, and MRP) in HepG2-HIF-2α cells and PLC/PRF/5-HIF-2α cells were higher than those of the control group ($p<0.05$).

Conclusion: In hepatoma cells, HIF-2α can induce multidrug resistance of liver cancer by upregulating the expression of MDR1, LRP, and MRP.

B26 Necessity of ZFHX3 for hypoxia-induced HIF-1α/VEGF axis in the promotion of angiogenesis and tumor growth in hepatocellular carcinoma. Changying Fu[1,2], Na An[1,2], Jun A[1,2], Mingcheng Liu[1,2], Zhiqian Zhang[2], Liya Fu[1], Xinxin Tian[2], Baotong Zhang[3], Jin-Tang Dong[1,2,3]. [1]Department of Genetics and Cell Biology, College of Life Sciences, Nankai University, Tianjin, China, [2]Southern University of Science and Technology, School of Medicine, Shenzhen, Guangdong, China, [3]Emory Winship Cancer Institute, Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, GA, USA.

As the most common form of primary liver cancer, hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer death worldwide. In HCC and gastric cancer, alpha fetoprotein (AFP) is not only a well-established biomarker, it also functionally impacts cell proliferation, migration, and other aspects. Hypoxia is a common feature of HCC, and interestingly, *AFP* expression is downregulated by the hypoxia in HCC cells via the binding of HIF-1α to *AFP*'s promoter. Zinc finger homeobox factor 3 (ZFHX3), a large transcription factor with 23 zinc fingers and multiple other domains, was originally identified as AT-motif binding factor 1 (ATBF1) that binds to the promoter of *AFP* to suppress its expression in HCC cells. Although *ZFHX3* is frequently mutated in advanced prostate cancer and deletion of *Zfhx3* in mouse prostates induces mouse intraepithelial neoplasia and promotes prostatic tumorigenesis induced by *Pten* loss, similar effects between ZFHX3 and HIF-1α on the repression of *AFP* transcription suggest that ZFHX3 could have a different function in HCC from that in prostate cancer. We therefore tested whether ZFHX3 plays a role in HCC development. Using publicly available databases, we compared *ZFHX3* expression between HCC and normal liver tissues and found that the expression of *ZFHX3* mRNA was higher in cancer tissues than in normal tissues. In addition, higher expression of *ZFHX3* correlated with poorer disease-free survival in HCC, suggesting an oncogenic role of ZFHX3 in HCC. IHC staining of HCC tissue microarrays confirmed the upregulation of ZFHX3 in HCC. Interestingly, higher levels of *ZFHX3* correlated with higher levels of HIF-1α in human HCC, and a series of analyses in HCC cells indicated that hypoxia indeed upregulated *ZFHX3*, and the underlying mechanism involved the binding of HIF-1α to the promoter of *ZFHX3* to activate *ZFHX3* transcription. Increased ZFHX3 appeared to enhance the stability of HIF-1α under hypoxia. Biochemically, ZFHX3 interacted with HIF-1α, and the interaction was necessary for HIF-1α to induce the transcription of VEGF, an essential factor for HIF-1α to promote angiogenesis. In vitro and in vivo functional experiments indicated that ZFHX3 indeed played an important role in angiogenesis, as knockout or knockdown of *ZFHX3* attenuated the effects of hypoxia and HIF-1α on the expression of VEGF in HCC, tube formation in endothelial cells, angiogenesis in HCC xenograft tumors, and tumor growth of HCC cells in nude mice. Collectively, these findings suggest that in HCC, ZFHX3 is upregulated by hypoxia to coordinate with HIF-1α in the stimulation of angiogenesis and promotion of tumor growth in HCC. The HIF-1α/ZFHX3 interaction could provide a promising therapeutic opportunity for the treatment of HCC.

B27 Messenger RNA hypermethylation fuels cancer-promoting inflammation in hepatocellular carcinoma. Jiajie Hou[1], He Zhang[2]. [1]The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China, [2]HKU-SZH, Shenzhen, China.

Dynamic N^6-methyladenosine (m^6A) modification was previously identified as a ubiquitous post-transcriptional regulation that affected mRNA homeostasis. However, the m^6A-related epitranscriptomic alterations and functions remain elusive in human cancer. Here we identify the post-transcriptional outcomes of m^6A-methylome in hepatocellular carcinoma (HCC). In a transcriptomic dimension, HCC exhibits a characteristic gain of m^6A modification in tandem with an increase of mRNA expression. Loss of YTH domain family 2 (YTHDF2) predicts poor classification and prognosis of HCC patients, and correlates with m^6A abundance. YTHDF2 silenced in human HCC cells or depleted in mouse hepatocytes evokes inflammatory tumor proliferation and disrupts vessel normalization. Mechanistically, YTHDF2 exerts its

suppressive function by processing the decay of m^{6}A-containing interleukin 11 (IL11) and serpin family E member 2 (SERPINE2) mRNAs. Although YTHDF2 is transiently activated in response to hypoxia, its transcription succumbs to hypoxia-inducible factor-2α (HIF-2α). Importantly, YTHDF2 is essential for HIF-2α antagonist (PT2385)-mediated tumor inhibition. Together, our findings highlight profound implications for YTHDF2-reprogramed m^{6}A-methylome in treating HCC. This study specified the suppressive potential of YTHDF2 in cancer-promoting inflammation and established a context of metabolic-epigenetic regulation where hypoxia imposes a cancer-specific procedure of m^{6}A-mRNA editing. Importantly, restoring YTHDF2 expression defined m^{6}A decoration as a tumor-suppressive mechanism. Despite the sensitivity of destabilizing IL11 and SERPINE2 mRNAs in response to tumor hypoxia, YTHDF2 expression is readily silenced in the presence of active HIF-2α. In accordance, HIF-2α blockade improves YTHDF2-mediated suppression on m^{6}A-mRNA stability and inflammatory cancer behaviors, leveraging this metabolic-epigenetic axis toward therapeutic opportunities in human HCC.

LUNG CANCER

B28 Role of YAP in Axl-mediated resistance to an EGFR tyrosine kinase inhibitor. Hirohito Yamaguchi. Qatar Biomedical Research Institute, Ar-Rayyan, Qatar.

YAP is the transcription coregulator that is downstream of Hippo signaling pathway and plays a critical role in cancer. Because our previous mass spec analysis showed that Axl is the potential binding protein of YAP, we investigated the role of Axl in YAP regulation. Coimmunoprecipitation and Western blot analysis verified the interaction between YAP and Axl, and the interaction was enhanced by Axl ligand GAS6 stimulation. Moreover, we found that YAP is phosphorylated by GAS6 stimulation in vivo. Axl overexpression increased YAP transcriptional activity, and YAP overexpression increased colony formation activity in soft agar, which was further enhanced by coexpression of Axl. In EGFR tyrosine kinase inhibitor (TKI) sensitive lung cancer cells, YAP was downregulated in response to TKI treatment, and overexpression of YAP attenuated TKI sensitivity. Moreover, knockdown of Axl enhanced TKI-induced YAP downregulation while activation of Axl attenuated it. Axl overexpression attenuated TKI sensitivity, which was reversed by YAP knockdown. Together, these results suggest that Axl regulates YAP functions, which is critical for Axl-mediated TKI resistance.

B29 MUC3A induces PD-L1 and reduces tyrosine kinase inhibitor effects in EGFR-mutant non-small cell lung cancer. Yuan Luo, Shijing Ma, Shan Peng, Yingming Sun, Yan Gong, Conghua Xie. Zhongnan Hospital of Wuhan University, Wuhan, China.

Therapeutic antibodies blocking programmed death-ligand 1 (PD-L1) brought striking regression of non-small cell lung cancer (NSCLC). However, only 20% of patients respond to the αPD-L1 therapy. Therefore, novel pathways to induced PD-L1 expression and αPD-L1 sensitivity are to be investigated. MUC3A is a transmembrane mucin with 2 epidermal growth factor (EGF)-like domains in the extracellular segment. Tissue microarray of 92 patients' lung tumor samples indicated that high levels of MUC3A were positively correlated with poor prognosis and PD-L1 expression. In the TCGA database, we found that the levels of PD-L1 were independent of the protein levels of EGFR, but correlated with EGFR mutation. Activation of epidermal growth factor receptor (EGFR) signaling pathway was also reported to induce PD-L1 expression in NSCLC. Our results showed that induction of PD-L1 by EGF stimulation was only detected in EGFR-mutated NSCLC cell lines, such as H1975 and PC-9, but not in H1299 and H460. Knockdown of MUC3A in H1975 and PC-9 reduced PD-L1 expression, as well as PI3K/Akt and MAPK pathways. In addition, MUC3A deficiency sensitized EGFR-mutated NSCLC cells to tyrosine kinase inhibitors, such as gefitinib and AZD-9291. More interestingly, knockdown of MUC3A decreased EGFR protein levels, but not mRNA levels. Our findings suggested that MUC3A deficiency improved EGFR-mutated NSCLC sensitivity to tyrosine kinase inhibitors via increasing EGFR protein stability and decreasing PDL1 expression through PI3K/Akt and MAPK pathways. In the present study, we demonstrated that the transmembrane mucin MUC3A increased EGFR stability. Therefore, MUC3A induces PD-L1 and reduces tyrosine kinase inhibitor effects via EGFR modulation in NSCLC, suggesting potential implications for a novel immunotherapeutic approach.

B30 Association of systemic inflammation with survival in limited-stage small-cell lung cancer. Ruihan Yang, Wei Ji, Xiangliang Liu, Lu Zhang, Xiao Chen. Cancer Center, the First Hospital of Jilin University, Changchun, Jilin, China.

Objective: This study was designed to investigate the baseline clinical characteristics of peripheral blood neutrophil-to-lymphocyte ratio (NLR) in patients with newly diagnosed small-cell lung cancer (limited period) and to explore the relationship between baseline characteristics and chemotherapy efficacy.

Methods: This was a retrospective analysis of 44 patients who underwent first-line chemotherapy for small-cell lung cancer (limited period) in the First Hospital of Jilin

University from 2010 to 2016, and collected NLR clinical information before first-line chemotherapy. Patients were divided into 2 groups by NLR ratio (NLR calculation method: NLR was higher than 2nd tertile: 3.2), NLR <3.2 was low NLR group, and NLR ≥3.2 was high NLR group. The survival curves of progression-free survival (PFS) and overall survival (OS) were plotted by Kaplan-Meier method using SPSS (version 22.0) software. The clinical features of high NLR group and low NLR group and their effects on PFS value and OS value were observed.

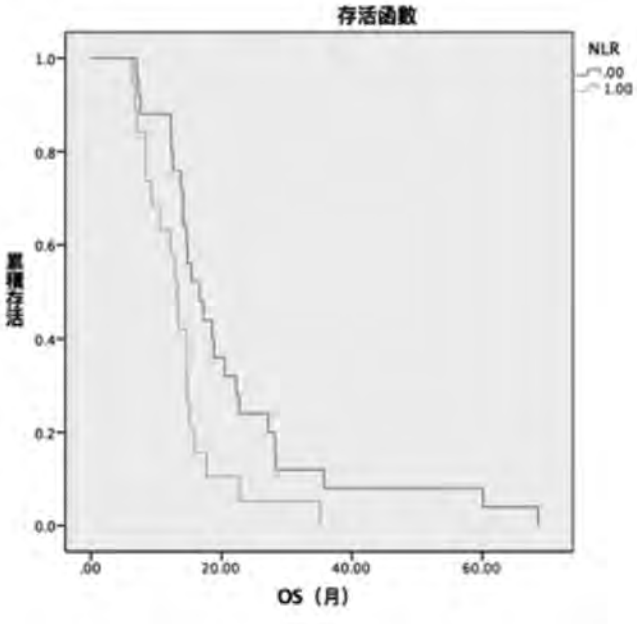
Results: Of the 44 patients, 25 (56.8%) had NLR <3.2 and 19 (44.2%) had NLR ≥3.2. The median OS of the high NLR group and the low NLR group were 13.2 months and 16.6 months, respectively (P < 0.05), and the difference was statistically significant, suggesting that patients with NLR <3.2 had longer OS.

Conclusions: High NLR is associated with poor survival in limited-stage small-cell lung cancer.

整體比較

	卡方	df	顯著性
Breslow (Generalized Wilcoxon)	5.711	1	.017

NLR不同層次的存活分配相等檢定。



B31 The efficacy of anti-PD-1/PD-L1 immunotherapy in KRAS-mutant non-small cell lung cancer is correlated with inflamed phenotype and increased immunogenicity.
Chengming Liu, Jie He. National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Background: KRAS mutation represents the common oncogene driver detected in approximately 30% of nonsquamous non-small cell lung cancer (NSCLC), conferring a poor prognosis without clinically effective targeted therapy. Immune checkpoint inhibitors against programmed cell death-1 (PD1)/programmed cell death ligand 1 (PD-L1) have brought survival benefits to KARS-mutant NSCLC patients while changing paradigm of cancer therapy. Yet, the underlying association between KRAS mutation and immune response remains largely unclear.

Methods: We performed an integrated analysis on the immunotherapeutic data concerning KRAS-mutant NSCLC from multiple sources including pool-analysis of clinical trials, immunotherapeutic data public from the cBioportal cohort, and $Kras^{G12C}$-mutant lung adenocarcinoma mouse model to estimate the relative efficacy of PD-1/PD-L1 inhibitor versus docetaxel in KARS-mutant NSCLC. A pool-analysis was set to clarify the correlation between KRAS mutational status and PD-L1 expression. Meanwhile, analysis of the Cancer Hospital/Institute, Chinese Academy of Medical Sciences (CICAMS) cohort of NSCLC patients harboring KRAS mutation was set to classify tumor microenvironment based on PD-L1 expression and $CD8^+$ tumor-infiltrating lymphocytes (TILs). In addition, we analyzed the mutation profiles based on the repository database to explore the relationship between KRAS mutational status and tumor mutation burden (TMB). Gene set enrichment analysis (GSEA) was used to determine potentially relevant gene expression signatures correlated with KRAS mutation.

Results: Two pool-analysis confirmed that compared to KRAS-wild-type patients, PD-1/PD-L1 inhibitors resulted in better objective response rate (OR=1.52, 95% CI: 1.15–2.01, P=0.004) in KRAS-mutant NSCLC patients, and improved overall survival in comparison to chemotherapy with docetaxel (HR=0.64, 95% CI: 0.43–0.95, P=0.03) in previously treated KRAS-mutant NSCLC patients. Publicly available trial data from a cohort of eight advanced KRAS-mutant NSCLC patients prescribed pembrolizumab showed that all patients enjoyed a durable clinical benefit (progression-free survival > 6 months) during treatment. Importantly, we found that anti-PD-L1 monoclonal antibodies (anti-PD-L1 mAbs) significantly restrained tumor growth versus docetaxel in $Kras^{G12C}$-mutant lung adenocarcinoma mouse model, while the combinatory administration of PD-L1 mAbs with docetaxel did not increase antitumor response. Moreover,

pool-analysis of 28 public studies suggested that patients with KRAS mutation had increased PD-L1 expression (OR=1.96, 95% CI: 1.41–2.71, P<0.0001). The combined analysis of PD-L1 and $CD8^+$ TIL in the IHC-detected group showed an increase of TILs and an augmented proportion of dual-positive ($PD\text{-}L1^+/TIL^+$) in the KRAS-mutant group, suggesting patients with KRAS mutation demonstrate an inflamed phenotype. Furthermore, the data from The Cancer Genome Atlas (TCGA) and Broad dataset verified a prominently increased mutation burden and a high rate of transversion/transition (Tv/Ti) in KRAS mutation, which indicates KRAS mutation is accounted for augmenting tumor immunogenicity in NSCLC. Further analysis focused on the potential molecular mechanism revealed that KRAS mutation manifested various defects of DNA repair.

Conclusions: Our results provided evidence that KRAS-mutant NSCLC patients show remarkable clinical benefit to anti-PD-1/PD-L1 immunotherapy. Also, we pointed out a complex relationship among KRAS mutation, inflamed phenotype and tumor immunogenicity.

B32 Application of whole body phase angle for evaluation of prognosis of patients with NSCLC. Kaiwen Zheng, Wei Ji, Xiangliang Liu, Lingyu Li, Wei Li, Jiuwei Cui. Cancer Center, First Hospital of Jilin University, Changchun, Jilin, China.

Purpose: Our intent is to examine the predictive role of whole body phase angle on NSCLC patients who received molecular targeted therapy.

Methods: Clinical materials of 79 NSCLC patients were prospectively and consecutively collected in The First Affiliated Bethune Hospital of Jilin University from January 1st, 2015 to July 31st, 2017. 79 cases were EGFR gene mutation-positive and received molecular targeted therapy. Electrical impedance at 50kHz was measured to calculate the whole body phase angle. Based on the 2nd of trisectional quantiles range of whole body phase angle (5.1°), patients were categorized into 2 grades: low whole body phase angle group (<5.1°) and high whole body phase angle group (≥5.1°). Kaplan-Meier analysis and log-rank analysis were done to compare survival rate of 2 groups.

Results: Log-rank test showed that the progression-free-survival (PFS) of patients in low whole body phase angle group and high whole body phase angle group was statistically different (mean 8.89 vs. 13.14, 95%CI 6.91-10.85 vs. 11.18-15.11, P=0.009) (Fig. 1). Multivariate analysis showed that whole body phase angle (HR=0.52, 95%CI 0.33-0.83, P=0.006) (Table 1) was an independent prognostic factor in patients with NSCLC. Patients in low whole body phase angle group obtained shorter PFS.

Conclusions: Whole body phase angle is a good predictor of prognosis in NSCLC patients with EGFR gene mutation-positive who received molecular targeted therapy.

Fig. 1 Kaplan-Meier survival analysis of low/high whole body phase angle group.

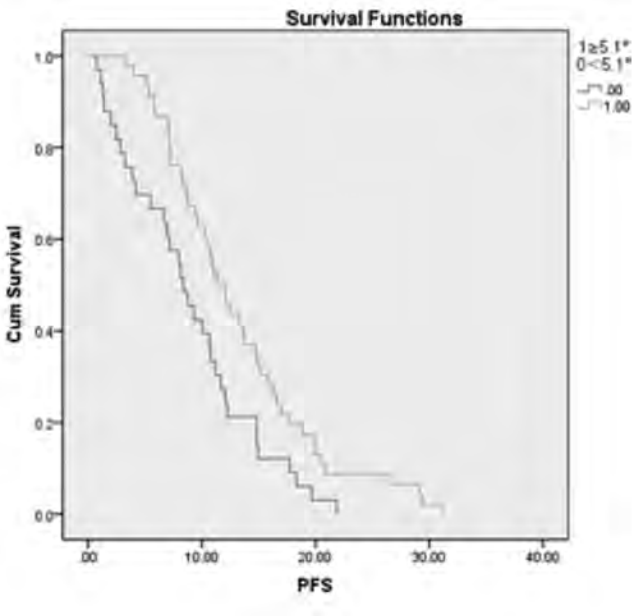


Table 1. Cox regression analysis

	Univariable model				Multivariable model			
Factors	HR	95%CI		P	HR	95%CI		P
Gender	1.189	0.751	1.882	0.460				
Age	1.006	0.986	1.027	0.533				
Whole body phase angle	0.522	0.329	0.829	0.006	0.522	0.329	0.829	0.006

B33 Application of neutrophil/lymphocyte ratio in prognosis of patients with small-cell lung cancer of extensive stage. Wei Ji, Kaiwen Zheng, Xiangliang Liu, Lingyu Li, Wei Li, Jiuwei Cui. Cancer Center, The First Affiliated Hospital of Jilin University, Changchun, Jilin, China.

Objective: To explore whether the preoperative neutrophil/lymphocyte ratio (NLR) can be used as effective prognostic predictors in patients with extensive small cell lung cancer (SCLC).

Materials and Methods: 121 cases of newly diagnosed extensive SCLC were analyzed retrospectively in The First Affiliated Bethune Hospital of Jilin University from January 1 ,2015, to July 31 ,2017. 109 cases were followed, with a follow-up rate of 90.04%. Prognostic factors such as gender, age, and NLR in baseline were analyzed. According to the 2nd of trisectional quantiles range, all the patients were

divided into low NLR group (NLR<3.30) and high NLR group (NLR≥3.30). The survival rate was calculated by Kaplan-Meier analysis, and the difference between groups were compared using log-rank test. Cox regression analysis was used to analyze the factors that may affect the prognosis of the patients.

Results: Log-rank test showed that the overall survival (OS) of patients in low NLR group and high NLR group were statistically different (mean 13.68 vs. 10.35, 95%CI 12.11-15.26 vs. 8.60-12.10, P=0.011) (Fig. 1). Multivariate analysis showed that NLR (HR=1.68, 95%CI 1.12-2.52, P=0.012) was an independent prognostic factor in patients with extensive SCLC (Table 1). Patients in low NLR group obtained longer survival.

Conclusion: NLR is a good predictor of prognosis in patients with SCLC of extensive stage.

Fig. 1 Kaplan-Meier survival analysis of low/high NLR group.

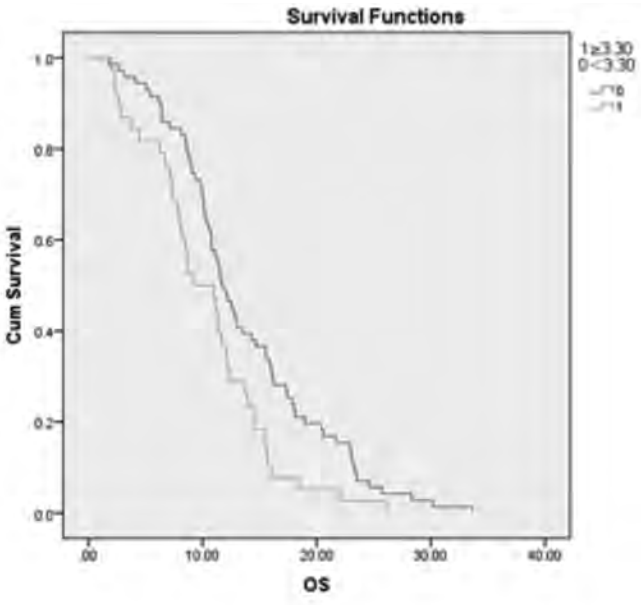


Table 1. Cox regression analysis

	Univariable model				Multivariable model			
Factors	**HR**	**95%CI**		**P**	**HR**	**95%CI**		**P**
Gender	0.976	0.653	1.460	0.907				
Age	1.017	0.994	1.040	0.160				
NLR group	1.679	1.121	2.516	0.012	1.679	1.121	2.516	0.012

B34 Post-treatment lymphocyte-to-monocyte ratio as a predictor of response and survival among patients with advanced EGFR wild-type non-small cell lung cancer. Lu Zhang, Xiangliang Liu, Ruihan Yang, Xiao Chen. Cancer Center, The First Hospital of Jilin University, Changchun, Jilin, China.

Background: Peripheral blood-based parameters (e.g., lymphocyte to monocyte ratio, LMR) have been studied as surrogate measures of intratumoral immunity that reflect a host's immune response, which may be a potential prognostic marker in cancers. We retrospectively analyzed the relevant cases and explored prognostic significance of the pretreatment or post-treatment lymphocyte-to-monocyte ratio in patients with advanced EGFR wild-type non-small cell lung cancer.

Methods: A total of 106 NSCLC patients with EGFR wild-type in Jilin University First Hospital were eligible for retrospective analysis between March 2011 and March 2015. Potential prognostic factors such as age, gender, metastasis, histology, and response to chemotherapy were analyzed. The peripheral lymphocyte, monocyte, and LMR were collected at baseline and the end of second cycles of chemotherapy. Univariate and multivariate Cox regression analyses were performed to determine the associations of the LMR and clinical features with overall survival (OS) and progression-free survival (PFS).

Results: Among 108 cases, the counts of baseline lymphocyte (L0) or post-treatment (L2) were respectively 1.81±0.61 and 1.84±0.62. The pretreatment monocyte (M0) or post-treatment (M2) were 0.51±0.23 and 0.44±0.18. M2 was significantly lower than M0 (P=0.001). The pretreatment LMR (LMR0) or post-treatment (LMR2) were 4.55±3.3 and 4.80±2.33, which there was statistical differences (P<0.05). M0 and LMR2 significantly correlated with the response of treatment with two cycles of chemotherapy and OS, PFS. The objective response rate (ORR) of the lower M0 group was 35.8%, significantly higher than in high M0 group (17.8%) (P<0.05). The ORR of higher LMR2 group was 35.5% and ORR of lower LMR2 group was 19% (P<0.05). The patients with lower Mo or higher LMR2 had the satisfactory survival (Fig. 1).

Conclusions: This study suggested that a higher post-treatment LMR level was associated with a favorable response and outcomes among advanced EGFR wild-type non-small cell lung cancer patients. The pretreatment peripheral monocyte also the potential predictors.

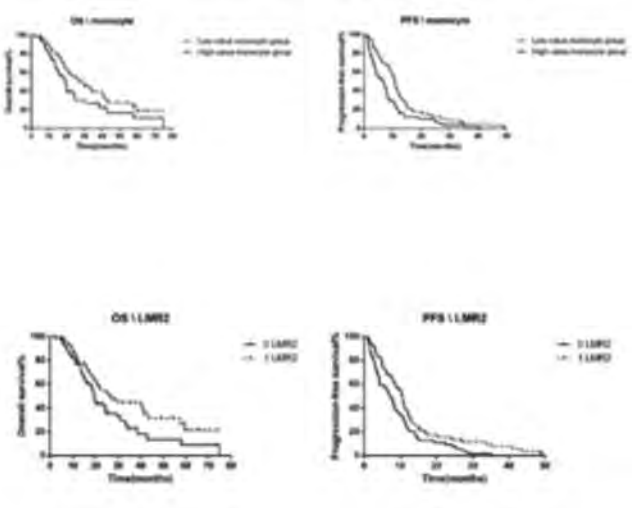


Fig. 1
Compared with high Mo group, the median PFS and OS patients with lower Mo, respectively, were 10.61 vs. 6.93 months (P<0.05) and 21.00 vs. 16.76 months (P = 0.05). Compared with patients with lower LMR2, median PFS and OS in higher LMR2 group were 10.46 vs. 7.26 months(P<0.05) and 20.73 vs. 18.2 months (P<0.05).

B35 Downregulating Nrf2 by tangeretin reverses multiple drug resistance cancer: In vivo and in vitro study. Xing-Xing Fan, Ying Xie, Liang Liu. State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Macau.

No matter in chemotherapy or molecularly targeted therapy, drug resistance is the main obstacle encountered by current cancer clinical treatment. However, the underlying mechanisms remain to be elucidated. Elevated reactive oxygen species (ROS) has been described to be closely associated with drug resistance. Therefore, targeting the abnormal redox signaling may provide a novel anticancer strategy to selectively kill resistant cancer cells. In this study, we aim to investigate the role of ROS in both chemotherapy and molecularly targeted drug resistance cancer and whether tangeretin can enhance the efficacy of chemotherapy on drug-resistant NSCLC cells by downregulating Nrf2 signaling pathway. Compared with normal cells and nonresistant cancer cells, the ROS and its key regulator Nrf2 were remarkably upregulated in drug-resistant NSCLC cells either with efflux transporters overexpression or molecularly targeted mutation. Knockdown of Nrf2 to further enhance ROS level substantially increased the percent of apoptotic cells and sensitized cancer cells to anticancer drug treatment. Tangeretin (TG), a flavonoid isolated from citrus peels, efficiently inhibited the growth of drug resistant cancer cells in both in vitro and in vivo studies. The underlying mechanism studies revealed that TG significantly induced Nrf2 degradation and thus suppressed Nrf2 signaling pathway, as well as the expression of ATP-binding cassette (ABC) transporter: P-glycoprotein (P-gp). Targeting Nrf2 to enhance ROS and reduce ABC transporter has promising potential for cancer patients with drug resistance.

Acknowledgments: This work was supported by grants from the Science and Technology Development Fund of Macao (Project code: 003/2018/A1) to Xing-Xing Fan.

B36 In situ detection of PD-1^{+}CXCL13^{+}CD8^{+} T cells in non-small cell lung cancer. Na Li, Bingqing Zhang, Niyati Jhaveri, Zhifu Zhang, Xin Wang, Hongzhe Sun, Ying Zhou, Courtney Anderson, Xiao-Jun Ma. Advanced Cell Diagnostics, Newark, CA, USA.

Recent reports identified one subset of intratumoral CD8^{+} cytotoxic T lymphocytes (CTLs) in non-small cell lung cancer (NSCLC) that are PD-1^{high} with distinct molecular and functional properties. Strikingly, these cells produce very high levels of CXCL13 mRNA and protein, which may mediate immune recruitment. Furthermore, the presence of PD-1^{high} CD8^{+} T lymphocytes are strongly predictive for both response and survival in NSCLC patients treated with PD-1 blockade. Thus, it is of great value to develop a practical biomarker assay to specifically detect these cells in formalin-fixed, paraffin-embedded (FFPE) tumor biopsies. In this study, we combined the highly sensitive and specific RNAscope multiplex fluorescent RNA in situ hybridization (ISH) assay detecting *CXCL13* and *PD-1* mRNAs with immunochemistry (IHC) detecting CD8 protein in a single tissue section to directly visualize PD-1^{+}CXCL13^{+}CD8^{+} T lymphocytes in NSCLC tissue. Two NSCLC tissue microarrays (TMAs) consisting of 63 independent patient FFPE samples were stained with full automation using the Leica BOND RX instrument. The resulting slides were scanned, and the images were analyzed using the Perkin Elmer Phenochart software. 57 of the 63 TMA cores were available for image analysis. Each tissue core was first examined under 4X magnification, then snapshot images of three independent 40X fields with enriched CD8^{+} cells (if present) were taken. CD8+ cells, CXCL13+ cells, and PD-1+ cells in each snapshot were counted. Every snapshot contained both stromal and tumor regions. 43 samples contained high (>20) CD8+ CTLs whereas 14 samples contained low (≤20) CD8+ CTLs across the three snapshots. Interestingly, PD-1^{+}CXCL13^{+}CD8^{+} cells were detected in both high and low CTL tumors. Five of 57 tumors carried high percentages of PD-1^{+}CXCL13^{+}CD8^{+} cells (>10% of CD8+ CTLs), with three from high CTL tumors and two from low CTL tumors. These results demonstrate that this fully automated multiplexed RNAscope dual ISH/IHC assay allows for colocalization of RNA and protein biomarkers in single cells with morphologic context. The ability to detect RNA and protein in a single slide-based

assay enables immune profiling applications to include biomarkers such as secreted proteins and noncoding RNAs that are difficult or impossible to detect by IHC.

MALE-SPECIFIC CANCERS

B37 Collaboration between PTEN and CHD1 in prostate cancer progression. Mengyao Wang[1], Ruixin Liu[1], Mei Wang[1], Tanu Shenoy[2], Liuzhen Zhang[1], Hong Wu[1,2]. [1]The MOE Key Laboratory of Cell Proliferation and Differentiation, School of Life Sciences, Peking-Tsinghua Center for Life Sciences, Beijing Advanced Innovation Center for Genomics, Peking University, Beijing, China, [2]Department of Molecular and Medical Pharmacology, University of California Los Angeles, Los Angeles, CA, USA.

Background: Loss of PTEN and CHD1 are two of the most common recurrent genomic alterations found in human prostate cancers. *CHD1* and *PTEN* mutations are mutually exclusive in primary cancer stage but are overlapped in metastatic tumors. Previous study demonstrates that *PTEN* and *CHD1* double deletion cause synthetic lethality in human prostate cancer cell lines in vitro.

Methods: We established a prostatic epithelial cell-specific *Chd1* and *Pten* double knockout mouse model. By comparing phenotypes associated with *Pten* or *Chd1* single and *Pten/Chd1* double deleted prostates, we aimed to investigate the collaboration between PTEN and CHD1 in prostate cancer progression in vivo.

Results: At earlier stage (5-10 weeks), prostates with *Pten/Chd1* double deletion have increased cell proliferation and tumor size when compared with *Pten* or *Chd1* single deleted prostates, which suggests that PTEN and CHD1 loss may not cause a synthetic lethal in vivo. However, double knockout prostates at 30 weeks start to show dark necrotic areas, which continuously expand to the entire prostate when animals reach one year old. Gene expression analysis showed increased TNFα, IFNα and γ response pathways in the *Pten/Chd1* double deleted prostates, accompanied by increased cleaved caspase and P-MLKL, signatures for apoptotic and necroptotoc pathway activations. We also detected increased immune cell infiltration in the tumor microenvironment during cancer progression in double knockout mice.

Conclusions: We found that *Chd1* deletion in the *Pten* null prostate epithelium could trigger cell proliferation and tumor growth, suggesting that CHD1 and PTEN double deletion may not cause cell-intrinsic lethality in vivo. Our study suggests that the crosstalk between PTEN and CHD1 may regulate the immune response and necroptosis pathways, which in turn trigger the cancer cell-extrinsic immune responses and necroptosis.

No conflict of interest.

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NEW DRUG DEVELOPMENT

B38 Lapatinib, a tyrosine kinase inhibitor (TKI), enhances AKT activation in MDA-MB231 triple-negative breast cancer cells—a new possible mechanism for resistance to TKI-targeted therapy. Parham Jabbarzadeh Kaboli[1,2], King-Hwa Ling[3,4], Zhangang Xiao[1,2]. [1]Laboratory of Molecular Pharmacology, Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou, Sichuan, China, [2]South Sichuan Institution for Translational Medicine, Luzhou, Sichuan, China, [3]Department of Genetics, Harvard Medical School, Boston, MA, USA, [4]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia.

Introduction: Triple-negative breast cancer (TNBC) is a diverse and heterogeneous group of breast tumors that lack estrogen and progesterone receptors and HER2 expression. Epidermal growth factor receptor (EGFR), which is a well-known tyrosine kinase receptor, is expressed in 50% of TNBC cases. Although chemotherapy with tyrosine kinase inhibitors (TKIs) has been recognized as the most important therapeutic method against TNBCs, TNBC patients acquire resistance to TKIs and EGFR targeted therapy. MDA-MB231 is a triple-negative breast cancer cell line known to be resistant to tyrosine kinase inhibitors, such as lapatinib. To figure out the mechanism of resistance to lapatinib, we studied the effects of lapatinib on downstream proteins of PI3K and MAP kinase pathways in both MDA-MB231 ($EF^-PR^-HER2^-$) and MCF-7 ($EF^+PR^+HER2^+$) breast cancer cell lines. Here, we wish to highlight the unexpected result we obtained from our study due to lapatinib-treated TNBC cells.

Methods: Using molecular modeling, ELISA, flow cytometry, and Western blotting, we analyzed and compared the

effects of lapatinib and berberine (BBR) on MDA-MB231 and MCF-7 cells. The kinase activity of p38, ERK1/2, EGFR, and AKT targeted by different concentrations of both compounds. For Western blotting, p-AKT and p-EGFR antibodies were diluted as previously reported. Phosphorylated proteins were analyzed in duplicate.

Results: While BBR reduced the level of both p-EGFR and p-AKT in both cell lines, the level of EGFR in MDA-MB231 was dramatically lower than that in MCF-7 cells. Although reduced EGFR expression is a known feature of MDA-MB231 cells, other alternative signaling proteins at downstream of EGFR signaling have also been implicated in MDA-MB231 cellular resistance to EGFR inhibitors. In lapatinib-treated MDA-MB231 cells, we detected a decrease in the level of p-EGFR and an increase in the level of p-AKT that may explain the effects of lapatinib on AKT. However, BBR successfully suppressed both EGFR and AKT in MDA-MB231 cells. In contrast to MDA-MB-231 cells, MCF-7 cells express Her2, Her3, and EGFR. Her3 and Her2 interact with EGFR to transduce EGF signaling to MAPK and downstream targets. In case of acquired resistance to lapatinib, BBR can reverse this resistance in MDA-MB231 cells. The mechanisms by which MDA- MB231 cells respond to lapatinib and its effect on p-AKT require further examination. BBR greatly sensitizes breast cancer cells by suppressing AKT and human Smoothened receptor. AKT inhibitors also sensitize tumor cells to stimulate apoptosis. We tested lapatinib against AKT activation in MDA-MB231 cells. AKT is an oncogenic protein located downstream of EGFR signaling. Therefore, AKT overactivation will impede EGFR targeted therapy. We observed that lapatinib could strongly increase the level of AKT activation compared to untreated and BBR-treated MDA-MB231 cells. We repeated the experiments four times to ascertain the observation. We observed a reduced level of p-EGFR but increased level of p-AKT when the cells were treated with lapatinib. Multikinase inhibitors such as sorafenib and berberine, and more specifically, AKT inhibitors seem to be good choices to overcome lapatinib resistance in TNBC cells.

Conclusions: Although lapatinib inhibits EGFR, we found MDA-MB231 cells were resistant to lapatinib by overactivation of AKT. Activation of AKT upon lapatinib treatment has not been previously reported. Since MDA-MB231 cells are reported as TKI-resistant cells, this outcome shows a new hope for overcoming lapatinib resistance in cancer treatment. Thus, using AKT inhibitors in combination with lapatinib is suggested for the future studies.

B39 The Rho kinase inhibitor H1152 preferentially kills tumor cells that overexpress the Myc oncoprotein by disabling the motor kinesin-like protein MKLP2. Qiong Shi, Chenglu Yang, Ting Zhang, Jing Zhang, Dun Yang. J. Michael Bishop Institute of Cancer Research, Chengdu, China.

Overexpression of the Myc oncoprotein causes tumors in mice and occurs frequently in a variety of human malignancies, but the potential of the protein as a therapeutic target is compromised by the threat of side effects and a current lack of practical pharmacologic Myc inhibitors. We have previously reported a synthetic lethal interaction between overexpression of Myc and disablement of the Aurora-B mitotic kinase. Reasoning that disablement of a component either upstream or downstream in the Aurora-B signaling pathway might also have synthetic lethal interaction with overexpression of Myc, we screened a chemical drug library in a pair of isogenic cell lines. We discovered that the Rho kinase inhibitor H1152 selectively killed cells that overexpress Myc, as opposed to their control cells. The synthetic lethal interaction was effective against a variety of human cancer cell lines both in vitro and in xenograft models, but the lethality was not due to inhibition of the Rho kinases, the established H1152 target. Instead, H1152 apparently inhibited the motor kinesin-like protein MKLP2, because both Aurora-B and INCENP failed to relocate from chromosomes to the central spindle in cells treated with the agent. This failure apparently disturbed both the assembly of the mitotic spindle microtubules during late mitosis and the localization of MKLP1, PLK1, and PRC1 to the central spindle and the midbody. These abnormalities, in turn, prevented completion of cytokinesis. After suffering cytokinetic failure, cells developed polyploidy and died eventually. Collectively, these data identify H1152 as a novel MKLP-2 inhibitor, uncover a new synthetic lethal strategy to kill cells that overexpress Myc, and discover overexpression of Myc as a predictive biomarker for H1152.

Funding: This work is supported by the J. Michael Bishop Institute of Cancer Research.

B40 Novel thiosemicarbazones downregulate the proto-oncogene, c-Met, via lysosomal degradation and intracellular shedding. Kyung Chan Park, Bekesho Geleta, Lionel Yi Wen Leck, Patric J. Jansson, Zaklina Kovacevic, Des R. Richardson. Molecular Pharmacology and Pathology Program, Discipline of Pathology and Bosch Institute, The University of Sydney, Sydney, NSW, Australia.

Introduction: The proto-oncogene, c-Met, drives tumor progression (1) and induces development of resistance in various cancers (2,3). The c-Met signaling pathway can also rescue cancer cells when other receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), are inhibited (4). Thus, c-Met is an important therapeutic

target for cancer treatment. The novel class of di-2-pyridylketone thiosemicarbazones (DpT) anticancer agents have shown potent antitumor and antimetastatic activities in multiple advanced solid tumors (5,6). These agents exert their anticancer effects by inhibiting key oncogenic signaling pathways (7). We recently demonstrated that these agents downregulate EGFR expression in pancreatic cancer through lysosomal degradation (8). Considering the close association of EGFR with c-Met, it was crucial to examine the effects of the DpTs on this important RTK.

Methods: DU145 prostate cancer cells were treated with either di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT) or di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) to examine their effects on c-Met expression. Western blot analysis and reverse transcription polymerase chain reaction (RT-PCR) were performed to measure protein and mRNA levels of c-Met, respectively. Immunofluorescence and confocal microscopy were utilized to detect the lysosomal localization of c-Met in response to these compounds and the effects of lysosomotropic agents, NH_4Cl, methylamine (MA) and chloroquine (CLQ), on preventing the downregulation of c-Met. Lysosomal localization of c-Met was examined by colocalizing c-Met with lysosomal associated membrane protein 2 (LAMP2). Further, a broad-spectrum metalloproteinase inhibitor, EDTA, or a proteasome inhibitor, MG132, was used in combination with Dp44mT to assess the role of metalloproteinases or the proteasome, respectively, in c-Met downregulation by Dp44mT. ELISA was also used to measure soluble c-Met.

Results: For the first time, we demonstrate that Dp44mT and DpC markedly downregulate total c-Met protein levels and its phosphorylated forms, while also inhibiting activation of its major downstream effector, Gab1. In contrast, *MET* mRNA levels remained unchanged when cells were treated with these agents. Incubation of cells with the lysosomotropic agents, NH_4Cl, MA, and CLQ partially rescued c-Met expression, suggesting Dp44mT and DpC induce lysosomal degradation of c-Met. A metalloproteinase inhibitor, EDTA, abolished the effect of Dp44mT on c-Met expression, suggesting that metalloproteinase-mediated shedding could be involved in c-Met downregulation by Dp44mT. The levels of soluble c-Met decreased after incubation of cells with Dp44mT, which suggested that a Dp44mT-induced c-Met shedding process may occur inside cells, e.g., within lysosomes.

Conclusion: This study demonstrates for the first time that the novel thiosemicarbazones, Dp44mT and DpC, downregulate the proto-oncogene, c-Met, via lysosomal degradation and potentially through intracellular shedding. These results highlight the potential of Dp44mT and DpC as promising new anticancer agents for advanced tumors.

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B41 Achieving better delivery for cancer therapy: From inorganic nanoparticles to self-assembled peptide nanosphere. Yin Feng. Peking University Shenzhen Graduate School, Shenzhen, China.

Tumor is one of the most serious diseases and one of the leading causes of human death worldwide. Thanks to the emerging therapeutics and technologies for the goal to cure cancer, the life spans of many cancer patients are significantly extended. Despite the great success, new therapies are still urgently needed, especially for malignant cancer. Nanomedicine is an emerging field that utilizes nanoparticles technology concept for advanced therapy and diagnoses of cancers. In our previous reports, we fabricated various multifunctional nanoparticles as drug delivery systems for cancer therapy, such as gold nanorods, mesoporous silica nanoparticles, two-dimensional (2D) nanomaterials MoS_2, graphene oxide, black phosphorus and aggregation-induced emission luminogen (AIEgen) (1-6). Although inorganic nanoparticles are also promising drug carriers due to their facilely functional surface and high drug loading efficiency, the hard degradation in vivo has caused serious safety concerns, which impede their therapeutic applications. As naturally biocompatible molecules, peptides can be rationally designed as efficient carriers for drug delivery. In our recent reports, we have developed two novel methodologies for drug delivery: (1) We stabilized the HDAC substrates acetylated H3K56 peptide with the N-terminal helix nucleating template strategy developed by our group and covalently conjugated the peptide with a hydroximic acid. The peptide/small molecule hybrid showed a significantly enhanced therapeutic window and offered promising opportunities for cancer therapy (7); (2) we utilized our developed Met crosslinking strategy to construct a peptide–siRNA co-assemble system. The peptide–siRNA complex rapidly forms by addition of siRNA and shows high cellular uptake, low cytotoxicity, and facile biodegradation (8,9).

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B42 Fluphenazine, an antipsychotic agent, inhibits brain metastases and lung metastases of triple-negative breast cancer. Fuyan Xu, Yong Xia, Yuquan Wei, Luoting Yu. Department of Rehabilitation Medicine, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy, Chengdu, China.

Background and Purposes: A major obstacle to successful breast cancer treatment is the metastasis to other organs of the body, including liver, bones, lungs, and brain. Brain metastasis is an end-stage disease of breast cancer progression. Traditional treatment options such as surgery, radiation, and chemotherapy showed minimal efficacy, and the overall survival of these patients is on the order of months. The incidence of brain metastases in patients with metastatic triple-negative breast cancer (TNBC) is approximately 40%. Many drugs used to treat primary TNBC cannot cross the blood-brain barrier (BBB), leading to therapeutic resistance. Therefore, finding new and effective drugs that could reach therapeutic concentrations in the brain is essential for improving the survival and life qualities of patients with brain-metastatic TNBC. Antipsychotic drugs easily penetrate the BBB and many papers have reported their anticancer potentials. Several publications have reported the anticancer abilities of fluphenazine (Flu), an FDA-approved antipsychotic drug. It has entered clinical trials for treating cancer. The above information drives us to assess its potential for treating TNBC and brain metastasis.

Experimental Procedures: MTT was used to assess the inhibitory effects of Flu on TNBC cells' viabilities. Next, we assessed the effects of Flu on cell cycle distribution, apoptosis, mitochondrial membrane potential ($\Delta\Psi m$), and reactive oxygen species (ROS) levels in cells by flow cytometry. The effects of Flu on TNBC cell migration and invasion were investigated in vitro using transwell. Pharmacokinetics studies were carried out in mice to determine the concentration of Flu in the blood and brain. Then, we treated the subcutaneous xenografts in the mice with Flu via intraperitoneal injection at 8 mg/kg/day. Moreover, we established murine models of brain metastasis, liver metastasis, and bone metastasis by intracarotid injection, splenic injection, and intratibial injections, respectively, and evaluated the efficacy of Flu on these metastatic growths. Side effects and toxicities of Flu to the mice were evaluated.

Results: Flu inhibited survival of several metastatic TNBC cell lines with IC_{50} values less than 15 μM after 72 hours' treatment. 4T1 cells and MDA-MB-231 cells were selected for further studies. Flu induced G0/G1 cell cycle arrest,

which was associated with decreased expression of cyclin-dependent kinase (CDK) 2, CDK4, cyclin D1, and cyclin E, and upregulation of p21 and p27. In addition, Flu induced apoptosis of 4T1 cells and MDA-MB-231 cells. It decreased $\Delta\Psi m$ and increased ROS levels in cancer cells. Besides, Z-LE(OMe)HD(OMe)-FMK, a caspase-9 inhibitor, and the antioxidant N-acetyl L-cysteine partially rescued Flu-induced apoptosis, implying that it probably induced the mitochondria-mediated apoptosis. Pharmacokinetic studies in the mice showed Flu was highly distributed in the brain with a brain/plasma drug concentration ratio above 25 for at least 24 hours after dosing. Flu moderately suppressed the tumor growth in a subcutaneous xenograft mouse TNBC model. In addition, Flu strongly inhibited spontaneous lung metastasis. Importantly, Flu exhibited good antimetastatic potential in a mouse brain metastasis model with an inhibition rate of 85%. However, Flu did not suppress experimental bone and liver metastasis. Moreover, Flu did not cause serious side effects in the mice. It did not cause significant changes of hematologic and serum biochemical parameters in the mice. Histologic examinations of heart, liver, kidney, and brain did not show obvious signs of pathologic changes.

Conclusions: The encouraging results of our study have laid the foundation for repurposing Flu to treat TNBC with brain and lung metastases, which currently lack any effective treatment options.

B43 Progress of MBRI-001, a deuterium-substituted plinabulin, as a potent anti-microtubule agent for anticancer. Zhongpeng Ding[1], Mingxu Ma[1], Shixiao Wang[3], Jianchun Zhao[1, 3], Wenbao Li[1,2,3]. [1]School of Medicine and Pharmacy, Ocean University of China, Qingdao, China, [2]Innovation Center for Marine Drug Screening and Evaluation, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China, [3]Marine Biomedical Research Institute of Qingdao, Qingdao, China.

Plinabulin is a marine natural product, which has its binding site of targeting microtubules near the colchicine site. The combination of plinabulin and docetaxel has been pushed into phase III clinical trial for treatment of NSCLC. MBRI-001 is a deuterium-substituted plinabulin derivative. The total synthesis yield of MBRI-001 was 11% in eight linear steps. Among the five discovered crystal forms of MBRI-001, Form E (composed of MBRI-001 and H_2O) was the most stable at different temperature and humidity. The analysis of co-crystal structure of MBRI-001 with tubulin (PDB:5XI5) showed that MBRI-001 bound in the interface region between α-tubulin and β-tubulin, which was similar to plinabulin (PDB: 5C8Y). The antiproliferative activities of MBRI-001 were observed at a low nanomolar level for a variety of cancer cell lines. Immunofluorescence and cell cycle assay demonstrated that MBRI-001 could inhibit the formation of microtubules and induce G2/M arrest through the downregulation of cyclin B1 (CCNB1). In vivo PK studies indicated that its pharmacokinetic properties were better than that of plinabulin. MBRI-001 could be distributed rapidly and widely in various tissues, of which the concentration in lung was remarkably higher than other tissues in rat body. The excretion study indicated that most of MBRI-001 might be decomposed and excreted as metabolites. In in vivo studies, MBRI-001 has shown a significant efficiency without apparent toxicity by intravenous injection (alone or combination administration) in different mice models for the treatment of lung cancer, liver cancer, and other cancers. More significantly, the combination treatment of MBRI-001 with gefitinib or sorafenib exhibited a higher antitumor effect in comparison with the monotherapy of MBRI-001 in vivo. Therefore, we believe MBRI-001 could be developed as a promising anticancer agent in near future.

B44 Theranostic applications of CD38 nanobodies on multiple myeloma. Yongjuan Zhao, Ya Jie Chen. National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

CD38, a 45kD single-transmembrane glycoprotein, is a multifunctional signaling enzyme, which acts as a receptor transducing signals to modulate cell functions such as adhesion, differentiation, and proliferation, as well as an enzyme catalyzing the metabolism of cyclic ADP ribose, cADPR, and nicotinic acid adenine dinucleotide phosphatenicotinic acid, NAADP, which are two calcium messengers. CD38 expresses much higher levels in several hematologic malignant cells, including multiple myeloma (MM), than those of normal cells, which leads CD38 to be considered as a good drug target for these diseases. We developed a series of nanobodies against the catalytic C-terminal domain of CD38, which bind CD38 at three distinct epitopes seen in the X-ray co-crystal structures. Based on one of the nanobodies, Nb-1053, we engineered an immunotoxin by fusing it with a bacterial toxin PE38. It shows selective cytotoxicity against multiple myeloma cell lines and patient-derived myeloma cells with an EC_{50} as low as 10^{-11} molar. By coupling the nanobody with a highly potent small-molecule toxin, MMAE, a nanobody drug conjugate was produced. It shows dose-dependent cytotoxicity on CD38 high expression cell lines and has no toxicity to the CD38 knockout cells. Using another nanobody Nb-1G3, we constructed chimeric antigen receptor T cells (CARTs) against CD38. The CARTs selectively lyse $CD38^+$ MM cell lines and primary MM cells from patient, while showing slight cytotoxicity against normal white blood cells with CD38 expression. On the animal model subcutaneously

inoculated with MM cells, the CARTs inhibited tumor growth. With a pair of nanobodies recognizing two different epitopes of CD38, Nb-1053 and Nb-551, a dual epitopes protein Identification (DepID) assay was developed that detected soluble CD38 as low as 10 pg/mL. By this method, we were able to precisely quantify the levels of soluble CD38 in the plasma of MM patients, which were significantly higher than those from healthy donors. We further showed that the increased plasma levels of soluble CD38 correlated with the progress of MM. These results indicate that CD38 nanobodies are versatile in developing new agents for diagnosis and therapeutics of multiple myeloma.

B45 Development of in vitro methodologies to study the behaviour of targeted drug delivery systems to triple-negative breast cancer. Sepideh Khazeni, Pegah Varamini. School of Pharmacy, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia.

Statement of the Problem: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype. Due to the lack of sex-hormone receptors and HER2 overexpression, tumor does not respond to the available targeted drugs. Hence, it is crucial to investigate the design of effective targeted drug delivery system (DDS) for the treatment of TNBC. In this study, we have developed different methods to test the behavior of different DDS based on the luteinizing hormone-releasing hormone receptor (LHRH-R) targeting.

Methodology and Theoretical Orientation: We studied the overexpression of LHRH-R by immunohistochemistry analysis using advanced confocal laser scanning microscopy (CLSM) on three different TNBC cells and normal breast cell line. Uptake of different DDSs conjugated with LHRH-R ligand has been investigated by using CLSM and IncuCyte® in these cell lines. By using small interfering RNA (siRNA) against LHRH-R sequences, we knocked down the LHRH-R in TNBC cell line with 85% knockdown efficiency and compared the uptake of LHRHD-DDS in silenced LHRH-R to wild-type cells by CLSM.

Findings: We observed the overexpression of LHRH-R in TNBC cells but not the control. We detected the uptake of LHRH-based DDS from TNBC cells by CLSM and IncuCyte®. Silencing LHRH-R with siRNA has decreased the uptake of LHRD-DDS in silenced LHRH-R cells.

Conclusion and Significance: In conclusion, we have shown LHRH-based DDSs were selectively uptaken through LHRH-R overexpressed TNBC cells. These findings indicate that LHRH-R ligands are promising carriers to use for future targeted drug design for the treatment of TNBCs. Furthermore, our developed methodologies allowed us to effectively investigate the in vitro behavior of this targeted DDS as a part of the preclinical studies.

B46 Preclinical evaluation of a novel antibody inhibitor of SEMA4D reduces tumor growth, progression, and metastasis in pediatric osteosarcoma. Branden Smeester. University of Minnesota, Minneapolis, MN, USA.

Osteosarcoma is the most common malignant bone tumor found in children and adolescents and is associated with many complications including metastases. With no known precursors to osteosarcoma, treatment options are extremely limited. Adjuvant chemotherapy and surgical resection are standard therapies, but treatment efficacy still remains poor for over one third of osteosarcoma patients. Although our understanding of the mechanisms underlying tumor development, progression, and metastasis is improving, the complex nature of the bone tumor microenvironment presents unique challenges in identifying novel drug targets and treatment strategies. Recently, a new pathway has been identified using the *SB* mutagenesis system in mice implicating semaphorin-4D (*SEMA4D)* in osteosarcoma. During normal bone homeostasis, osteoclasts express high levels of Sema4d, whereas osteoblasts do not. Thus, it is possible that mis-expression of SEMA4D in osteoblasts might give rise to a subset of osteosarcomas. VX15/2503 (Vaccinex, Inc.) is a highly novel, immunomodulatory monoclonal antibody that specifically targets SEMA4D (CD100), a receptor and soluble protein known to be involved in immune modulation and regulation of normal bone formation. Here we report ongoing results using VX15/2503, SEMA4D mechanistic studies, and data contributing to a phase1/2 Trial of VX15/2503 in pediatric patients with relapsed or refractory solid tumors.

B47 Engineering clinically relevant mutations into disease specific cell types proves useful for therapeutics discovery in neurofibromatosis type-1 related cancers. Kyle B. Williams[1], Bryant Keller[2], Alex Larsson[2], Mark Sokolowski[2], David A. Largaespada[2]. [1]University of Minnesota Masonic Cancer Center, Minneapolis, MN, USA, [2]University of Minnesota, Minneapolis, MN, USA.

Genome engineering technologies, such as CRISPR/Cas9, offer exciting opportunities to build more relevant cell line-based models to advance cancer research. Historically, the majority of small molecules and drugs identified by screening efforts fail when advanced toward clinical development. We believe many of these failures arise from context and cell type-dependent factors missed in those efforts. Genome engineering allows generation of clinically relevant mutations into cells of the correct tissue type for the cancer being studied. We have utilized these principles to build models of tumors that arise in a prevalent cancer predisposition syndrome known as neurofibromatosis type 1 (NF1) and used these models for therapeutics discovery with synthetic lethal pharmacogenomic screens. NF1 is a

common genetic disorder caused by mutations in the tumor suppressor gene *NF1*. NF1 results in formation of benign Schwann cell tumors (neurofibromas), which can cause significant pain and mobility problems. These can progress to malignant peripheral nerve sheath tumors (MPNSTs), the most deadly of all soft tissue sarcomas and a leading cause of death among NF1 patients. The majority of NF1 MPNSTs develop within pre-existing plexiform neurofibromas. Given that plexiform neurofibromas and MPNSTs arise within the Schwann cell linage, we developed a drug discovery pipeline to identify targeted therapeutics for treating NF1-related neoplasia. Using CRISPR/Cas9, we created immortalized human Schwann cell lines that are deficient for the *NF1* gene. ~80% of all MPNST harbor loss of function mutations in Polycomb Repressive Complex 2 (PRC2) genes, such as *SUZ12*, which is highly suggestive that perturbation of epigenetic homeostasis plays a role in malignant transformation of neurofibromas. Dysregulated chromatin remodeling caused by the loss of PRC2 in MPNSTs likely confers novel vulnerabilities, which could be exploited therapeutically. As such, we also built models of this more "MPNST-like" state by generating loss-of-function mutations in *SUZ12* alone or in combination with the *NF1* loss. This has given us distinct models of benign neoplasia and malignant cell types, with matched isogenic parental lines for comparison in screening efforts. Our small-molecule screening efforts identified compounds showing selective toxicity towards either *NF1* deficient cells or *NF1*, *SUZ12* deficient double mutant lines. Two clinically interesting candidates showed efficacy in vivo, utilizing both an NF1 genetically engineered mouse (GEM) model and a human MPNST xenograft model. These were the cardiac glycoside digoxin and a PP2A inhibitor (LB-100). Both prolonged the life of the GEM model, controlled tumor growth, and rapidly shrank established human MPNST xenografts. Work with the *NF1 -/-, SUZ12 -/-* engineered human Schwann cells revealed new classes of drugs strongly selective at targeting these MPNST-like cells, in particular HDAC inhibitors. When tested in vivo the FDA-approved HDAC inhibitor vorinostat showed striking efficacy through controlled tumor growth and dramatically increased lifespan in a human MPNST xenograft model deficient for both *NF1* and *SUZ12*. These pharmacogenomic screens leveraged synthetic lethal interactions with *NF1* and *SUZ12* to identify therapeutics for the treatment of NF1-related neoplasia. Digoxin is of particular note given its well-understood safety profile, and it has shown synergy with MEK inhibition for treatment of metastatic melanoma in clinical trials. The discovery of vorinostat as an effective agent in treating models of MPNST is equally exciting, as to date there are no effective or approved targeted therapies for MPNST. Results from our studies are forming the basis for clinical trials we hope to propose for the treatment of MPNSTs and aggressive plexiform neurofibromas.

B48 The eltrombopag antitumor effect on breast cancer with HuR-dependent manner. Yuying Zhu, Xiyan Yang, Liuqing Yang, Jiange Zhang. Shanghai University of Traditional Chinese Medicine (SHUTCM), Shanghai, China.

Background: HuR is an mRNA-binding protein that regulates cell proliferation, apoptosis, and angiogenesis by promoting mRNAs stabilization in many cancers. High expression of HuR causes poor prognosis of malignancy, highlighting the potential of HuR inhibitors as antitumor drugs. Here we established a HuR-RNA^{VEGF-A} high-throughput screening (HTS) system based on fluorescence polarization (FP) technique, from which a clinically used drug, eltrombopag (ELB), was selected out. ELB is a thrombopoietin receptor agonist, while we discovered its new target and mechanism for antitumor effect.

Methods: FP technique was applied for establishment of the HTS system, electrophoretic mobility shift assay (EMSA) was used for further confirmation of positive hit, and AutoDock 4.2 program was employed for simulating ELB and HuR interaction. MTT assays, RNA-seq analysis, and flow cytometry were used for exploring ELB's effect to 4T1 cells. qRT-PCR, DRB pulse chase experiments, RNA immunoprecipitation, and luciferase assays were used to study regulation of ELB with HuR-dependent manner in 4T1 cells and RAW264.7 cells. Cell scratch assays and in vitro Matrigel angiogenesis assays were used for detecting impact of ELB-treated RAW264.7 supernatants to HUVEC cells. Finally, tumor allograft experiments in mice were adopted to study the in vivo inhibition of tumor growth and angiogenesis by ELB.

Results: The thrombopoietin receptor agonist ELB was screened out from the HuR-RNA^{VEGF-A} HTS system, and we demonstrated it with high efficiency to inhibit HuR binding to its target mRNAs. Our data showed that ELB could reduce VEGF-A, MMP9, and Bcl2 mRNAs with an HuR-dependent manner in mouse breast cancer cells 4T1, and more importantly, ELB could also inhibit mRNAs stabilization of VEGF-A and MMP9 in macrophage RAW264.7 cells, and thus inhibit angiogenesis-related functions of the downstream vascular endothelial cells (HUVEC). The in vivo antitumor activity of ELB was also demonstrated in mice bearing 4T1 cell allografts, and the tumor angiogenesis-inhibiting effect was also observed. With these data, a new mechanism for ELB's antitumor effect was confirmed.

Conclusion: A new mechanism for ELB's antitumor effect was described, whereby inhibiting angiogenic factors in macrophage cells with HuR-dependent manner, the angiogenesis in tumor tissue of breast cancer was inhibited.

B49 Glyco-methods to predict and circumvent chemoresistance. Nahid Razi[1], Nissi Varki[2], Seyed Monemian[3]. [1]AccuDava Inc., La Jolla, CA, USA, [2]University of California San Diego, La Jolla, CA, USA, [3]Southern California Medical Group, Kaiser Permanente, Lancaster, CA, USA.

Platinum-chemotherapy (pt-CHEMO) is the mainstay of first-line drugs in treating epithelial cancers such as ovarian, lung, testis, head/neck, and cervix. However, chemoresistance has remained the main reason for treatment failure and the major cause of death for nearly half of all chemotherapy patients. The ability to predict chemoresistance and circumventing the problem is an undeniable, unmet need in today's cancer care. To address this problem, we have previously developed a novel glycan method to predict chemoresistance. Our method is based on the identification of two cell surface glycan motifs (glycomarker-1 and -2) on ovarian carcinoma, whose cell surface expressions are positively correlated with responses to pt-CHEMO, i.e., very low expression or absence of the glycomarkers indicate that the tumor will be resistant to chemotherapy (Patent #7585503, PCT pending patent # 20150024409, Divisional pending patent #15/385629). Advanced triplex immunofluorescent (3x-IF) has been developed to quantify the glycomarkers' expression on formalin-fixed, paraffin-embedded (FFPE) tumor sections. Preliminary validation on ovarian cancer patients revealed staggering 92% and 70% correlation between glycomarkers-1 and -2 expression levels, respectively, with patients' response to chemotherapy. Funded by the National Cancer Institute (NCI-USA), our novel predictive tests are currently being validated on 140 ovarian cancer specimens from untreated patients, whose responses to chemotherapy are known. Further evidence indicates that this method will potentially apply to other cancer types that express the glycomarkers. With additional NCI funding support, the utility of our method is also being tested on non-small cell lung (NSCL) cancer. To define the glycomarkers' role in chemoreactivity, we have carried out a series of diverse experiments, on chemosensitive vs. chemoresistant cell isotypes, and observed that 1) treating chemosensitive cells with cisplatin, carboplatin, and paclitaxel, caused a cell surface glycan alteration, accompanied with drug internalization. Notably, the drug-induced glycan alteration was not seen in chemoresistant phenotypes. 2) Chemoresistant cells treated with a fluorescent cisplatin (F-pt) accumulated F-pt on their surface, a phenomenon not observed on chemosensitive cells. 3) Interestingly, the accumulated F-pt on chemoresistant cells disappeared when chemoresistant cells were treated with F-pt along with an exogenous selected glycoenzyme, implying that the glycoenzyme activity potentiates the drug uptake. Trace metal mass spectrometry and colony-forming assay confirmed the increase in drug internalization by exogenous glycoenzyme and enhancing cytotoxicity. These findings lay the foundation for our hypothesis that the in situ glycan modification is the signaling event to start the glycan pathway of drug internalization. We are testing the glycoenzyme's utility to enforce the drug internalization in chemoresistant tumors, thereby overcoming the tumor's drug resistance. This is an unprecedented approach to improve chemotherapy in chemoresistant patients.

Conclusion: This work introduces a novel glycan-mediated concept in cancer treatments, linking the specific cell membrane glycans to a patient's response to chemotherapy. Our investigations have resulted in:

1) Developing the first direct *predictive test for chemotherapy* to provide guidance for treatment strategy, and helping physicians with informed decision making to avoid ineffective drugs on chemoresistant patients.

2) Defining the utility of a selected glycoenzyme in developing unprecedented therapeutic methods to circumvent chemoresistance, giving hope to the most vulnerable cancer patients.

B50 Automated 3D spheroid culture media exchange using a novel MultiFlo™ FX accessory. Dongping Guo, Peter Banks, Brad Larson. BioTek Instruments, Inc., Winooski, VT, USA.

Introduction: Spheroidal 3D cellular structures are a mainstay in many research areas, including oncology and toxicology1. Culturing cells in 3D provides a more in vivo-like environment, allowing cells to maintain high viability when cultured for extended time periods. To maintain the highest levels of viability within untreated cells and ensure that observed effects are solely from treatment, media exchanges and redosing are necessary throughout the experiment, particularly in in vitro tests lasting weeks. Media exchanges with cell models that do not rely on adherence to labware can be daunting if performed by hand, even when performed on a single plate. Multichannel pipettes must remove and dispense media at an extremely slow rate, and care must be taken to keep pipette tips away from the actual spheroids. Through incorporation of the AMX™ Automated Media Exchange module on the MultiFlo™ FX Multi-Mode Dispenser, risk of accidental spheroid removal from wells is eliminated. Spent media is automatically removed and replaced with fresh media only or fresh media containing treatment concentrations.

B51 Nucleic acid aptamers: Potential diagnostic and therapeutic tools for leukemia. Yuan Tan[1], Yuejin Li[1], Zigang Dong[2], Faqing Tang[1]. [1]Department of Clinical Laboratory, Hunan Cancer Hospital & The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, China, [2]Hormel Institute, University of Minnesota, Austin, MN, USA.

Leukemia immunotherapy using synthetizing antibodies to target cluster of differentiation (CD) molecules is very popular; nevertheless, it has inevitable cytotoxicity. Recently, various reports have focused on nucleic acid aptamers, a class of high-affinity nucleic acid ligands. Aptamers purportedly serve as "chemical antibodies," have negligible cytotoxicity and low immunogenicity, and are widely used in the diagnosis and therapy of diseases, especially leukemia. In leukemia diagnosis and therapy, nucleic acid aptamers display a specific and accurate character because of their specific recognition sites on leukemia through unique three-dimensional conformations. Additionally, it could be chemically modified to resist nuclease degradation, renal clearance and improve binding affinities. Moreover, aptamers can be linked with existing detection and imaging techniques to improve sensitivity and selectivity. In this review, we summarize aptamers' preparation, chemical modification, and conjugation, and discuss that CD molecules commonly used in leukemia diagnosis and therapy are conjugated with aptamers and their application in diagnosis and treatment of leukemia. Finally, the application prospect of aptamers in fusion gene is also introduced. (This work was supported in part by the National Natural Science Foundation of China [81872226, 81502346], Hunan Province Natural Science Foundation of China [2018JJ6131, 2019JJ40175], Changsha Science and Technology Project [kg1801107].)

B52 Targeting cancer-related intracellular PPIs with stabilized peptide. Zigang Li. Peking University Shenzhen Graduate School, Shenzhen, China.

Continuous efforts have been invested in developing constrained peptides for modulating PPIs by enhancing the peptides' helicity. A precisely positioned in-tether chiral center was found to be able to modulate a peptide's helicity and cell permeability (1). This chirality induced helicity (CIH) concept was then translated into a N-terminus aspartic acid linkage (TD) for more facile peptide synthesis (2). The stabilized peptides constructed with these methods were utilized to target different intracellular protein targets including MDM2, ERα, and HDAC family proteins both in vitro and in vivo (3-5). Notably, PROTAC strategy could also be applied with stabilized peptides (6).

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B53 A revolutionary technology to solve FFPE sample preparation bottleneck problems in cancer research and diagnosis. Tom C. Xu. SenseCare Medicals, Inc., Pleasanton, CA, USA.

Introduction: FFPE (formalin-fixed, paraffin-embedded) sample is one of the most important DNA and RNA sources for cancer research and clinical diagnosis by next-generation sequencing (NGS). Fast and accurate genetic profiling of FFPE cancer samples is crucial to provide information for targeted therapies. However, a slide of FFPE sample has only about a 4μm x 0.2-0.5cm^2 volume, which is very small and delicate to handle. Extracting DNA from FFPE currently is a tedious and less reproducible process. The limited quantity and poor quality of DNA thus obtained from FFPE are major bottlenecks for cancer research and diagnosis using NGS and PCR. Here we report a one-step, highly efficient FFPE sample preparation method to solve the problems.

Methods: Traditional FFPE DNA library preparation is laborious and time-consuming. It involves following steps: (1) deparaffinization with xylene, which is toxic; (2) wash in ethanol; (3) protease K digestion; (4) lysis; (5) DNA extraction and purification by columns or beads; (6) PCR amplification of the purified DNA to yield DNA libraries for sequencing. We have developed a new method to amplify DNA from FFPE tissue directly without any pretreatment steps as described above. The amplified DNA was successfully used for targeted DNA sequencing in NGS.

Results: DNA was amplified by PCR from FFPE tissues directly without any pretreatment, resulting in high-quality DNA libraries for next-generation sequencing. The DNA libraries were successfully sequenced. We validated the new FFPE sample preparation method using Illumina sequencing platforms. A currently widely used commercial FFPE DNA extraction kit was used to prepare the same DNA libraries for side-by-side comparison. The libraries were analyzed by Bioanalyzer. The new method produced much higher library yields than the traditional one. For sequencing conformity, in general, >90% variants detected from traditional FFPE libraries were also detected from the new libraries. However, the new FFPE method had higher sensitivity since it detected more mutations than traditional methods. Advantages of the new method include: no deparaffinization or any pretreatment needed; require only a sample of 1/8 to 1/4 of a FFPE slide; one tube reagent does it all, and directly generates high-quality DNA library from FFPE tissue; greatly simplifies the FFPE NGS work flow with low cost and fast turnaround time.

Conclusions: We have developed a new method to make FFPE DNA library preparation much easier, simpler, and low cost. This new method will make NGS more straightforward and cost effective in cancer clinical diagnosis and treatment. It should become a very useful tool for molecular biology research and clinical diagnostics.

B54 Advancing mass spectrometry-based large-cohort proteomics for precision medicine—an International Cancer Moonshot multiple-site study. Yue Xuan[1,2], Nicholas W. Bateman[3], Sebastien Gallien[2,15], Yue Zhou[16], Niyati Parikh[3], Mo Hu[16], Pedro Navarro[1], Yuju Chen[5], Albert Sickmann[6], Bernd Wollscheid[7], Connie R. Jimenez[8], Martin R. Larsen[9], Hu Zhou[10], Siqi Liu[11], Zhinan Chen[12], Thomas Kislinger[13], Ben Crossett[14], Brian Hood[3], Reta Birhanu Kitata[5], Christin Lorenz[6], Christina Loosse[6], Sandra Goetze[7], Sander Piersma[8], Davide Chiasserini[8], Muhammad Tahir[9], Hongwen Zhu[10], Guixue Hou[11], Xiuxuan Sun[12], Andrew Macklin[13], Amanda Khoo[13], Benjamin L. Parker[17], Stuart J. Cordwell[17], Thomas P. Conrads[3,4]. [1]Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany, [2]Thermo Fisher Precision Medicine Science Center, Cambridge, MA, USA, [3]Gynecologic Cancer Center of Excellence, Murtha Cancer Center, Uniformed Services University of the Health Sciences, Bethesda, MD, USA , [4]The Inova Schar Cancer Institute, Annandale, VA, USA , [5]Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan, [6]Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, [7]Institute of Molecular Systems Biology (IMSB), Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland , [8]Dept. Medical Oncology, Cancer Center Amsterdam, VU University Medical Center, Amsterdam, Netherlands, [9]Dept. Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark, [10]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China, [11]BGI-SHENZHEN, Shenzhen, Guangdong Province, China, [12]Cell Engineering Research Center/National Key Discipline of Cell Biology, The Fourth Military Medical University, Xi'an City, China, [13]Princess Margaret Cancer Centre, Toronto, ON, Canada, [14]Sydney Mass Spectrometry, The University of Sydney, Sydney, NSW, Australia, [15]Thermo Fisher Scientific, Paris, France, [16]Thermo Fisher Scientific (China) Co. Ltd., Shanghai, China, [17]School of Life and Environmental Science, The University of Sydney, Sydney, NSW, Australia.

Introduction: To successfully elevate discovery proteomics to translational research in the pipeline of precision medicine, large-cohort studies are essential in discovery and verification of protein biomarkers. Apart from sensitivity and specificity, to reproducibly and reliably quantify large numbers of proteins in different laboratories remain challenges. To address these challenges, we present a high-throughput and streamlined analytical workflow using high-resolution MS1-based quantitative data-independent acquisition (HRMS1–DIA) mass spectrometry. The HRMS1-DIA workflow is standardized with well-defined experimental steps and systematically applied to a set of test samples. The study was benchmarked across multiple Cancer Moonshot sites worldwide utilizing identical instrument platforms, procedures, and software, and demonstrated to be stable in a 24/7 operation mode for 7 consecutive days.

Methods: All experiments were performed on a Q Exactive HF mass spectrometer using a standard method consisting of full-scan MS with interspersed DIA acquired for qualitative analysis. Chromatographic separations were performed using capillary flow with a 60-minute gradient for high-throughput analysis. The samples consisted of three separate proteomes (HeLa, yeast, and *E. coli*) mixed in various proportions and prepared onsite following a standardized protocol. For the initial quality assessment, a HeLa digest served as a quality control (QC) sample. An exhaustive spectral library was created for each proteome by performing LC-MS/MS analysis of multiple off-line high pH reverse phase fractions. Additional quality control steps were taken by spiking each sample with a set of reference peptides to normalize retention times.

Preliminary Results: A high-throughput analytical workflow using high-resolution MS1-based quantitative data-independent acquisition (HRMS1–DIA) mass spectrometry was standardized with well-defined experimental steps and systematically applied to a set of test samples. The study was benchmarked across multiple Cancer Moonshot sites worldwide utilizing similar instrument platforms, procedures, and software, and was demonstrated to be stable in a 24/7 operation mode for 7 consecutive days.

A HELA cell digest quality control (QC) sample was routinely analyzed. Two mixed proteome samples with different ratios of three proteomes (HELA, Yeast, and *E. coli*) were analyzed to evaluate the label-free quantitation performance. >280 QC files and >380 mixed proteome sample files were acquired by 11 participating laboratories. With the 1-hour capillary LC-HRMS1 DIA workflow, >5,000 protein groups from >40,000 peptides were consistently identified from the QC sample across all sites and all days (1% FDR). The peptide intensity correlation among all QC files from the entire study was 0.9, demonstrating ultrahigh interlaboratory and interday reproducibility. The normalized median coefficient of variation among all identified precursors across 7 days was <20% at the same site. With the label-free quantitation sample, >7,200 up to ~8,600 protein groups were identified (1% FDR) across all 11 labs. In addition to the 1% FDR, a rollup strategy was developed to filter none-reliable peptides from protein quantitation, successfully enhancing the quantitative precision to >0.9. As a result, an average of >6,000 protein groups from the mixed proteome sample, including >4,000 human proteins, ~2,000 yeast proteins, and >300 *E. coli* proteins, were precisely quantified among all labs and all days. ~80% protein groups were identified and quantified in common across all the laboratories, and >85% were quantified in common across different days at the same site. Empirical label-free quantitation ratios of the three mixed proteomes accurately reflected the ratios anticipated at each site. This international, multisite harmonization study demonstrates that the HRMS1-DIA workflow is a highly reproducible and robust label-free quantitative workflow enabling high-throughput and deep proteome profiling for large-cohort studies, generating reproducible results across different laboratories in a longitudinal mode.

Novel aspect: Multisite reproducibility, robustness, and method transfer evaluation for HRM MS-based global proteome profiling using a standardized DIA analytical workflow.

B55 Local measurements of hypoxia and cell metabolism in a synthetic tumor. Yihua Zhou[1], Robert H. Austin[2], Junle Qu[1]. [1]Shenzhen University, Shenzhen, China, [2]Princeton University, Princeton, NJ, USA.

Tumors are characterized as swamps: abnormal and disordered tissue masses with highly stressful conditions of hypoxia, low pH, low-nutrient conditions due to a combination of rapid cell growth, lack of vasculature, and altered metabolism. While for normal cells that combination would be lethal, for cancer cells it provides a genotoxic environment they are adapted to. We have explored the oxygen and metabolic dynamics of cancer cells in a chemotherapy gradient in a synthetic vitro tumor swamp using a 3-dimensional passive diffusion tumor model. Localized oxygen concentrations are measured using a phosphorescence lifetime imaging (PLIM) technology-based oxygen sensor developed at the Shenzhen Key Laboratory of Optoelectronic Devices and Systems, while metabolism can be simultaneously measured using the fluorescence lifetime imaging (FLIM) of NADH within the cancer cells.

B56 A passive diffusion ecology reveals the emergence of polyploidal resistant cancer cells. Robert H. Austin[1], Ki-Chih Lin[1], Gonzalo Torga[2]. [1]Princeton University, Princeton, NJ, USA, [2]Johns Hopkins Medical Institute, Baltimore, MD, USA.

Once prostate cancer (PCa) becomes clinically apparent at distant sites it is lethal, and metastatic PCa continues to kill more than 29,000 men per year in the United States with similar rates in the developed world. The vast majority of tumors develop resistance to all known natural and synthetic drugs, while normal cells do not have this capability. While there are many mechanisms that have been demonstrated to contribute to tumor cell drug resistance, how and why these mechanisms are part of the evolution of cancer remain undefined. Resistance to a therapy may be solely cell intrinsic and therefore be present in a treatment-naïve setting. We have developed a micro-fabricated tumor ecosystem to study cell genotype and phenotype during therapeutic stress over months of time in a complex environment. This permits the observation and quantitation of cellular changes in a mix of habitats over an extended period of time with controlled gradients for temperature, pH, nutrients, and oxygen tension. The micro-fabricated ecosystem using static diffusive drug gradients is built on a re-sealable platform that allows resampling of individual cells longitudinally over time and space. We will present results that demonstrate that polyploidy emergence seems to be the primary driver of the emergence of drug resistance in a complex environment over several weeks of time.

B57 Automated scratch tool, high-contrast brightfield, and fluorescence kinetic imaging. Dongping Guo. Brad Larson BioTek Instruments, Inc., Winooski, VT, USA.

The ECM is a complex cellular scaffold found throughout the body that consists of collagen, cell-adhesive glycoproteins, and other proteins. ECM supports adhesion and intracellular communication networks, and also facilitates cellular migration, including directionality, as a collective group (1). This type of migration is a significant aspect of wound healing and also of tumor metastasis, which is the primary cause of cancer morbidity and mortality (2). Additionally, stromal cells, such as fibroblasts, are responsible for depositing components of the ECM, and in the solid tumor

microenvironment, influence cancer cells in migration, invasion, and other tumorigenic processes (3). Because of the important role that ECM and stroma play in vivo, each should be included when performing in vitro cell migration studies to increase the relevance of generated data. Here we demonstrate the use of a novel, automated tool to create consistent and reproducible scratch wounds in 2D cell monolayers formed on the bottom of a microplate. We use a single cancer cell model as well as a coculture of fibroblasts and cancer cells, each plated in collagen-coated microplate wells to more closely simulate the in vivo tumor microenvironment and facilitate cell migration. The tool fits into any size laminar flow hood and features an interchangeable pin manifold for use with 24- or 96-well microplates, as well as a preprogrammed, hands-free cleaning and decontamination protocol to reduce the risk of buildup and contamination. Following scratch wound generation, the plate is transferred to BioTek's automated imagers or the BioSpa™ Live Cell Imaging System to kinetically monitor cell migration using high-contrast brightfield and fluorescence imaging.

B58 Extracellular matrix gene expression and cytotoxic T-lymphocyte infiltration in the tumor microenvironment in non-small cell lung cancer. Na Li, Hongzhe Sun, Xin Wang, Ying Zhou, Zhifu Zhang, Courtney Anderson, Xiao-Jun Ma. Advanced Cell Diagnostics, Newark, CA, USA.

Immunotherapy has proven to be a powerful antitumor therapy, harnessing the body's own immune system to target and kill tumor cells. However, immunotherapy is not successful in all cancer patients due to both intrinsic nonresponsiveness and adaptive resistance. Developing predictive biomarkers and understanding mechanisms of resistance are major goals of the immuno-oncology community. The extracellular matrix (ECM), an important factor for promoting tumor growth, survival, and migration of tumor cells, can also act as a physical barrier to prevent immune cell infiltration and promote tumor immune escape. Components of the ECM such as COL11A1, COL4A1, and LOXL2 have been shown to be associated with cancer progression. Furthermore, new data suggest that TGFβ activation leads to upregulation of ECM genes in cancer-associated fibroblasts and immune suppression. However, it remains poorly understood which cells in the tumor microenvironment (TME) are the sources of ECM gene expression and how they are related to tumor-infiltrating cytotoxic T lymphocytes (CTLs). In this study, we employed a highly sensitive and specific RNAscope in situ hybridization (ISH) duplex assay to directly visualize the tissue distribution of cells expressing *COL4A1*, *COL11A1, LOXL2, and TGFB1* in relation to tumor infiltrating CTLs in non-small cell lung carcinoma (NSCLC). NSCLC tissue microarrays (TMAs) consisting of 63 independent patient FFPE tumor samples were analyzed using this ISH assay with the following probe combinations: Hs-CD8/Hs-IFNG, Hs-CD4/Hs-FOXP3, Hs-LOXL2/Hs-COL4A1, and Hs-TGFB1/Hs-COL11A1. We observed *COL4A1* expression in both tumor and tumor-associated stromal cells in different samples. In contrast, *COL11A1* was only expressed in tumor-associated stromal cells. Interestingly, high *COL4A1* expression was associated with high CD8+ T-cell infiltration, whereas high *COL11A1* expression was associated with poor CD8+ T-cell infiltration. In addition, tumor expression of *TGFB1* was positively correlated with *COL11A1* expression. These data depict a complex landscape of ECM gene expression and their relationship to T-cell infiltration in the tumor and TME. Taken together, these results demonstrate that the RNAscope assay provides a powerful approach to directly examine the interactions between tumor, ECM, and T-cell immune infiltration, and offers advantages over immunohistochemistry (IHC) for identifying the cellular sources of secreted proteins such as ECM components in the TME.

B59 Electron paramagnetic resonance imaging. Michal Gonet[1], Adam Plewinski[2], Marek Murias[3]. [1]Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland, [2]Wielkopolska Centre for Advanced Technologies, Animal Facility, Poznan, Poland, [3]Poznan University of Medical Science, Department of Toxicology, Poznan, Poland.

Hypoxia is widely recognized as a major player in the tumor microenvironment that is associated with poor prognosis and patient survival rates. Hypoxia has been implicated in cellular resistance to front-line chemotherapeutics such as doxorubicin, cisplatin, carboplatin, and paclitaxel. As radiation therapy is oxygen-dependent, hypoxic tumor regions are additionally protected from the DNA damage caused by ionizing radiation. Hypoxia is, therefore, a major problem in chemotherapy and radiotherapy of cancer, and it is a major problem in photodynamic therapy of cancer. Until today, imaging hypoxia in human cancers has been accomplished with positron emission tomography (PET) and with blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI). Radiotracer techniques involve reductive retention of tracer in hypoxic cells in tumors. Electron paramagnetic resonance (EPR) spectroscopic images, in which a quantitative oxygen partial pressure (pO2) is obtained directly from the spectral line width or relaxation times T1 and T2 of a suitable spin probe, do not have the limitation of the above-mentioned techniques. The EPR techniques for in vivo imaging of oxygen in the tissues and tumors of living animals have been developed over the past decade and become to recognized as the gold standard in the measurement of the absolute level of oxygen in tissue. In our work imaging was performed on TM 600 ERI tomograph

(Novilet, Poland) capable of performing continuous wave (CW) with multiharmonic (MH) detection or rapid scan (RS) mode with a 1kHz field modulation. The loop-gap resonator with a 3.4 cm diameter dedicated to imaging whole mice. 3D and 4D functional imaging was conducted on C57bl/6J mice aged 3-8 weeks. As a spin probe, 100ul of 90mM OX063 was used. This is the first report showing in vivo imaging of oxygen concentration with sub-millimeter resolution and obtained in sub-minutes time scale, performed in vivo on mice using advanced EPR imaging technology such as multiharmonic analysis and fast imaging protocol. Presented results confirm the effectiveness of application EPR tomography for in vivo imaging of oxygen concentration in murine tumor models. Spatial and temporal resolutions of obtained images allow to monitor the temporary changes of the oxygen concentration and introduce new standards in vivo oxygen imaging in tumor tissue and oxygen-dependent anticancer therapies.

B60 A high-throughput assay for screening natural products that boost NK cell-mediated killing of cancer cells. Zihang Xu[1],* Xiaowen Zhu[1],* Chunpu Zou[1], Xiao Chen[1], Lin Su[1], Chenyuan Gong[1], Wanyi Ng[1], Zhongya Ni[1], Lixin Wang[2], Xuewei Yan[1], Yangzhuangzhuang Zhu[1], Xiaoning Jiao[1], Chao Yao[1,2], Shiguo Zhu[1,2]. [1]Laboratory of Integrative Medicine, School of Basic Medical Sciences, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [2]Department of Immunology and Pathogenic Biology, School of Basic Medical Sciences, Shanghai University of Traditional Chinese Medicine, Shanghai, China. *These authors contributed equally to this work.

Background: As central effector lymphocytes of the innate immune system, natural killer (NK) cells can eliminate transformed cells and virally infected cells. Malignant cells have developed strategies to escape from NK cell immunosurveillance. Administration of natural compounds represents a promising approach for antitumor immunotherapy, which may enhance the NK cell activity via multiple mechanisms. Therefore, it is necessary to establish approaches to evaluate the cytotoxicity of NK cells. Several methods have been developed to determine the NK cell activity, including 51Cr-release assay, CD107α degranulation assay, and IFN-γ secretion assay. However, most of these approaches require complicated operation and time-consuming incubation procedures and are not available for high-throughput screening.

Methods: In the present study, a luminescence-based assay was developed that could concurrently detect NK cell-mediated cytotoxicity, NK cell viability, and tumor cell viability through the quantitation of ATP, which signals the proportion of metabolically active cells. Expanded NK cells were cocultured with H1299 cells and then treated with candidate compounds, while IL-2 was employed as a positive control. After 24-h coincubation, luciferase and its substrate were added into the culture system, followed by luminescence detection.

Results: 2,880 natural products were screened, which were extracted from a variety of plants. We found that 406 compounds could increase the cytotoxicity of NK cells by more than 20%, and 222 compounds could increase the proliferation of NK cells by more than 20%. Moreover, our data also showed that 42 compounds directly suppressed the proliferation of H1299 cells by more than 50%.

Conclusion: Collectively, our findings provided valuable insights into the further identification of natural products that could enhance the tumoricidal activity of NK cells. In addition, our newly established ATP-based method was a valuable and information-rich screening tool to investigate the biologic effects of natural products on both NK cells and tumor cells.

OTHER

B61 MEK inhibitor trametinib does not prevent the growth of anaplastic lymphoma kinase (ALK)–addicted neuroblastomas. Ganesh Umapathy[1], Jikui Guan[1], Dan E. Gustafsson[1], Niloufar Javanmardi[2], Diana Cervantes-Madrid[1], Anna Djos[2], Tommy Martinsson[2], Ruth H. Palmer[1], Bengt Hallberg[1]. [1]Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [2]Department of Clinical Pathology and Genetics, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Activation of the RAS-RAF-MEK-ERK signaling pathway is implicated in driving the initiation and progression of multiple cancers. Several inhibitors targeting the RAS-MAPK pathway are clinically approved as single- or polyagent therapies for patients with specific types of cancer. One example is the MEK inhibitor trametinib, which is included as a rational polytherapy strategy for treating EML4-ALK–positive, EGFR-activated, or KRAS-mutant lung cancers and neuroblastomas that also contain activating mutations in the RAS-MAPK pathway. In addition, in neuroblastoma, a heterogeneous disease, relapse cases display an increased rate of mutations in ALK, NRAS, and NF1, leading to increased activation of RAS-MAPK signaling. Cotargeting ALK and the RAS-MAPK pathway is an attractive option, because monotherapies have not yet produced effective results in ALK-addicted neuroblastoma patients. We

evaluated the response of neuroblastoma cell lines to MEK-ERK pathway inhibition by trametinib. In contrast to RAS-MAPK pathway-mutated neuroblastoma cell lines, ALK-addicted neuroblastoma cells treated with trametinib showed increased activation (inferred by phosphorylation) of the kinases AKT and ERK5. This feedback response was mediated by the mammalian target of rapamycin complex 2–associated protein SIN1, resulting in increased survival and proliferation that depended on AKT signaling. In xenografts in mice, trametinib inhibited the growth of EML4-ALK-positive non-small cell lung cancer and RAS-mutant neuroblastoma but not ALK-addicted neuroblastoma. Thus, our results advise against the seemingly rational option of using MEK inhibitors to treat ALK-addicted neuroblastoma.

B62 ALKALs are in vivo ligands for ALK in the neural crest and derived cells. P. Mendoza-Garcia[1,2], A. Fadeev[3,5], U. Irion[3], J. Guan[1], K. Pfeifer[1], S. Wiessner[4], F. Serluca[4], A. Pratap Singh[3,4], C. Nüsslein-Volhard[3], R. Palmer[1]. [1]Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden, [2]Molecular Biology, Umea University, Umea, Sweden, [3]Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany, [4]Novartis Institutes for Biomedical research (NIBR), Cambridge, MA, USA, [5]Max-Planck-Institut für Infektionsbiologie, Berlin, Germany.

The receptor tyrosine kinase (RTK) anaplastic lymphoma kinase (ALK) has a key role in neuroblastoma development, and so it is considered as an important therapeutic target. ALK is mainly expressed in central and peripheral nervous systems among mammals; however, its physiologic role remains still unclear. The recent in vitro identification of ALKAL proteins (FAM150, AUG) as potential ligands for human ALK and the related LTK RTKs has offered new clues and therefore new questions regarding the role of ALKALs in combination ALK/LTK RTKs during development and tumorigenesis. In this study, we have investigated the role of zebrafish Alkal proteins for activation of the endogenous LTK, which is similar to the human ALK in sequence and domain structure. Zebrafish LTK (DrLtk) controls the development of iridophores, neural crest-derived pigment cells, making *Danio rerio* an excellent system to study ALK/LTK involvement in neural crest regulation in vivo. In addition, we used *Drosophila* eye patterning and cell culture-based assays to corroborate our results. Here we show that zebrafish Alkals potently activate both DrLtk and HsALK, and overexpression of DrAlkals causes ectopic iridophore development, consistent with the loss of iridophores observed in loss of function alleles for DrAlkals. Overall, this work shows in vivo evidence of activation of ALK/LTK receptors by ALKAL proteins, as well as a conserved role for ALKAL proteins in activation of ALK RTKs family in the development of the neural crest.

B63 A novel approach for the study of oncogenesis: Secondary primary malignancies associated with approved therapeutic mAbs. Henry Hongrong Cai. PAREXEL International, Waltham, MA, USA.

Introduction: Discovering the mechanism for oncogenesis is critical to the development of robust therapies for the treatment of cancers. Due to the specificity of the interaction between antigen and antibody, the secondary primary malignancies (SPMs) that occurred during therapeutic monoclonal antibody (mAb) treatment may provide insight into the inciting events.

Drug Label Review: As of February 15, 2019, 92 therapeutic mAbs have been approved by US FDA. All approved labels have been reviewed. In addition to several anti-TNF (tumor necrosis factor) mAbs for nononcology indications, for which increased rates of malignancy were reported, four other therapeutic mAbs were associated with treatment emergent malignancies. In a placebo-controlled clinical study with CINQAIR (reslizumab), an anti-IL-5 antibody for the treatment of asthma, 6/1,028 (0.6%) patients receiving CINQAIR had at least 1 malignant neoplasm reported compared with 2/730 (0.3%) patients in the placebo group. In a study with EMPLICITI (elotuzumab) (N=635), an anti-SLAMF7 (signaling lymphocytic activation molecule F7) antibody for the treatment of multiple myeloma, invasive SPMs were observed in 9% of patients treated with EMPLICITI and 6% of patients who did not receive EMPLICITI. In a controlled trial with OCREVUS (ocrelizumab), an anti-CD20 antibody for treatment of multiple sclerosis, breast cancer occurred in 6 of 781 females treated with OCREVUS and 0 of 668 females treated with placebo or REBIF. In a clinical study with XOLAIR (omalizumab), an anti-Ig E antibody for the treatment of asthma, malignant neoplasms were observed in 20 of 4127 (0.5%) XOLAIR-treated patients compared with 5 of 2236 (0.2%) control patients.

Conclusion: 1. IL-5, SLAMF7, CD20, and Ig E signaling pathways may be related to malignancies; 2. investigation of SPM adverse drug reactions may provide a novel approach to the understanding of oncogenesis.

B64 Inhibition of cathepsin D suppressed multiple malignant features of glioblastoma cells. Zhiyuan Zhu, Karrie Mei Yee Kiang, Ning Li, Pingde Zhang, Stephen Yin Cheng, Gilberto Ka Kit Leung. Division of Neurosurgery, Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong.

Background: Cathepsin D (CTSD) is a lysosomal aspartic protease involved in protein degradation and programmed cell death. High serum CTSD level has been reported as a potential biomarker for poor prognosis in glioblastoma patients. Its functions in glioblastoma remain unclear. Here,

we assessed the significance of CTSD in the malignant feature of glioblastoma.

Methods: The Cancer Genome Atlas (TCGA) and REpository for Molecular BRAin Neoplasia DaTa (REMBRANDT) database were used to evaluate the expression profile prognostic prediction value of CTSD in glioma patients. GEO microarray database GSE16011 were analyzed to distinguish differential expressed genes between GBM patients who had high and low CTSD expression. Geno ontology (GO) enrichment and KEGG pathway analysis were performed on gene ontology consortium (www.geneontology.org). We used the online analysis tool String(https://string-db.org/cgi/network.pl) to get the protein-protein interaction network of CTSD. We used siRNAs to knock-down CTSD in D54 GBM cells. Real-time PCR and Western blot were used to confirm the knock-efficiency of siRNAs. MTT assay was used to measure cell proliferation and TMZ cytotoxicity. Wound healing and transwell assay were used to evaluate the cell migration and invasion ability.

Results: CTSD is upregulated in GBM patients and is an unfavorable survival marker. We used data from REMBRANDT to evaluate the expression of CTSD in glioma patients and normal brain samples. Results showed that GBM patients had significantly higher CTSD mRNA level than the normal brain tissue. CTSD expression level in oligodendroglioma and astrocytoma patients had no significant difference when compared with normal brain. Then we used the online server GEPIA to analyze TCGA dataset of GBM. The results also demonstrated that CTSD was higher expressed in GBM patients than normal. Furthermore, Kaplan-Meier analysis showed that GBM patients with higher CTSD level have a shorter survival time (median cut-off), which indicates that CTSD might be a prognostic marker for poor overall survival in GBM patients.

Differentially expressed genes and related biologic processes associated with CTSD in GBM patients. We analyzed the differential gene expression profiles and the functional properties associated with CTSD in GBM. Data of 159 GBM patients from GSE16011 were included. Differential expressed genes between $CTSD^{high}$ and $CTSD^{low}$ groups were clustered. GO enrichment analysis showed that these genes were associated with several essential tumor-related biologic processes, such as angiogenesis, response to hypoxia, cell migration, and apoptotic. KEGG pathway analysis demonstrated that enriched pathways including ECM-receptor interaction, lysosome, focal adhesion, phagosome and protein digestion, and absorption. Moreover, we found the potential functional partners of CTSD by using string analysis, which includes CTSA, CTSB, STK11, ESR1, TP53, AGT, CD74, ATP6AP2, TPO, and COL18A1. The protein-protein interaction network of CTSD was illustrated.

Knock-down of CTSD suppressed cell migration, invasion of GBM cells and enhanced the efficacy of TMZ. The functional roles of CTSD in GBM cells were studied by knocking-down of CTSD in D54 GBM cell line. After confirmation of the knocking-down efficiency, cells transfection with si-scr and si-CTSD were subject to subsequent experiments. Cell proliferation assay showed that si-CTSD group had lower cell proliferate than si-scr group. Further, knocking-down of CTSD significantly decreased the migration and invasion abilities of GBM cells. MTT assay was used to evaluate the cytotoxicity of TMZ, and results showed that CTSD silencing enhanced the cytotoxicity of TMZ in GBM cells.

Conclusion: CTSD is highly expressed in GBM patients and indicates poor overall survival. Targeting CTSD might be a potential therapeutic strategy for GBM patients. The differential gene expression profile and biologic pathways associated with CTSD provide valuable information for the future study of the roles of cathepsin D in GBM.

B65 Downregulation of NRASq61 and its value in the prognosis in melanoma. Raj Kumar[1], Zhenyu Ji[1], Bobby Y. Reddy[1], Jenny Ching-Ni Njauw[2], Anpuchelvi Rajadurai[2], and Hensin Tsao[1]. [1]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA.

Introduction: Melanoma is potentially life-threatening skin cancer affecting men and women worldwide. *BRAF V600E* and *NRASQ61* are important mutations in the MAP kinase pathway, which have been validated as a driver oncogene in cutaneous melanoma. *NRASQ61*-mutant melanomas are highly resistant to available therapies. Although *BRAFV600E-mutant* melanomas respond to targeted therapies and immune checkpoint inhibitors, the response is often short-lived and followed by resistance, similar to that of *NRAS*-mutant melanomas. The aim of this study is to elucidate the prognostic and clinical significance of *NRASQ61*-driven melanoma.

Methods: The expression patterns of *NRASQ61* in melanoma and primary immortalized PMEL cell lines were assessed via qPCR and Western blotting. We created Tet-On isogenic cell models that contain mutations in driver oncogenes. These functionally validated models were used in 2-D and 3-D formats for screening in *NRASQ61* degradation and inhibition studies. Linear regression analysis was carried out to analyze the prognostic value of *NRASQ61* degradation in melanoma cell lines.

Results: Slow growth phenotype of SK-MEL119($NRAS^{Q61}$) melanoma cell lines were more closely associated with downregulation of NRASQ61 expression as compared with BRAFV600E ($P < 0.001$). Moreover, isogenic Tet-On $NRAS^{Q61}$ dependent A375($BRAF^{V600E}$) mutant cells also showed

growth retardation upon downregulation of NRASQ61R/K with cycloheximide (1ug/ml) expression, as compared with vector controls. Linear regression cell viability curves revealed that the overall cell growth was poor in cells with diminished level of NRASQ61 compared to the high-level cell models. Proteasome clamp assay demonstrated that NRASQ61 is a very labile protein and quickly undergoes degradation as compared to the more stable BRAF protein. Our data suggest that interference in the expression of NRASQ61 at transcription or translational level results in significant loss of unstable NRASQ61 endogenous protein and this would be beneficial to *NRASQ61*-melanoma patients. *NRASq61* dependency may potentially severe as an independent clinical and prognostic factor in patients with melanoma.

Conclusion: In summary, our studies found that NRASQ61R is a labile oncoprotein. Downregulation of *NRASQ61* expression with novel inhibitors may be a breakthrough in the management of *NRASQ61*-mutant melanoma.

AUTHOR INDEX

G

H

I

J

K

AUTHOR INDEX

M

N

O

P

Q

R

S

AUTHOR INDEX

X

Y

Z

AUTHOR INDEX

SUBJECT INDEX

SUBJECT INDEX

I

J

K

L

M

N

SUBJECT INDEX

T

U

V

W

Y

Z

NOTES

NOTES

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