
A02 Therapeutic targeting of the histone demethylase KDM4 subfamily in breast cancer.

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Breast cancer is a heterogeneous disease, consisting of tumors with varying pathologic and molecular characteristics. The primary biological subtypes of breast cancer include estrogen receptor–positive (ER+) tumors (luminal A and B), tumors that are human epidermal growth factor receptor 2 (HER2) –enriched, and tumors that are ER/PR-negative (basal-like). These molecular determinants have significant effects on disease pathophysiology, clinical outcome, and treatment response. ER+ tumors are generally associated with better clinical prognosis, whereas basal-like tumors are associated with higher rates of metastasis and death. The treatment for basal breast cancer consists of standard chemotherapy regimens, as no effective molecularly targeted therapy has been developed. New therapeutic options are likely to result from a growing understanding of both genetic and epigenetic abnormalities that participate in the different types of breast cancer, which helps to identify new subtype-specific targets for therapy.

Aberrant histone lysine methylation, controlled by histone lysine methyltransferases and demethylases, plays significant roles in cancer initiation and progression. Previously, we demonstrated that histone demethylase KDM4C (also known as GASC1 and JMJD2C) is significantly amplified and overexpressed in aggressive basal-type breast cancer. The KDM4C/GASC1 protein belongs to the KDM4 family of histone demethylases, in which KDM4A, B, and C share more than 50% percent of sequence identity. KDM4A, B, and C contain JmjN, JmjC, PHD and Tudor domains, while the KDM4D protein lacks C-terminal PHD and Tudor domains. Jumonji domains of KDM4 mainly catalyze the demethylation of tri- and dimethylated histone 3 lysine 9 (H3K9me3/me2) and lysine 36 (H3K36me3/me2) marks, thus regulating chromatin structure and gene expression. The goal of this study is to analyze genomic anomalies and expression levels of KDM4 demethylases in different types of breast cancer, and to explore the therapeutic potential of a novel KDM4 demethylase inhibitor for treating aggressive breast cancer. First, we analyzed KDM4 demethylases expression in a panel of breast cancer cell lines and primary human tumors. We found that amplification and overexpression of KDM4A, C, and D were more prevalent in aggressive, basal-type breast cancer, while KDM4B overexpression was more prevalent in Luminal breast cancer. We further assessed global methylation (H3K4, H3K9 and H3K36) levels by using western blotting. We found that global levels of H3K9me3/me2 levels were lower in basal breast cancer cell lines. Using a novel KDM4 demethylase inhibitor, we demonstrated that inhibiting KDM4 demethylase activity blocks proliferation and cancer phenotypes of basal breast cancer. More importantly, transcriptome analyses revealed that KDM4 inhibitor can suppress expression of multiple genes that are critical in controlling cell growth and proliferation in breast cancer. In summary, our data indicate that KDM4 histone demethylase members might have distinct functions in promoting breast cancer development

and progression, and that they are promising new targets for the epigenetic therapy of breast cancers.

A03 Understanding the tumorigenicity of Phosphatase of Regenerative Liver-2 (PTP-PRL-2). E. Kostantin[1,2], S. Hardy[1], N. Uetani[1], M.L. Tremblay[1,2]. [1]Rosalind and Morris Goodman Cancer Research Centre, Montréal, Canada, [2]Department of Biochemistry, McGill University, Montréal, Canada.

The three **P**hosphatase of **R**egenerative **L**iver (PRL-1, -2, -3) enzymes have been identified as key contributors to tumor progression and metastasis in several human cancers, yet the molecular basis of their pro-oncogenic property is unclear. Our previous study identified the CNNM3 magnesium transporter as key binding partner of PRL2 in an evolutionarily conserved complex that regulates the intracellular magnesium concentration. Our discovery that PRL-2 controls magnesium levels, has led us to study its mechanism of action in complex with CNNM3, which could explain its oncogenic activity when highly expressed. More precisely, we examined if PRL-2 tumorigenicity is dependent on the interaction with CNNM3.
To do so, we generated several recombinant CNNM3 point mutants to identify PRL-2 interaction sites and their binding affinity was screened using pull-down assays. From those pull-down experiments, we infected cancer cell lines to stably express either wild type proteins or point mutants inhibiting the interaction. Proliferation was assayed using CyQUANT DNA dye, and anchorage independent tumor proliferation capacity was characterized by soft agar assay. Xenografts were performed with FVB mice injected with mouse cancer stable lines expressing our proteins of interest in order to investigate tumorigenic potential.
Here, we show that a novel point mutation targeted in the CBS domain of CNNM3 was able to completely abolish the interaction with PRL-2. Since CNNM3 is a magnesium transporter, cancer cell lines expressing the mutation had significantly reduced proliferation. Supporting the role of this complex in cancer progression, the point mutation lowered the colony number of breast cancer cell line in anchorage independent soft agar assays. Most importantly, xenograft tumor assays expressing CNNM3 mutant that does not associate with PRL-2 drastically lowered tumor formation and size compared to wild type CNNM3 expression.
In summary, exploring further details of this newly uncovered pathway would be a major breakthrough in understanding oncogenic activity of PRLs, and represents a new potential class of targeted therapeutics in cancer.

A04 CD147 regulates malignant testicular germ cells apoptosis/survival through both extrinsic and intrinsic apoptotic pathways. Chaoqun Wang[1], Hao Chen[1, 2], Hsiao Chang Chan[1]. [1]Epithelial Cell Biology Research Center, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, [2]The Second People's Hospital of Shenzhen, Shenzhen, PR China.

Testicular germ cell cancer is one of the most common types of cancer in men between the ages of 15 and 34 years. In the testis, the processes of germ cell proliferation and apoptosis are tightly controlled to maintain proper tissue homeostasis. Apoptosis, a programmed cell death, is thought to facilitate the removal of abnormal germ cells and to reduce the risk of cancer. Cluster

of differentiation 147 (CD147), also named basigin or extracellular matrix metalloproteinase inducer (EMMPRIN), is a transmembrane glycoprotein, which has been reported to be highly expressed in various different types of cancer, including testicular germ cell cancer. Our previous studies have shown the involvement of CD147 in regulating spermatocyte apoptosis; however, the detailed mechanism remains elusive. In this study, we aimed to clarify the molecular mechanism underlying CD147-regulated testicular cancer cell survival/apoptosis with the mouse testicular germ cell cancer cell line P19 and human testicular germ cell cancer cell line NCCIT. We found that the cleaved caspase-8 was dramatically increased in anti-CD147 treated P19 and NCCIT cells, indicating that interference with CD147 induced activation of extrinsic apoptosis in testicular cancer cells. Further, the pro-apoptotic factors P53 and Bax were up-regulated and the anti-apoptotic factor Bcl-2 was down-regulated in anti-CD147 treated testicular cancer cells, suggesting that CD147 is also involved in regulating intrinsic apoptosis in testicular cancer cells. In addition, NFκB signaling, which is known to protect cancer cells from apoptosis, was down-regulated in the anti-CD147 treated cancer cells. These findings support an important role of CD147 in regulating testicular cancer cell survival, inhibition of which may promote apoptosis.

A05 Regulation of Spindle Dynamics and Mitotic Fidelity by BCCIP. Steven Huhn, Jingmei Liu, Huimei Lu, Xing Feng, Zhiyuan Shen. CINJ Rutgers University, New Brunswick, NJ, The Rutgers Cancer Institute of New Jersey, Department of Radiation Oncology, Robert Wood Johnson Medical School, Rutgers, the State University of New Jersey, New Brunswick, NJ.

During mitosis, the mitotic spindle apparatus is responsible for the faithful distribution of chromosomes between daughter cells. Compromised function of the mitotic spindle is a sufficient impetus of chromosome instability and aneuploidy, features associated with both tumor establishment and evolution. BCCIP, a p21 and BRCA2 interacting protein, is also intimately linked to chromosome instability and aneuploidy, yet the mechanisms through which BCCIP safeguards genomic stability are not completely understood. Here, we describe a novel role for BCCIP in regulating spindle microtubule dynamics during mitosis. We demonstrate that BCCIP accumulates at spindle poles and spindle fibers in dividing cells where it binds to tubulin and stabilizes microtubules. BCCIP loss results in the disappearance of the stable microtubule marker, K40-acetyl-tubulin, and sensitizes cells to the chemotherapeutic spindle poison, taxol. We show that this function is a mediated by BCCIP's central conserved domain. Loss of BCCIP function destabilizes spindle microtubules, causing improper and/or weak kinetochore attachments, resulting in a mitotic delay. Collectively, these data suggest an important role of BCCIP in ensuring the fidelity of mitosis through the regulation of microtubule dynamics.

A06 Cellular senescence induction by NBR1 silencing. Hee Suk Kim, Jeongwon Sohn. Department of Biochemistry and Molecular Biology, Korea University College of Medicine, , Seoul, Korea.

NBR1 (Next to BRACA1 gene) is known as an autophagy receptor which functions by binding both to LC3 and ubiquitin. We found that silencing NBR1 induced cellular senescence in several human cancer cell lines including MCF-7. Cellular senescence was shown by the characteristic flattened morphology and an increased SA-β-gal activity. Cellular senescence induction was

dependent on p53 and p21, in that knocking down p53 or p21 prevented it. Oxidative stress as well as DNA damage was involved in this process. ATM played an essential role in senescence induction through activation of AKT and mTOR. NBR1 silencing increased ROS generation in MCF-7 cells, and treatment of cells with apocynin or silencing NADPH oxidase blocked senescence induction, indicating the role of NADPH oxidase in NBR1 silencing-induced senescence induction. Oxidative stress was upstream of ATM, since apocynin or tiron dimished ATM activation. ER stress was induced by NBR1 silencing as shown by enhanced ElF2α phosphorylation and Bip expression. Salubrinal, an inhibitor of ER stress blocked senescence suggesting the role of ER stress in senescence induction. Furthermore, knocking down expression of PERK, IRE1 or ATF6 prevented activation of ATM, as well as accumulation of p53 and p21. NBR1 was reported to regulate p38 activity, and we also found evidence of the role of p38 in NBR1 silencing-induced senescence. Our data indicate that silencing NBR1 can induce cellular senescence in p53-wild type human cancer cell lines through p38 activation, ER stress, ROS generation by NADPH oxidase and a DNA damage response.

A07 Death effector domain1/2-contained procaspase-8 (1-215) fragment induces apoptosis of osteosarcoma cells. Zhengding Wu, Bin Xiang, Guanlin Wang and Kwen-Jen Chang. Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, Yunnan, China.

Apoptosis is a fundamental process to multicellular organisms that enables control of cell population and the removal of unwanted, damaged or potentially harmful cells. Caspases, a family of aspartate-specific cysteine proteases, play a critical role in the execution phase of apoptosis and are the key effectors responsible for many of the dramatic morphological and biochemical changes of development and immuno surveillance. Caspases in the death receptor-mediated apoptosis pathway can be divided into two classes: (1) initiator caspases, with long pro-domains, such as caspases-8/10 and -9, which either directly or indirectly activate (2) effector caspases, such as caspases-3, -6, and -7. As caspase-8 activates all known caspases in vitro, it is a prime candidate for an initiator caspase in many other forms of death receptor-mediated apoptosis. Procaspase-8 is a 55 kDa cysteine protease of 480 amino acids protein, and contains two death effector domains (DED) and a catalytic protease domain. Peocaspase-8 requires multiple steps for activation that include oligomerization and proteolysis cleavage. Active caspase-8 consists of a tetramer with two large and two small subunits. Procaspase-8(1-215) contains mainly two death effector domains (DED1/2). It is unknown whether procaspase-8(1-215) can mediate cell apoptosis and death. Therefore, we performed following experiments. First, we established the vector pCMV6-procaspase-8(1-215)-GFP and pCMV6-procaspase-8(1-215) containing *procaspase-8(1-215)* by gene cloning. Second, we performed MTT assay to study cell apoptosis, and to use flow cytometry to confirm cell apoptosis mediated by the transfection of vector pCMV6-procaspase-8(1-215)-GFP or pCMV6-procaspase-8(1-215). We also performed the western blots to study the mechanism of apoptosis induced by pCMV6-procaspase-8(1-215)-GFP transfection.

The U2OS cells transfected with pCMV6-procaspase-8(1-215)-GFP were all died within 24 hours, but transfected with mock vector pCMV6-AC-GFP were all lived well. We treated U2OS cells either with necrostatin-1(necrosis inhibitor) or with Z-VAD-FMK (Caspase inhibitor), respectively,

when cells were transfected with vector pCMV6-procaspase-8(1-215)-GFP. Our result showed that cells treated with necrostatin-1 died off almost， while the viability of cells treated with Z-VAD-FMK was increased remarkablely. To confirm these results, we performed MTT assay using transient transfection. The results showed that the cell viability of pCMV6-procaspase-8(1-215)-GFP transfection was about 35% and the cell viability of pCMV6-AC-GFP was about 80%. After treated with Z-VAD-FMK, the cell viability of after pCMV6-procaspase-8(1-215)-GFP transfection was increased to about 77%. Therefore, Z-VAD-FMK inhibited cell death induced by pCMV6-procaspase-8(1-215)-GFP. The apoptosis rate was also determined by flow cytometry. The result showed that the apoptosis rate of the cells transfected with pCMV6-procaspase-8(1-215) was about 58%. After treated with Z-VAD-FMK, the apoptosis rate was decreased to 16% which is similar to the apoptosis rate (17%) of the cells transfected with mock vector pCMV6-AC. The western blot results also showed that caspase-3 was actived by pCMV6-procaspase-8(1-215)-GFP vector transfection and inactived by Z-VAD-FMK treatment. Therefore, we can draw out our conclusion that pCMV6-procaspase-8(1-215)-GFP induced cell death through apoptosis pathway. The possible mechanism induced by pCMV6-procaspase-8(1-215)-GFP transfection will be discussed.

A08 JNK1/2 activation by an extract from the roots of *Morus alba* L. reduces the viability of multidrug-resistant MCF-7/Dox cells by inhibiting YB-1-dependent MDR1 expression. Youn Kyung Choi[1], Sung-Gook Cho[1], Hyeong Sim Choi[1], Yee Jin Yun[1], Min Kyoung Kim[1], Ah Jeong Kim[1].
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Introduction: Cancer cells acquire anticancer drug resistance during chemotherapy, which aggravates cancer disease. MDR1 encoded from multidrug resistance gene 1 mainly causes multidrug resistance phenotypes of different cancer cells. In this study, we demonstrate that JNK1/2 activation by an extract from the root of *Morus alba* L. (White mulberry) reduces doxorubicin-resistant MCF-7/Dox cell viability by inhibiting YB-1 regulation of MDR1 gene expression.
Materials and Methods: To examine anti-cancer effects of *Morus alba* L, we performed the experimental methods such as MTT, RT-PCR, western blot, luciferase assay, immunofluorescence assay, rhodamine 123 efflux assay and chromatin-immunoprecipitation assay.
Results and discussion: When MCF-7 or MCF-7/Dox cells, where MDR1 is highly expressed were treated with an extract from roots or leaves of Morus alba L., respectively, the root extract from the mulberry (REM) but not the leaf extract (LEM) reduced cell viabilities of both MCF-7 and MCF-7/Dox cells, which was enhanced by cotreatment with doxorubicin. REM but not LEM further inhibited YB-1 nuclear translocation and its regulation of MDR1 gene expression. Moreover, REM promoted phosphorylation of c-Jun NH2-terminal kinase 1/2 (JNK1/2) and JNK1/2 inhibitor, SP600125 and rescued REM inhibition of both MDR1 expression and viabilities in MCF-7/Dox cells. Consistently, overexpression of JNK1, c-Jun, or c-Fos inhibited YB-1-dependent MDR1 expression and reduced viabilities in MCF-7/Dox cells.

Conclusion: In conclusion, our data indicate that REM-activated JNK-cJun/c-Fos pathway decreases the viability of MCF-7/Dox cells by inhibiting YB-1-dependent MDR1 gene expression. Thus, we suggest that REM may be useful for treating multidrug-resistant cancer cells.

A09 Herbal extract SH003 suppresses tumor growth and metastasis of MDA-MB-231 breast cancer cells by inhibiting STAT3-IL-6 signaling. Sung-Gook Cho[1], Youn Kyung Choi[1], Sang-Mi Woo[1], Kangwook Lee[1], Kim Min Kyoung[1]. [1]Laboratory of Clinical Biology and Pharmacogenomics and Center for Clinical Research and Genomics, Institute of Oriental Medicine, Kyung Hee University, Republic of Korea.

Introduction: Cancer inflammation promotes cancer progression, resulting in a high risk of cancer. Here, we demonstrate that our new herbal extract, SH003, suppresses both tumor growth and metastasis of MDA-MB-231 breast cancer cells via inhibiting STAT3-IL-6 signaling path.
Materials and Methods: Our new herbal formula, SH003, a mixed extract from *Astragalus membranaceus*, *Angelica gigas* and *Trichosanthes kirilowii* Maximowicz. To examine anti-cancer effects of SH003, we performed the experimental methods such as animal experiments, MTT, migration assay, invasion assay, anchorage-independent soft agar assay, western blot, luciferase assay and immunofluorescence assay.
Results and discussion: Our new herbal formula, SH003, mixed extract from Astragalus membranaceus, Angelica gigas, and Trichosanthes kirilowii Maximowicz, suppressed MDA-MB-231 tumor growth and lung metastasis in vivo and reduced the viability and metastatic abilities of MDA-MB-231 cells in vitro. Furthermore, SH003 inhibited STAT3 activation, which resulted in a reduction of IL-6 production.
Conclusion: We conclude that SH003 suppresses highly metastatic breast cancer growth and metastasis by inhibiting STAT3-IL-6 signaling path.

A10 Effect of Sipjeondaebo-tang on cancer-induced anorexia and cachexia in CT-26 tumor-bearing mice. Sang-Mi Woo[1], Hyeong Sim Choi[1], Kang Wook Lee[1], Yee-Jin Yun[1], Ah Jeong Kim[1]. [1]Laboratory of Clinical Biology and Pharmacogenomics and Center for Clinical Research and Genomics, Institute of Oriental Medicine, Kyung Hee University, Republic of Korea.

Introduction: Cancer-associated anorexia and cachexia are a multifactorial condition described by a loss of body weight and muscle with anorexia, asthenia, and anemia. Moreover, they correlate with a high mortality rate, poor response to chemotherapy, poor performance status, and poor quality of life. Cancer cachexia is regulated by proinflammatory cytokines such as interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor- α (TNF- α). In addition, glucagon like peptide-1 (GIP-1), peptide YY (PYY), ghrelin, and leptin plays a crucial role in food intake.
Materials and Methods: In this study, we investigated the therapeutic effects of one of the traditional herbal medicines, Sipjeondaebo-tang (Juzen-taiho-to in Japanese; SJDBT), on cancer anorexia and cachexia in a fundamental mouse cancer anorexia/cachexia model, CT-26 tumor-bearing mice.

Results and discussion: SJDBT was more significantly effective in a treatment model where it was treated after anorexia and cachexia than in a prevention model where it was treated before anorexia and cachexia on the basis of parameters such as weights of muscles and whole body and food intakes. Moreover, SJDBT inhibited a production of IL-6, MCP-1, PYY, and GLP-1 and ameliorated cancer-induced anemia.
Conclusion: Therefore, our in vivo studies provide evidence on the role of SJDBT in cancer-associated anorexia and cachexia, thereby suggesting that SJDBT may be useful for treating cancer-associated anorexia and cachexia.

A12 Hypoxia-induced upregulation of AQP1 in prostate cancer cells. Lu Tie[1], Ning Lu[1], Xue-Jun Li[1]. [1]Department of Pharmacology, School of Basic Medical Sciences, Peking University, Beijing, China.

Background: Aquaporin-1 (AQP1) is a glycoprotein that mediates osmotic water transport, its expression has been found to correlate with tumour stage in some tumours. However, the mechanism by which AQP1 protein expression is regulated in tumor cells remains to be fully elucidated. We hypothesized that hypoxia might play an important role in AQP1 induction during tumorigenesis and at the late stages of tumor development. Methods: Isotonic and serum-free hypoxic models were used to investigate AQP1 expression in PC-3M human prostate cancer cells. Results: AQP1 expression was up-regulated by density-induced pericellular hypoxia and cobalt(II) chloride ($CoCl_2$)-induced hypoxia at the transcriptional level. Moreover, phosphorylation of p38 mitogen-activated protein kinase (MAPK) was induced by density-induced pericellular hypoxia and $CoCl_2$-induced hypoxia, specific inhibitors of p38 MAPK could concentration-dependently block those effects of hypoxia on AQP1 expression. Intracellular calcium ion (Ca^{2+}) and protein kinase C (PKC) were shown to be responsible for the activation of p38 MAPK pathway. In addition, AQP1 induction in dense cultures was dependent on lowered oxygen (O_2) tension. In high cell density culture, certain secretory proteins might induce AQP1 expression indirectly. Conclusion: These findings suggest that AQP1 could be induced by hypoxia at transcription level, and the regulation of AQP1 in PC-3M cells is dependent on calcium, PKC and p38 MAPK, as well as low oxygen tension.

A15 Use of a novel mutagenesis approach for the discovery of tumor-specific regulations of angiogenesis in melanoma. Indhu Subramanian[1,2], Emily S. Fuller[1], Susan Smith[3], Jennifer Froes[1], Viive M. Howell[1*], Anthony W. Ashton[2*]. [1]Bill Walsh Translational Cancer Research Laboratory, [2]Perinatal Research Laboratory, AND [3]Raymond Purves Research Laboratories, Kolling Institute of Medical Research, University of Sydney, St Leonards, NSW, Australia. **Denotes equal contribution of the authors.*

Background: Growth of solid tumors beyond $1mm^3$ requires the recruitment of new blood vessels (angiogenesis). Multiple studies have correlated angiogenesis with aggressive tumor behavior, poor prognosis and poor survival. This “angiogenic switch” is believed to be due to the release of a multitude of factors from the tumor, most notably VEGF-A; however, this process is not well understood and may also be due to the readiness of the endothelium to respond to these factors. Observations that tumor vessels are phenotypically different to those in normal tissues have

prompted speculation that tumor endothelium itself is different. Moreover, if identified, the factors responsible for these differences could be targeted to inhibit tumor growth, without compromising tissue homeostasis.

Aim: To manipulate the endothelial genome using a mutagenesis strategy to alter levels of tumor angiogenesis and, through comparison with the levels of angiogenesis in normal tissues, to delineate genes that may play a specific role in tumor angiogenesis.

Methods: Sleeping Beauty (SB) is a random insertional mutagenesis system for genetically modifying cellular behavior. Mutation of the endothelial genome was achieved by using the Tie-2 promoter/enhancer in a CRE transgenic mouse model to activate SB. Immunohistochemistry (IHC) for SB confirmed the endothelial-specific nature of this activation. Allografts of B16F10 malignant melanoma cells were implanted in 8-10 week-old mice. Mice were sacrificed 10 or 14 days post implantation and tissues formalin fixed, paraffin embedded and analyzed histologically. Physiologic and pathologic angiogenic potential was quantified by the measurement of micro-vessel density in heart and tumor tissue, respectively, using immunohistochemistry for CD31 to identify blood vessels and LYVE-1 to identify lymphatic vessels.

Results: Immunohistochemistry and PCR were used confirm the endothelial-specific nature of transgene expression in CRE+ mice. No significant difference in overall growth or organ weight was observed between CRE+ (n=44) and wild type (WT; n=23) mice, demonstrating that activation of the SB mutational system did not alter development and weight gain in transgenic mice. Upon challenge with a sub-cutaneous allograft of melanoma cells approximately 30% of CRE+ mice developed tumors significantly different in size to those in WT mice. In many of these mice the relationship between vascular density and tumor size was perturbed indicating that the SB-mediated mutagenesis altered angiogenic (CD31) and lymph-angiogenic potential (LYVE-1). In the clear majority of CRE+ mice the level of physiological angiogenesis (in the heart) was not different to WT animals indicating that only pathological angiogenesis was being altered.
Conclusion: Changes to the endothelial genome via SB insertional mutagenesis: (1) alters the angiogenic response, which directly translated into altered tumor growth; (2) perturbs the known correlation between tumor size and vascularity. This novel approach has never been used in a tissue-specific directed phenotype screen of disease. Future work will involve sequencing of the mutated genes to identify new targets for anti-angiogenic therapies that may progress the therapeutic strategies for the treatment of malignant melanoma.

A16 Steroid sulfatase regulates the integrin signaling pathway in human cervical cancer cells. Dong-Jin Ye, Nahee Park, Yeo-Jung Kwon, Sangyun Shin, Mihye Hong, and Young-Jin Chun. College of Pharmacy, Chung-Ang University, Seoul, Korea.

Steroid sulfatase (STS) is responsible for the hydrolysis of aryl and alkyl steroid sulfates. STS has a pivotal role in regulate the level of estrogen and androgen responsible for growth of hormone dependent tumor, such as breast or prostate cancer. But the molecular function for tumor growth of STS is still not clear. To elucidate possible role of STS on cancer cell proliferation, we investigated whether STS is able to regulate integrin signaling pathway. In this study, we

observed that overexpression of STS in HeLa cells induces the expression of integrin β1 and fibronectin, a ligand of integrin α5β1 at protein and mRNA levels. Dehydroepiandrosterone (DHEA), one of the main metabolite of STS, also induces mRNA and protein level of integrin β1 and fibronectin. We found that STS expression and DHEA enhance phosphorylation of focal adhesion kinase (FAK) at tyrosine 925 residue. Moreover, phosphorylation of ERK at threonine 202 and tyrosine 204 residues was also induced, indicating that STS may activate Ras/Raf/MEK signaling pathway. In conclusion, these results suggest that STS expression and DHEA may enhance Ras/Raf/MEK signaling through upregulation of integrin β1 and activation of FAK.

A17 Re-expression of HPV16 E2 in SiHa (human cervical cancer) cells potentiates NF-κB activation induced by TNF-α concurrently increasing senescence and survival. Devan Prabhavathy, Karthik Subramanian and Devarajan Karunagaran. Department of Biotechnology, Bhupat and Jyoti Mehta School of Biological Sciences, Indian Institute of Technology Madras, Chennai, India.

Cervical neoplastic progression is initiated by high-risk human papilloma virus (HR-HPV) infection followed by viral integration and episome clearance in most cases. However, about 12.5% of cervical carcinomas contain viral transcripts from only episomal HR-HPV and coexistence of episomal and integrated forms of HPV is reported with a higher proportion in HPV16-positive malignancies. HPV E2 is one of the early genes known to repress the expression of another early gene E6 with a consequent increase in p53 levels. Although HPV infection is necessary, several host factors or conditions such as cytokines and chronic inflammation play an equally important role in cervical cancer progression. Since reinfection of the same or different HPV types or reactivation of the latent virus are known to occur it was of interest to study the effects of E2 re-expression in the presence of an inflammatory factor, TNF-α, in human cervical cancer cells. The results from this study show that E2 inhibits endogenous E6 gene expression analyzed by qPCR and sensitizes SiHa cells to TNF-α-induced NF-κB activation assessed in a luciferase reporter gene assay. Under this condition there was an increase in the expression of p53, p21, p27 and p16 analyzed by qPCR and senescence associated-β-galactosidase activity also increased indicating that TNF-α augments E2-mediated senescence. Re-expression of E2 expression with TNF-α treatment resulted in an increase in the expression of anti-apoptotic Bcl2 protein and other pro-survival genes like cyc D1, survivin and hTERT. Concomitantly, E2+TNF-α combination increased the survival of SiHa cells by positive changes in viability, proliferation and colony formation. E2-induced apoptotic tendency shifted towards senescence in presence of TNF-α by arresting the cells at both G_0/G_1 and G_2/M phases, thus enhancing cell survival. Another new observation in this study is the significant up regulation of key senescence messaging factors regulated by NF-κB namely IL-6, IL-8, HMGA1 and HMGB1 in E2-transfected cells treated with TNF-α and thus provides a mechanistic basis for the observed increase in cell survival. HPV16 E2 protein could contribute to the HPV oncogenic potential in presence of TNF-α by synergistically activating NF-κB and enhancing the expression of cell survival factors. The results attribute an important role to E2 in sustaining tumorigenesis through the regulation of senescence in the presence of cytokines and these data have implications in cancer therapy.

A18 IKKα attenuates arsenite-induced hepatoma death via promoting p53-dependent autophagy. Xixing Tan, Ming Gao, Meiru Hu, Lun Song*. Department of Stress Medicine, Beijing Institute of Basic Medical Sciences, 27 Taiping Road, Beijing, P. R. China.

The IκB kinase (IKK) complex play a critical role in the activation of the NF-κB pathway under physiological and pathological conditions. The two catalytic subunits of the IKK complex, IKKα and IKKβ, share structural similarity but trigger NF-κB activation by different mechanisms. Besides the NF-κB proteins, IKKα and IKKβ also target a variety of other substrates and therefore are involved in many physiological and pathological processes through NF-κB-independent mechanisms. In the previous studies, we focus on elucidating NF-κB-independent roles of IKKα and IKKβ under stress conditions, and the results indicate that IKKα and IKKβ are able to mediate growth arrest, apoptosis or inflammatory responses via regulating a variety of downstream signaling molecules that are unrelated to NF-κB (J Cell Biol, 2006; Exp Cell Res, 2008; BBA-Mol Cell Res, 2010; Mol Cell Biochem, 2011; Nucleic Acids Res, 2012). In the current study, we identified the novel function of IKKα in promoting cytoprotective autophagy in the arsenite-treated human hepatoma cells, which is unrelated to IKKβ and NF-κB activities. The induction of autopahgy depended on the activation of p53 and upregulation of its downstream target DRAM expression upon arsenite exposure. Blocking p53 activation or DRAM upregulation dramatically inhibited the autophagic flux and therefore increased HepG2 cells sensitivity to arsenite. Furthermore, IKKα functioned as the critical activator for p53 upon arsenite exposure. Overexpression of IKKα significantly increased the levels of p53 activation, DRAM expression as well as autophagy induction. On the contrary, blocking kinase activity of IKKα inhibited the p53/DRAM pathway activation and the upregulation of autophagy. In the attempt to figure out the functional link between IKKα and p53, we found that the presence of IKKα was important to the induced activation of ATR/LKB1 signal transduction pathway, which was the upstream signaling event in mediating p53 activation in the arsenite-treated HepG2 cells. Therefore, these data indicate that IKKα/ATR/LKB1/p53 pathway activation functions as a protective mechanism in antagonizing arsenite-induced apoptosis via inducing autophagy. Interestingly, arsenite exposure downregulated IKKα expression in the HepG2 cells, which effect was almost absent in the normal human diploid hepatic cells. Moreover, downregulation of IKKα resulted from autophagy-dependent degradation of this protein. Overexpression of IKKα significantly overcame the apoptotic response in the arsenite-treated HepG2 cells. Taken together, we conclude that IKKα attenuates arsenite-induced apoptosis in HepG2 cells by inducting p53-dependent autophagy. And then the autophagy-dependent feedback degradation of the cytoprotective IKKα is a critical event to mediate the cytotoxic effect of arsnite.

A19 The PRC1.1 complex as a potential drug target. Sarah E. Junco[1]; Alex Taylor[1]; Micah D. Gearhart[2]; P. John Hart[1]; Matthew Hart[1]; Vivian J. Bardwell[2]; Chongwoo A. Kim[1]. [1]University of Texas Health Science Center at San Antonio, San Antonio, Texas, [2]University of Minnesota, Minneapolis, MN.

Polycomb Group (PcG) proteins form large multiprotein complexes that mediate epigenetic modifications of histones to cause transcriptional repression. Many of the PcG gene targets are tumor suppressor genes. A recently identified complex, known as the BCOR complex or PRC1.1,

contains the proteins BCOR (a Bcl-6 co-repressor), PCGF1 and RING1B (ubiquitin ligases and canonical PcG proteins), KDM2B (also known as FBXL10, a lysine demethylase) and SKP1. Recently, KDM2B has been shown to be necessary for the initiation and maintenance of acute myeloid leukemia (AML). We believe that the oncogenic properties of KDM2B are due to its activity within the PRC1.1 complex. This makes this lysine demethylase an excellent drug target. We have determined the crystal structure of the fbox and LRR regions of KDM2B in complex with SKP1, PCGF1 and BCOR (KSPB complex) and measured the binding affinities for components of the complex. Analysis of the structure reveals regions of hydrophobicity on the surface of KDM2B that may be utilized as drug targets. We have begun to optimize a high throughput screen of drugs against the KSPB complex to determine if any of the drugs will disrupt the KSPB interaction.

A20 The mechanisms of miR-*602 and miR-363* in the regulation of head and neck squamous cell carcinoma metastasis. Wantao Chen[1], Qiang Sun[1,2], Jianjun Zhang[1].
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Purpose: Dysregulated microRNAs (miRNAs) play an important role in many malignant tumors. However, elucidating the roles of miRNAs in cancer biology, especially in epithelial cancers, remains an ongoing process. In this study, we identified the differentially expressed miRNAs and investigated the specific mechanisms of the miR-602and miR-363.
Methods: The miRNA arrays of miRNomes in 8 human normal oral mucosa, 8 head and neck squamous cell carcinoma (HNSCC) tissues with non-lymph node metastasis and 8 HNSCC tissues with lymph node metastsis were carried out. We validated the results of miRNA array in a large number of clinical samples by using real-time PCR. Expression levels of the candidated miRNAs were analyzed for association with clinicopathological parameters by using the Kaplan-Meier survival analysis. The cell growth, cell cycle distribution, invasion and migration potential, and clone formation were observed to detect the functions of the miRNAs in epithelial cancer cells. The cell line xenograft mouse models were used to valudate the effects on cancer growth *in vivo*. The target genes of the miRNAs were predicted by bioinformatics methods and confirmed by dual luciferase report analysis system. The interactions between genes and proteins were confirmed by Chromatin Immunoprecipitation Assay (CHIP). The DNA methylation status within gene promoter were evaluated through the induction of 5-aza-2'-deoxycytidine (5-Aza-dC) and by methylation specific PCR (MSP) in HNSCC cell lines and tissues.
Results: In this study, in the 77 HNSCC tissues with lymph node metastases, the expression level of *miR-602* was 6.70±0.37, whereas its expression level in the 52 tissues with non-lymph node metastasis was 4.81±0.39 (*p=0.013*). In patients with HNSCC, higher level of *miR-602* expression significantly correlates with worse overall survival (*p=0.039*). Ectopic expression of *miR-602* promoted cancer cell invasion and migration through suppressing *MAL* expression in cancer cells. We also explored the pro-metastatic effect of EHMT1 in epithelial cancer cells mainly through catalyzes H3K9me2 methylation at promoter of *MAL* genes. In addition, *miR-602* can function synergistically with *EHMT1* to enhance epithelial cancer cell invasion and migration. Thus, our findings indicate that increased copy number of the transcripts which include *EHMT1* and *miR-*

602 is a crucial stimulus for tumor invasion and migration of epithelial cancer. In the 34 HNSCC tissues with lymph node metastasis, the expression level of *miR-363* was 0.99±0.14, whereas its expression level in the 28 tissues with non-lymph node metastasis was 1.93±0.21 (*p=0.001*). Higher level of *miR-363* expression significantly correlates with better OS (*p=0.040*). We used bioinformatics and molecular methods to predict and prove that *miR-363* can directly target *podoplanin* (*PDPN*) in HNSCC. Furthermore, we provided evidence to demonstrate that *PDPN* dysregulation caused by down-regulation of *miR-363* contributes to HNSCC invasion and migration. And the results suggest that DNA promoter methylation as major factors were involved in silencing the *miR-363*.
Conclusions: These data reveal a key role of *miR-602* and *miR-363* in HNSCC invasion and metastasis and support the clinical links between differentially expressed miRNAs and HNSCC. This newly identified *EHMT1*/*miR-602-MAL* module provides a new idea to understand the processes of epithelial cancer cell invasion and migration, and may facilitate the development of potential diagnostic and therapeutic biomarkers against epithelial cancer.

A21 miR-300 inhibits epithelial to mesenchymal transition and metastasis by targeting Twist. Qin Xu. Shanghai Jiaotong University School of Medicine, Shanghai, China.

The epithelial-mesenchymal transition (EMT) is a crucial step in epithelial cancer invasion and metastasis. The aims of this study were to investigate and validate unidentified micro RNAs (miRNAs) that regulate EMT and to reveal their clinical relevance in epithelial cancer patients. By applying miRNA array screening in a natural epithelial-mesenchymal phenotype cell line pair and in a transforming growth factor β-induced EMT cell model, we found miR-153 was markedly downregulated in the cells that underwent an EMT. A close association was confirmed between inhibition of miR-153 and the EMT phenotype, as well as the invasive ability of epithelial cancer cells. Ectopic expression of miR-153 in mesenchymal-like cells resulted in an epithelial morphology change with decreased cellular invasive ability. On the contrary, transfection of a miR-153 inhibitor in epithelial-like cells led to a mesenchymal phenotype change. In vivo ectopic expression of miR-153 significantly inhibited tumor cell metastasis formation. Data from the dual-luciferase reporter gene assay showed, for the first time, that SNAI1 and ZEB2 were direct targets of miR-153. Inverse correlations were also observed between miR-153 and SNA1 and ZEB2 levels in oral cancer patients' samples. Furthermore, low expression level of miR-153 was found to be significantly related to metastasis and poor prognosis in oral cancer patients. These data demonstrate that miR-153 is a novel regulator of EMT by targeting SNAI1 and ZEB2 and indicate its potential therapeutic value for reducing cancer metastasis.

A22 Oncogenic Ras mutants differentially utilize PLC-dependent calcium flux and PKC activation for MAPK signalling. Cameron L. Pitt, Frank McCormick. University of California San Francisco, CA, USA.

Ras mutations drive approximately 30% of human cancers by aberrant regulation of the mitogen-activated protein kinase (MAPK) signaling cascade in gene expression, cellular growth and survival. While oncogenic Ras mutations are sufficient for constitutive GTP-loading and plasma membrane localization of the immediate MAPK effector Raf, it is unknown whether Ras

variants differentially recruit additional signaling proteins in scaffolding complexes required for MAPK hyperactivation. Phospho-regulation of Raf is a crucial second step in Ras-dependent MAPK signaling. Phospholipase C (PLC) is a key mediator of Raf phosphorylation by generation of diacylglycerol (DAG) and inositol trisphosphate (IP3) activating protein kinase C (PKC) and calcium signaling.
K-Raslox(H-Ras-/- ;N-Ras-/-;K-Raslox/lox;RERTert/ert) mouse embryonic fibroblasts (MEFs) were provided by the laboratory of Mariano Barbacid (CNIO, Madrid, Spain). Treatment with 4-hydroxytamoxifen (4-OHT) yields "Rasless" cells, devoid of the major endogenous alleles. These cells were lentivirally transduced with various Ras alleles for comparison. Western blot and co-immunoprecipatation were the primary signaling readouts. Intracellular calcium flux in response to extracellular stimuli, ion channel agonists or inhibitors was quantitatively measured using the fluorescent Ca^{2+} indicator Fluo-4 NW (Invitrogen).
Growth of "Rasless" MEFs can be restored by reconstitution with any of the major Ras alleles. "Rasless" cells are disconnected from growth factor or phorbol ester signaling and are deficient in producing a MAPK response. Though oncogenic Ras variants display constitutive GTP-loading, unaffected by upstream stimulation, EGF and PMA are both capable of increasing the MAPK output from the basal level. PLC activity is required to sustain basal MAPK signaling and necessary for the maximal response to stimuli. Mutant Ras alleles display differences in their extent of their dependency on PLC for generation of the MAPK response to stimulation. K-Ras mutants differentially bind Kinase Supressor of Ras (KSR) 1 and 2 scaffolding proteins and degrees of interaction can be modulated by PLC inhibition. Downstream of PLC, calcium signaling is sufficient to generate increases in MAPK signaling in mutant Ras-expressing cells. Further, the calcium-regulated chloride channel TMEM16A modulates the MAPK response to Ca^{2+} flux in a Ras mutation-dependent manner.
Though oncogenic Ras does not require growth factor-mediated activation, enhanced MAPK signaling depends on both Ras-GTP status for membrane recruitment of Raf and Raf activation by proximal factors. The latter step requires PLC activity via downstream PKC and calcium signaling. Due to conformational variation of mutant Ras proteins, variable signaling complexes are formed and proximal Raf agonists or inhibiting proteins are differentially regulated by KSR scaffolding interactions in response to stimulation or inhibition of PLC. The discovery that specific Ras mutants may be more susceptible to PLC inhibition or downstream modulation of calcium flux may provide a novel avenue for clinical blockade of growth signaling in certain Ras mutant cancers.

A23 AP-1 transcription factor JDP2 potentiates the generation of medulloblastoma cancer stem cells from induced pluripotent stem cells. Yokoyama, K. Kazushige[1], Ku, Chia-Chen[1], Lin, Shih-Han[1], Wuputra, Kenly[1], Miyoshi, Hiroyuki[1, 2], Nakamura, Yukio[2], Lin Chang-Shin[1]. [1]Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [2]RIKEN BRC, Tsukuba, Japan.

Transcription factor Jun dimerization protein (JDP2) plays roles in cell cycle regulation, cellular senescence, nuclear reprogramming and oncogenesis through the epigenetic control involved in cascades of $p19^{Arf}$-Mdm2-p53-p21-cyclin/CDK or $p16^{Ink4a}$-cyclin/CDK-RB-E2F. Clinical studies of medulloblastomas indicate that JDP2 might be a tumor suppressor gene candidate because

normal granule cells express significant levels of JDP2, whereas cancer cells do not. Thus, we generated three different induced pluripotent stem cells (iPSCs) from human medulloblastoma cancer cells (DAOY1) using Lenti-virus encoded standard 4 factors (4F; Oct4, Klf4, Sox2 and c-Myc), 2 factors (2F; Oct4 and JDP2) and I factor (JDP2). The original DAOY1 expressed three standard stemness genes like Oct4, Sox2 and Nanog, but did not show the alkaline phosphatase activity. By contrast, iPSCs expressed stemness genes and demonstrated the alkaline phosphatase activity. Moreover, we found that iPSCs enhanced the tumor progression as compared with DAOY1 in SCID mice. These results indicate that JDP2 is highly possible to play a critical role of tumor potentiating function of iPSCs to generate the cancer stem cells. We also found the role of JDP2 is concerned the signaling of Wnt signals such as the genes encoding LEF1 (lymphoid enhancer binding protein), TCF3. The cross talk of Wnt signal and LIF/JAK-STAT3-Oct4 will be critical for generation of cancer stem cells from medulloblastoma iPS cells.

A24 FUS/TLS targets mRNAs and miRNAs involving DNA damage response. Yueqin Zhou[1], Songyan Liu[1,2], Arzu Öztürk[1], Geoffrey G. Hicks[1]. [1]Manitoba Institute of Cell Biology; Department of Biochemistry and Medical Genetics; Regenerative Medicine Program, University of Manitoba, Winnipeg, Manitoba, Canada, [2] Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction: FUS/TLS is an RNA binding protein that can regulate gene expression by targeting RNA for pre-mRNA alternative splicing, mRNA transport or miRNA processing. The FUS/TLS gene is translocated in human liposarcoma and leukemia. In the translocated fusion protein, the RNA binding function of FUS is lost, suggesting the dysregulation of FUS-dependent RNA metabolism may play important roles in FUS-associated cancer. FUS is known to regulate DNA damage repair, an important cellular process which, when dysregulated, leads to genomic instability in cancer. However the molecular mechanism underlying FUS-regulated DNA damage repair is yet to be identified. The aim of this study is to identify FUS RNA targets involving DNA damage response and repair.

Method: FUS binding RNA in HeLa cells was identified by RNA crosslinking immunoprecipitation and next generation sequencing (CLIP-seq). mRNA expression profiling of $FUS^{+/+}$ and $FUS^{-/-}$ mouse embryonic fibroblasts (MEFs) were performed using Illumina bead chip array. miRNA expression of $FUS^{+/+}$ and $FUS^{-/-}$ MEFs was measured using NanoString miRNA expression assay. Bioinformatics analyses were performed using CisGenome for CLIP-seq, Gene Set Expression Analysis (GSEA) and Ingenuity Pathway Analysis (IPA) for mRNA and miRNA expression assays.

Results: FUS CLIP-seq analysis identified 1928 FUS CLIP clusters that are regions statistically enriched with multiple overlapping FUS CLIP tags, as compared to the control (FDR $\leq$ 0.05). These 1928 FUS CLIP clusters are mapped to 1149 pre-mRNAs. Focus were given to 382 RNA targets that fall into the Gene Ontology (GO) functional category of cellular response to DNA damage stimulus (GO: 0006974) in ours and other previously published FUS CLIP-seq data. IPA Analysis of these 382 genes revealed that BRCA1 breast cancer signaling, ATM signaling, DNA double strand break repair and cell cycle checkpoints are among the most enriched pathways. Remarkably, about 60% of the genes in the DNA double strand break repair homologous

recombination pathway and non-homologous end joining pathway are targeted by FUS. These data suggest that FUS may directly regulate the pre-mRNA processing of genes that are involved in DNA damage response and repair.
To identify whether FUS regulates the expression of its RNA targets, gene expression analysis was performed by microarray. The analysis revealed that 160 mRNAs (111 upregulated, 49 downregulated) are differentially expressed (FDR $\leq$ 0.05, fold change $\geq$ 1.5) in *FUS*$^{-/-}$ MEFs, as compared to *FUS*$^{+/+}$ MEFs. GSEA analysis of the mRNA expression data identified that five groups of gene sets with similar biological processes are significantly (P $\leq$ 0.001, FDR $\leq$ 0.05) underrepresented in *FUS*$^{-/-}$ MEFs. Among these five, the two largest groups (having the most gene sets) are related to DNA replication, recombination, and repair, and cell cycle. These data demonstrate that DNA damage repair and cell cycle regulation are primary cellular processes regulated by FUS.
The gene expression analysis also revealed miRNAs that are known to involve DNA damage response, such as miR-34b, are differentially expressed in *FUS*$^{-/-}$ MEFs, as compared to *FUS*$^{+/+}$ MEFs. Correspondingly, the expression of mRNA targeted by these miRNAs are changed in the opposite direction in *FUS*$^{-/-}$ MEF. These data suggest alternatively FUS may modulate miRNA biogenesis to control the expression of mRNAs that are involved in DNA damage response and repair.

Conclusion: This study identified FUS mRNA and miRNA targets that are related to DNA damage response and repair. The data suggest that RNA metabolism including pre-mRNA processing and miRNA biogenesis may represent novel mechanisms underlying FUS-dependent DNA damage response and repair. The study provides novel insight into the molecular mechanism underlying FUS-associated genomic instability and cancer.

A25 Repression of the human telomerase gene during differentiation of embryonic stem cells. De Cheng, Shuwen Wang, Yuanjun Zhao, Jiyue Zhu*. Pharmaceutical Sciences, Washington State University, Spokane, WA, USA.

Telomerase reverse transcriptase (TERT) is essential for maintaining telomeres and plays important roles in aging and tumorigenic processes. The TERT gene is stringently repressed in most of human somatic tissues, but not in mice (Jia et al, 2010). Silencing of the TERT gene in human somatic cells is critical for aging and tumor suppression. The TERT gene occupies a large genomic region and likely possesses multiple regulatory regions. We previously found that the human TERT (hTERT) gene underwent stronger repression than its mouse counterpart during differentiation of embryonic stem cells (ESCs) (Wang et al, 2009). Furthermore, inhibition of histone deacetylases by trichostatin A was able to alleviate this repression and induce telomerase activity, suggesting that this repression resulted from epigenetic modifications. To determine the *cis* elements required for hTERT repression, we developed a novel technical platform, recombinase-mediated BAC targeting (RMBT), for integrating single-copy BAC reporters into specified chromosomal sites. Using RMBT, we found that chromatinized BAC reporters containing corresponding genomic regions that include either *hTERT* or *mTERT* loci recapitulated their respective native genes during differentiation of mouse ESCs, indicating that *cis* regulatory elements were responsible for the species-specific hTERT repression. By

substituting the hTERT promoter in the human BAC reporter with the mouse promoter, or vice versa, we found that distal sequences, not the promoters, are critical for differential repression of the TERT gene upon cell differentiation. Indeed, the HSV TK promoter, an irrelevant promoter, underwent similar repression as the hTERT promoter in its genomic environment. By analyzing human and mouse chimera BAC reporters, we discovered that multiple distal elements were involved in the stringent hTERT regulation in somatic cells.
In summary, our data indicates that hTERT core promoter has robust activity in pluripotent stem cells, but its activity is stringently repressed upon cellular differentiation. This repression depends on its genomic and chromatin environment and is not specific to its core promoter sequence. Conversely, the mTERT genome locus is unable to repress its core promoter activity, resulting in its broader expression in mouse somatic tissues.

A26 The deubiquitinating enzyme USP24 regulates the apoptotic response to UV irradiation by directly targeting p53. Ling Zhang, Leah Nemzow, Hua Chen, Abigail Lubin, Xi Rong, Zhongyi Sun and Feng Gong*. Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, USA.

In response to cellular stress, the tumor suppressor p53 is stabilized and activated. One of the most important p53 tumor suppressor functions is its ability to activate apoptosis, and disruption of this process can promote tumor progression and chemoresistance. p53 regulation by ubiquitination and deubiquitination is important for its functions. In this study, we have identified USP24 as a novel deubiquitinase targeting p53 for deubiquitination in human cells. USP24 is required for basal level of p53 expression under unstressed conditions. Importantly, USP24 is up-regulated after UV damage and is essential for p53 stabilization and PUMA activation in vivo after UV irradiation. Consistent with the requirement of USP24 for p53-PUMA activation, USP24 depletion renders cells resistant to apoptosis after UV treatment. In addition, we showed that purified USP24 protein is able to cleave ubiquitinated p53 in vitro. Our data reveal that the USP24 deubiquitinase regulates the apoptotic response to DNA damage by directly targeting the p53-PUMA axis.

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we showed that purified USP24 protein is able to cleave ubiquitinated p53 in vitro. Our data reveal that the USP24 deubiquitinase regulates the apoptotic response to DNA damage by directly targeting the p53-PUMA axis.

A27 RPL41-induced lethal actin polymerization: a new anti-mitosis strategy. Yanchun Ma[1*], Wei Zhou[1*], Dong Ren[1], Wenli Ma[1], John H. Hartwig[2] , Sheng Xiao[1]. [1]Department of Pathology, [2]Division of Translational Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. *these authors contributed equally.

Anti-mitotic reagents are among the most effective anti-cancer drugs, although most patients eventually succumbed to diseases due to relapse/drug resistance. Ribosomal protein L41 (RPL41) is unique in that it is probably the smallest and also the most basic eukaryotic protein. RPL41 contains 25 amino acids and an amphiphilic a-helix WH2 domain, a common actin-interacting structure. A direct interaction between RPL41 and actin was confirmed by various pull down assays and a native PAGE analysis. In an *in vitro* actin polymerization assay using pyrene-conjugated actin, RPL41 accelerated in a dose-dependent fashion the rate of actin polymerization, by causing significant decrease of its critical concentration for polymerization. Electron microscopy also revealed robust F-actin bundles in reactions containing RPL41, consistent with a role in actin bundling. Cellular RPL41 was localized in several cytoskeleton structures including ruffles, lamellipodial protrusions and midzone/midbody region. Cells with RPL41 knockdown were deficient in cell spreading and cell attachment, lacked stress fibers and were deficient in forming serum-stimulated stress fibers. In addition, mitotic abnormalities were observed. These results suggested a role of RPL41 in multiple cell processes involving cytoskeleton rearrangements. RPL41 was down-regulated in many tumors, which may contribute to tumorigenesis and/or tumor cell dissemination via its roles in mitosis and cell attachment.
A synthetic RPL41 was cell permeable and induced necrotic cell death specifically in mitosis. RPL41-induced mitotic cell death was associated with massive actin polymerization (by as much as 20-fold as compared to control cells), increased DNase activity and DNA loss. In contrast, RPL41 induced a transient increase in filopodia and lamellipodia formation in interphase cells, although the overall actin polymerization was not significantly increased, nor was the RPL41-induced DNA loss and cell death. Further studies suggested that accelerated actin filament dynamics in mitosis allowed RPL41 to interfere. Mitosis is a stage of cytoskeleton remodeling and increased mitosis is a common feature in malignancy, which provides an excellent opportunity to interfere. Because RPL41 killed cells by mechanically impeding mitosis in a p53-independent manner, the use of synthetic RPL41 as an anti-cancer drug worth further evaluation.

A28 HNF4a is an important mediator for glucose-induced ChREBPa and ChREBPb transcription. Jian Meng, Ming Feng, Yemin Zhu, Dianqiang Yang, Yakui Li, Lifang Wu, Ping Zhang, Minle Li, Xuemei Tong. Dept. of Biochemistry and Molecular Cell Biology, Institute of Medical Science, Shanghai Jiao Tong University School of Medicine, Shanghai, P.R. China.

ChREBPa and ChREBPb are two isoforms of carbohydrate-responsive element binding protein (ChREBP) transcribed from two promoters. ChREBP is an important glucose-responsive transcription factor regulating glycolysis and lipogenesis in metabolic tissues and cancer cells. However, it remains unclear so far how glucose promotes ChREBPa and ChREBPb gene transcription. Among many transcription factors predicted to bind to ChREBPa and ChREBPb promoters, we found that ectopic HNF4a expression increases ChREBPa and ChREBPb transcription in 293T and HepG2 cells. Knocking down HNF4a not only greatly reduced ChREBPa and ChREBPb transcription under 0 mM and 25 mM glucose conditions, but also abolished the glucose-dependent ChREBPa and ChREBPb induction in HepG2 cells. We found that HNF4a directly bound to E-boxes located in intron 12 of ChREBPa gene and in the 400 bp promoter region of ChREBPb gene. ChREBPa was reported to directly bind to carbohydrate response element (ChoRE) of ChREBPb gene and promote ChREBPb transcription. We found that ChREBPa and HNF4a could additively increase ChREBPb transcription. Furthermore, we found that ChREBPa and HNF4a co-immunoprecipitated with each other in 293T cells. Therefore, our results not only demonstrated that HNF4a played an important role in mediating glucose-induced ChREBPa and ChREBPb transcription, but also revealed that ChREBPa and HNF4a could function in the same transcriptional complex and promote ChREBPb transcription.

A30 Gemcitabine up regulates ABCG2/BCRP and modulates the intracellular pharmacokinetic profiles of bioluminescence in pancreatic cancer cell BxPC3. Yue Sun, Mancang Gu, Junhuan Zheng, Junying Liu. College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China.

Gemcitabine can cause severe multidrug resistance (MDR) which greatly limits chemotherapy efficacy for pancreatic cancer. Even there are many possible mechanisms for gemcitabine induced MDR, the underlying mechanism is still unknown. Several reports had associated gemcitabine induced MDR with ATP-binding cassette (ABC) transporters which may lead to drug efflux and decreased intracellular drug accumulation. However, the lack of appropriate approaches to monitor the intracellular drug changing rates and amounts realtime *in vitro* and *in vivo* limits the further explanation the mechanisms of gemcitabine induced MDR. The purpose of our study was to employ bioluminescence imaging (BLI) to determine the intracellular pharmacokinetic profiles of pancreatic cancer cell in realtime. Hence, we investigated whether gemcitabine could modulate the intracellular kinetic behavior of D-luciferin, a specific substrate of ABCG2 as well as the possible molecular mechanism.
A firefly luciferase overexpression pancreatic cancer cell line $BxPC3^{luc}$ was employed as cell model. Under the condition of luciferin substrate unsaturated, D-luciferin concentration and photon bioluminescent signal followed a linear correlation (y = 389944x + 132430, R = 0.9944) (Fig1 A) when the intracellular D-luciferin level was unsaturated. Gemcitabine has no significant effect on luciferase activity compared with DMSO treated ($P > 0.05$) (Fig1 B). The cells were treated with gemcitabine at indicated concentrations for 24h and 48h followed by determination of the D-luciferin-dependent BLI in intact cells. The photon signaling intensity of each group was normalized by protein concentration and considered as relative BLI(BLI_{rel}). To investigate the pharmacokinetic profiles of D-luciferin in pancreatic cancer cells following by different time and dosage treatment of gemcitabine, The BLI_{rel} versus time curves were plotted

(Fig2, Fig3). The pharmacokinetic parameters such as area-under-curve (AUC), elimination rate constant (*K*) and mean resident time (MRT) were calculated according to the non-compartment model (Table1). As shown in results, gemcitabine treatment could significantly increase *K* and decrease AUC and MRT in a dose-response relationship manner compared with control group. The western blot result suggested that gemcitabine could up regulate the protein level of reflux pump ABCG2 comparing with control group (Fig4). Our data indicated that the faster elimination and lower accumulation of bioluminescent signal in BxPC3^{luc} cells according to gemcitabine treatment may associate with the up regulation of multidrug resistant protein ABCG2 by gemcitabine.
On conclusion our study revealed that gemcitabine might up regulate the activity of multidrug resistant protein ABCG2, hence increase the elimination rate of ABCG2 substrate D-luciferin, decrease D-luciferin's accumulation in pancreatic cancer cell BxPC3^{luc} according to a dose-response relationship manner. On the other hand, our bioluminescent model would be a useful method to directly observe the chemoresistant in realtime in vitro.

A36 Profound apoptotic effects of triple combination of a pan antagonoist of IAPs, an inhibitor c-FLIP biosynthesis and TRAIL on most solid tumor cells. Ying Huang, Xiang Yang, Guanlin Wang and Kwen-Jen Chang. Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming Yunnan, China.

Although proliferation of most tumor cells can be inhibited by high-dose cocktail chemotherapy, the treatment causes high risks of later toxicities for normal cells. More effective strategies are needed for the cancer curing. Tumor necrosis factor-related apoptosis-inducing ligand TRAIL is a potential new agent for tumor treatment due to its anti-tumor apoptosis selectivity and its nontoxicity for normal cells. But the disadvantage is that about 85% cancer cells show resistance to TRAIL. Most TRAIL resistant cancer cells have overexpressed IAPs (inhibitors of apoptosis proteins) and c-FLIP (cellular FADD-like interleukin-1□-converting enjyme [FLICE]-inhibitory protein). These proteins block TRAIL induced apoptosis by inhibiting these activities of executive caspase-3, 6, 7 and initiating caspase-8/10, respectively, in the extrinsic apoptosis pathway. In this study, we investigate the combined apoptotic effects of AT-406 (A) a pan antagonist of IAPs, rocaglamide (R) a translational inhibitor of biosynthesis of c-FLIP and TRAIL(T)
In our studies, we used MTT cell viability assay, Annexin 5 fluorescent stain and Western Blots analysis of proteins in the TRAIL-induced extrinsic pathway of apoptosis to investigate the apoptotic effects of TRAIL (T), AT-406 (A) and Rocaglamide (R), or c-FLIP specific siRNA on 20 cancer cell lines and normal Pulp cells, either alone or in double or ART triple combination. These cancer cell lines were originated from different types of human solid tumors, including breast tumors, lung tumors, prostate tumors, cervix cancers, ovary cancers, osteosarcoma, liver cancers, colorectal tumors and glioblastoma.
Results showed that all cancer cells exhibited minor to severe degree of resistance to TRAIL alone. Neither AT-406 nor Rocaglamide alone was sufficient to overcome the resistance of cancer cells to TRAIL-induced apoptosis. Synergistic actions of ART triple combination greatly improved the apoptotic effects of TRAIL in 16 out of 20 cancer cell lines, and did not show harmful effects to the normal pulp cells. One breast cancer cell line MCF-7 and all three glioblastomas remain resistant to ART triple combination. Western Bolts analysis showed the

expression of all proteins in the extrinsic apoptosis pathways including DR4/5, FADD, procaspases8/10, 3, c-FLIP$_{L/S}$ and XIAP in all cancer cells sensitive to ART triple combination. However, in normal pulp cells and cancer cells resistant to ART triple combination, the level of procaspase 8/10 was expressed in a very low level.
In conclusion, our data suggest that TRAIL can effectively induce apoptosis of most solid tumor cancer cells in the presence of an inhibitor of c-FLIP and a pan antagonists of IAPs to block the activities of both c-FLIP and IAPs. The data also suggest that both c-FLIP and IAPs are involved in the mechanism of resistance of solid tumor cancer cells to TRAIL-induced apoptosis. Thus, ART triple combination or similar combinations may be used to treat possible 90% of peripheral solid tumors in vivo. Furthermore, additional mechanism of resistance to apoptosis in brain tumor cells and normal pulp cells may involve low level expression of procaspase 8/10.

A37 Low level expression of procaspase8/10 contributes to the high resistance to apoptosis in a breast cancer cell line, brain tumor cells, and normal pulp cells. Xiang Yang, Ying Huang, Guanlin Wang, Kwen-Jen Chang. Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming Yunnan, China.

In the accompanied presentation, we have demonstrated that most peripheral solid tumor cells are sensitive to the apoptotic effects of triple combination (ART) of AT-406 (A), Rocaglamide (R) and TRAIL (T). TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand) is a member of the family of TNF-□-like cytokines and potential as an agent for tumor treatment due to its anti-tumor apoptosis selectivity and its nontoxicity for normal cells. AT-406 is a pan antagonist of inhibitor of apoptosis proteins (IAPs). Rocaglamide a natural product isolated from Aglaia species was previously shown to be a translational inhibitor of de novo synthesis of c-FLIP (cellular FADD-like (FLICE)-inhibitory protein). We found that the above ART triple combination is very effective in inducing apoptosis for most peripheral solid tumor cells, but ineffective in brain tumor cells, a breast cancer cell line MCF-7 and normal pulp cells. In this study, we further demonstrated that in addition to the high levels of expression of c-FLIP and IAPs, the expression of procasepase8/10 is extremely low in these cells resistant to ART triple combination treatment. Up-regulation of procasepase8 sensitized these cells to the apoptotic effect to ART triple combination treatment.
In this study, we used MTT cell viability assay for the apoptosis effect and Western Blots analysis of proteins in the TRAIL-induced extrinsic pathway of apoptosis to investigate the apoptotic effects of ART triple combination. These cancer cell lines are originated from different types of solid tumors, breast tumor, brain tumor cells, and normal pulp cells.
Results showed that the breast cancer cells MCF-7, brain tumor cells and normal pulp cells all showed high resistance to the ART triple combination treatment. Western Blots protein analysis revealed the very low levels of expression of procaspase8/10 in these cells, but high levels of expression of other proteins in the extrinsic apoptosis pathways including DR4/5, FADD, c-FLIP, procaspase9 and XIAP. DuP697, a COX-2 inhibitor, was shown to up-regulate the expression of procaspase8 in these cells and sensitized these cells to the apoptotic effects of the ART triple combination treatment.
In conclusion, our data in this and the accompanied presentations suggest that the ART triple combination of TRAIL/a pan antagonist of IAPs/a c-FLIP inhibitor can effectively induce apoptosis

of most peripheral solid tumor cancer cells. There are some solid tumor cells and most brain tumor cells are still resistant to the ART triple combination treatment. Up-regulation of procaspase8 by DuP697 sensitized these cells to the ART triple combination treatment. Normal pulp cells were also resistant to the ART triple combination treatment and became sensitive after DuP697 pretreatment to up-regulate the expression of procaspase8. Thus, low level expression of procaspase8/10 contributes to the development of high resistance to apoptosis in a breast cancer cell line, many brain tumor cells and normal pulp cells.

A38 Anti-tumor effect of a novel soluble recombinant human endostatin: Administered as a single agent or in combination with chemotherapy agents in mouse tumor models.

Zhihua Ren[1], Yanan Wang[2], Wenhong Jiang[3], Wie Dai[4], Yongping Jiang[1]. [1]Biopharmaceutical R&D Center, Chinese Academy of Medical Sciences & Peking Union Medical College, Suzhou, China, [2]Department of Laboratory Diagnosis, Suzhou Municipal Hospital Affiliated Nanjing Medical University, Suzhou, China, [3]Biopharmagen Corp., Suzhou, China, [4]Department of Environmental Medicine, New York University Langone Medical Center, NY, USA.

Background: Angiogenesis has become an attractive target in cancer treatment. Endostatin is one of the potent anti-angiogenesis agents. Its recombinant form expressed in the yeast system is currently under clinical trials. Endostatin suppresses tumor formation through the inhibition of blood vessel growth. It is anticipated that combined therapy using endostatin and cytotoxic compounds may exert a additive effect. In the present study, we expressed and purified recombinat human endostatin (rhEndostatin) that contained 3 additional amimno acid residues (arginine, glycine, and serine) at the amino-terminus and 6 histidine residues in its carboxyl terminus. The recombinant protein was expressed in *E. Coli* and refolded into a soluble form in a large scale purification process. The protein exhibited a potent anti-tumor activity in bioassays. Furthermore, rhEndostatin showed an additive effect with chemotherapy agents including cyclophosphamide (CTX) and cisplatin (DDP).
Methods: rhEndostatin cDNA was cloned into PQE vector and expressed in *E. Coli*. The protein was refolded through dialysis with an optimized protocol. To establish tumor models, nude mice were subcutaneously injected with human cancer cells (lung carcinoma A549, hepatocellular carcinoma QGY-7703, or breast cancer Bcap37). rhEndostatin and/or DDP was administered peritumorally to evaluate the rate of growth inhibition of A549 tumors. For the tumor metastasis model, mice were injected intravenously with mouse melanoma B16 cells. One day after tumor cell injection, a single dose of rhEndostatin, or in combination with CTX, was administered intravenously or at a site close to the tumor.
Results: rhEndostatin reduced the growth of A549, QGY-7703, and Bcap37 xenograft tumors in a dose dependent manner. When it was administered peritumorally, rhEndostatin exhibited a more potent inhibitory activity. Furthermore, rhEndostatin displayed an additive effect with CTX or DDP on the inhibition of metastasis of B16 tumors or growth of A549 tumors.
Conclusion: Soluble rhEndostatin exibibits a potent anti-tumor activity in mouse xenograft models and it also has an additive effect with CTX and DDP, implying possible applications in clinical settings.

A39 Lineage programming by MITF modulates EGFR signaling and therapeutic response. Zhenyu Ji, Hensin Tsao, Ching Ni Njauw, Michael Taylor, Keith Flaherty, Raj Kumar, Erin Chen. Massachusetts General Hospital, Harvard Medical School, Boston, USA.

Despite of common oncogenic mutations shared by human cancers, drug sensitivity varies depending upon tissue origin. Here we identified MITF, a master regulator of melanocytes lineage, as the determining factor that renders melanoma unique sensitivity to BRAF/MEK inhibitors. Acquired BRAF inhibitor (BRAFi) resistance after treatment in Melanoma shows a loss of MITF expression. Knockdown of MITF expression in melanoma cells contributes to resistance to BRAF/MEK inhibitor. On the contrary, Induced MITF expression in colon cancer or immortalized melanocytes with oncogenic BRAF(V600E) confers increased sensitivity to BRAF/MEK inhibitors. The sensitizing effect of MITF is attributed to its regulation of EGFR signaling components, including receptor and ligands. In addition, we demonstrated that ligand is required for BRAF/MEK inhibitor resistance even in the presence of EGFR overexpression or hyperphosphorylation.

A40 Functional and mutational landscape of BRCA1 for homology-directed repair and targeted therapy. Rachel Anantha[1], Srilatha Simhadri[1], Tzeh Keong Foo[1], Susanna Miao[2], Bing Xia[1]. [1]Department of Radiation Oncology, Rutgers Cancer Institute of New Jersey and Robert Wood Johnson Medical School, Rutgers, The State University of New Jersey, New Brunswick, NJ, [2]Department of Genetics, School of Arts and Sciences, Rutgers, The State University of New Jersey, Piscataway, NJ.

Inherited mutations in *BRCA1* predispose to breast cancer with high penetrance. BRCA1 plays a critical role in the homology recombination (HR)-mediated repair of DNA double strand breaks (DSBs), and the repair defect of *BRCA1*-mutant cells is being targeted with poly (ADP-ribose) polymerase inhibitors (PARPi) and platinum drugs for cancer treatment. While truncating mutations in BRCA1 are clearly associated with cancer risk and drug sensitivity, the functional and clinical relevance of the majority of missense variants remain unclear, which continue to pose a challenge for genetic counselling and treatment choice. We have developed relatively simple and sensitive assays to determine the DSB repair function and drug resistance of BRCA1 missense variants in human cancer cells. By functional profiling a panel of over 30 missense variants and mutations, we find that cellular HR function and cisplatin/PARPi resistance both require 3 highly conserved functional domains of BRCA1, the RING, coiled-coil and BRCT domains, which binds its key partners BARD1, PALB2 and CtIP/BRIP1/Abraxas, respectively. Certain variants of unknown significance (UVSs) outside of the above 3 domains also have moderate impacts on HR and drug sensitivity. Moreover, loss of ATM/ATR phosphorylation of BRCA1 moderately affects drug resistance without significant impact on the rate of HR or single strand annealing (SSA). Interestingly, loss of PALB2 binding to BRCA1 at the coiled-coil domain, while diminishing HR, substantially upregulates SSA, indicating that PALB2 controls DSB repair pathway choice downstream of BRCA1. These findings establish a functional and mutational landscape of BRCA1 for homology-directed DSB repair and drug resistance.

A41 Restoring expression of microRNA-7 by targeted bacterial minicells inhibits the

MAPK/ERK pathway and offers a novel therapy for adrenocortical cancer. A.R. Glover, J.T. Zhao, J. Weiss, N. Vanegas, J.C. Ip, G. Reid, B.G. Robinson, P.S.H. Soon, H. Brahmbhatt, J.A. MacDiarmid, S.B. Sidhu. Kolling Institute of Medical Research, University of Sydney, Royal North Shore Hospital and EnGeneIC Ltd, Sydney, Australia.

Background: Metastatic adrenocortical cancer (ACC) has a poor prognosis with limited treatment options. Pilot studies from our lab have identified a number of micro-RNAs, including microRNA-7 (miR-7) that are significantly under-expressed in ACC versus normal adrenal cortex, implying the involvement of microRNAs in the pathogenesis of ACC and its potential as a therapeutic target.
Aims: To establish the role and mechanisms of miR-7 as a tumour suppressor in ACC and to assess the role of miR-7 replacement therapy, delivered via bacterial minicells as a novel treatment.
Methods: miR-7 replacement was performed in ACC cell lines (H295R, SW-13) in 2D and 3D cell culture systems. All experiments were performed in triplicate and compared to a negative control scrambled microRNA (Ambion). A mouse xenograft model was established using the H295R cell line and primary cell cultures and systemic microRNA replacement therapy was administered in bacterially derived EGFR targeted EnGeneIC delivery vehicles (EDVs)/minicells.
Results: The role of miR-7 as a tumour suppressor in vitro was confirmed after replacement lead to a 63.2% reduction in cell proliferation and 27.9% reduction in cell migration ($P<0.05$). Cell cycle analysis showed this to be due to a 15.7% increase in G1 arrest ($P<0.05$) with no difference in the rates of apoptosis. miR-7 predicated targets, EGFR and C-Raf were found, following replacement to have reduced mRNA expression of 50% and 49% ($P<0.05$) . EGFR and C-Raf protein expression was also significantly reduced ($P<0.05$). Luciferase reporting assays confirmed EGFR and C-Raf seed binding sequences as miR-7 targets with a reduced activity of 46.2% for EGFR reporting activity and 38.9% for C-Raf reporting activity ($P<0.05$). miR-7 replacement therapy in a mouse xenograft model caused a 55.5% reduction in tumour growth compared to control treated mice with no evidence of toxicity and reduced mRNA expression of C-Raf, mTOR and CDK1 ($P<0.05$).
Conclusions: miR-7 acts a tumour suppressor in ACC by inhibiting the MAPK/ERK pathway and replacement therapy when delivered via targeted minicells offers a potential major advance in the treatment of this deadly malignancy.

A42 Synergistic antitumor interactions between MK-1775 and Panobinostat in preclinical models of pancreatic cancer. Guan Wang[1, 2*], Xiaojia Niu[1], Wenbo Zhang[1], J. Timothy Caldwell[3, 4], Holly Edwards[5, 6], Jeffrey W. Taub[2, 6, 7], Lijing Zhao[8], and Yubin Ge[1,5,6]. [1]National Engineering Laboratory for AIDS Vaccine, Key Laboratory for Molecular Enzymology and Engineering, the Ministry of Education, School of Life Sciences, Jilin University, Changchun, China, [2]Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI, USA, [3]MD/PhD Program, Wayne State University School of Medicine, Detroit, MI, USA, [4]Cancer Biology Program, Wayne State University School of Medicine, Detroit, MI, USA, [5]Department of Oncology, Wayne State University School of Medicine, Detroit, MI, USA, and [6]Molecular Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA, [7]Division of Pediatric Hematology/Oncology, Children's Hospital of Michigan, Detroit, MI, USA,

[8]Department of Pathophysiology, College of Basic Medical Sciences, Jilin University, Changchun, China.

Pancreatic cancer is the fourth most lethal cancer in the United States with a 5-year survival rate of 6%. Standard chemotherapeutic care involves treatment with gemcitabine, a nucleoside analogue, but offers only modest benefit. Furthermore, combinations of other cytotoxic agents with gemcitabine have, in general, offered little improvement. Thus, there is an obvious need to develop more effective options for the treatment of this disease.

MK-1775, the first potent and selective inhibitor of Wee1, has been investigated primarily as an agent that can target the G2/M checkpoint to exert toxicity specifically in p53-mutant cells. It has been shown that, when combined with DNA damaging agents, MK-1775 is able to abrogate the G2/M checkpoint and enhance apoptosis, although recent studies have questioned the p53-dependence of these effects. Importantly, it has been shown that Wee1 inhibition alone can induce DNA damage, likely through the induction of replication stress secondary to overactive CDKs and inhibition of DNA repair. MK-1775 has been demonstrated to synergize with CHK1 inhibitors in various malignancies and we have previously demonstrated that the pan-histone deacetylase inhibitor panobinostat can down-regulate CHK1. Accordingly, we hypothesize that panobinostat will down-regulate CHK1 and synergize with MK-1775 to enhance cell death in preclinical pancreatic cancer models.

In this study, we used pre-clinical pancreatic cancer models to investigate the effects of the combination of MK-1775 and panobinostat, and the mechanism by which panobinostat enhances MK-1775 induced cell death. MK-1775 treatment resulted in increased phosphorylation of H2AX and CHK1 as determined by Western blots, indicating that MK-1775 treatment causes DNA damage and activation of the CHK1 pathway, as well as S and/or G2/M cell cycle arrest, as determined by propidium iodide staining and flow cytometry analysis. Roscovitine, a pan-CDK inhibitor, antagonized MK-1775 growth inhibition in pancreatic cancer cells. In addition, roscovitine attenuated induction of apoptosis as well as H2AX and CHK1 phosphorylation, demonstrating the requirement of CDK activity for the antitumor activity of MK-1775 in pancreatic cancer cells. LY2603618, a CHK1 selective inhibitor, combined with MK-1775 resulted in synergistic antitumor activity in pancreatic cancer cells, confirming published reports. Panobinostat treatment caused down-regulation of the CHK1 pathway in pancreatic cancer cells, as indicated by down-regulation of p-CHK1 and p-CDC25C following panobinostat treatment. Panobinostat synergized with MK-1775 in all six of the cell lines tested, as measured by MTT assays, and enhanced MK-1775-induced apoptosis in the BcPC-3 cells. Finally, we demonstrate that the combined drug treatment results in significant growth delay of externally measurable tumor growth in a BxPC-3 xenograft mouse model.

Our study provides evidence that the combination of MK-1775 and panobinostat in pancreatic cancer cell lines and a xenograft mouse model has synergistic/cooperative antitumor activity. Panobinostat treatment down-regulates the CHK1 pathway, allowing MK-1775-induced DNA damage to accumulate and subsequently results in apoptosis in pancreatic cancer cells. Importantly, we demonstrate that, in some cell lines, the CHK1 pathway is able to overcome single agent Wee1 inhibition and maintain phosphorylation of CDK1. This demonstrates a potential mechanism of resistance to treatment with MK-1775 and emphasizes the importance

of combinations with agents such as panobinostat. Our results support the clinical development of MK-1775 and panobinostat combination for the treatment of pancreatic cancer.

A43 A novel BH3 mimetic efficiently induces apoptosis in melanoma cells through direct binding to anti-apoptotic Bcl-2 family proteins, including phosphorylated Mcl-1.
Ting Song [1], Yubo Liu [1, 2], Gaobo Chai [2], Xiaoyan Yu [2], Zhichao Zhang[1]. [1]State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, Dalian, People's Republic of China, [2] School of Life Science and Technology, Dalian University of Technology, Dalian, People's Republic of China.

Background: The Bcl-2 family modulates sensitivity to chemotherapy in many cancers, including melanoma, in which the RAS/BRAF/MEK/ERK pathway is constitutively activated. Mcl-1, a major anti-apoptotic protein in the Bcl-2 family, is extensively expressed in melanoma and contributes to melanoma's well-documented chemoresistance. Moreover, Mcl-1 can be phosphorylated at multiple sites that distinctly regulate Mcl-1 protein turnover and interactions with pro-apoptotic proteins. Because ERK1/2, which is a physiologic kinase that acts on the T163 site in Mcl-1, is activated in melanoma, Mcl-1 phosphorylation most likely plays a key role in the regulation of melanoma cell apoptosis.
Methods: Western bolt was used to evaluate the contribution of Bcl-2 family members to the cellular response of 3 melanoma cell lines to the well-established Bcl-2 inhibitors Gossypol, S1, and ABT-737. Viable cells were determined using Flow cytometry. MEK/ERK inhibitor PD98059 and JNK inhibitor SP600125 were applied to find out the kinases that act on Mcl-1. Gene silencing, PD98059 and stable transfection were used to confirm the link between Mcl-1 phosphorylation (T163) and resistance to BH3 mimetic drugs. Isothermal titration calorimetry was used to characterize the direct binding of Compound 6 to phosphorylated Mcl-1 (pMcl-1).
Results: Here, we provide the first evidence that Mcl-1 phosphorylation at T163 by ERK1/2 and JNK is associated with the resistance of melanoma cell lines to the existing BH3 mimetics gossypol, S1 and ABT-737, and a novel anti-apoptotic mechanism of phosphorylated Mcl-1 (pMcl-1) is revealed. pMcl-1 antagonized the known BH3 mimetics by sequestering pro-apoptotic proteins that were released from Bcl-2/Mcl-1. Furthermore, an anthraquinone BH3 mimetic, Compound 6, was identified to be the first small molecule to that induce endogenous apoptosis in melanoma cells by directly binding Bcl-2, Mcl-1 and pMcl-1 and disrupting the heterodimers of these proteins. Although compound 6 induced up-regulation of the pro-apoptotic protein Noxa, its induction was independent of Noxa.
Conclusion: These data confidently conclude that pMcl-1 is a therapeutic target for melanoma and facilitates resistance to known BH3 mimetics. Compound 6 therefore provides a unique tool to probe the structure and biological function of pMcl-1 at its T163 site.

A44 Induce antitumor immune responses by using codon optimized GM-CSF fused with HPV16-E7 as DNA base vaccine in TC-1 tumor model. Chu-Chi Lin, Chia-Yuan Chen, Yu-Sing Chen, Ta-Hsien Lee, Jiantai Timothy Qiu. Graduate institute of biomedical science, College of medicine, Chang-Gung University, Taoyuan, Taiwan, ROC.

The cervical cancer is caused by persistent infection in the human body via several high-risk type of human papilloma virus. In recent years, despite the preventive vaccine against HPV has been approved to come out and get a good effectiveness but in clinical still need therapeutic vaccine against persistent infection in women who have been infected with high risk HPV types. In order to enhance the antigen presenting ability of HPV vaccine produced, we modify codon usage of granulocyte-macrophages stimulating factor (GM-CSF) of mice but without changing amino acid sequence. As a consequent of modifying codon, it enhanced the expression amount of the protein. Granulocyte macrophage-colony stimulating factor (GM-CSF) is a potent immunomodulatory cytokine known to facilitate vaccine efficacy by promoting development and prolongation of both humoral and cellular immunity. Here we investigated a new approach by fusing codon optimized murine GM-CSF (cGM-CSF) with HPV-16 E7 as a novel DNA vaccine. Ectopic expression of cGM-CSF in different cell lines were significantly increased expression of functionally identical GM-CSF when compared expressing wild type GM-CSF. Consistently, vaccination with cGM-CSF-E7 induced stronger IFN-gamma production of HPV E7-specific $CD8^+$ T cells. Vaccinating mice with cGM-CSF-E7 DNA vaccine promoted better survival of recipients against lethal TC-1 challenge than vaccination with wild type GM-CSF-E7 did. Immunohistochemistry staining of paraffin-embedded tumor section from B6 mice with different DNA vaccination showed that more CD4 lymphocyte cells were infiltrated at cGM-CSF-E7 vaccinated group compared to the other immunized group. These results indicate that using of novel codon optimized GM-CSF may prove a practical molecular strategy for overcoming the limitations of DNA based vaccines.

A46 SKLB703, a novel active inhibitor with potent induce autophagy and antitumor efficacy in LL/2 Lewis lung cancer cells. You-Zhi Xu[1,2], Wen-jie Lu[2], Sheng-yong Yang[2], Ying-Lan Zhao[2].
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Autophagy was a response of cancer cells to anticancer therapies, inducing autophagy is a promising therapeutic approach to overcome cancer. We previously exhibited that SKLB703 (3-amino-N-(4-chlorobenzyl)-6-(3-methoxyphenyl)thieno[2,3-b]pyridine-2-carboxamide) inhibits the growth of several cancers such as inhibiting the growth of liver cancer both *in vitro* and *in vivo* and induced apoptosis with caspases-dependent manner after treated by SKLB703. Here we demonstated that SKLB703 induced autophagy and blocked the tumor growth. The specific mechanism of action of SKLB703 was investigated in a Lewis lung carcinoma cell line (LL/2). Our results shown that SKLB703-induced autophagy was followed with apoptotic cell death. With anticancer efficacy stimulated autophagy in LL/2 cells and after treated by SKLB703 for 24h, SKLB703 which was evidenced by the appearance of autophagic vacuoles, formation of acidic vesicular organelles (AVO), recruitment of autophagy-associated protein II chain 3 (LC3-II) to the autophagosomes and conversion and cleavage of LC3-I to LC3-II. Furthermore, SKLB703 up-regulated expression of Beclin 1 and promoted formation of the Atg12-Atg5 conjugate; meanwhile, p53 activation and the p-Histone H3 phosphorylation occurred after cell exposure to SKLB703 for 48h, suggesting that cell apoptosis had occurred simultaneously. Pharmacological inhibition of autophagy with 3-methyladenine (3-MA) increased cell apoptosis, suggesting that

SKLB703-induced autophagy played an enhanced role in tumor cell apoptosis. The Additional molecular level studies revealed that SKLB703 inhibited survival signaling by blocking activation of phosphorylation of Akt (p-Akt) and mTOR, also reduced p-mTOR downstream targets phosphorylation of p70 ribosomal S6 kinase (p-p70S6K), p-TSC, p-MAPK and p-AMPK, suggesting that the Akt/mTOR/p70S6K and the TSC/MAPK/AMPK, two major pathways regulate autophagy induced by nutrient starvation in many cancer cell, was involved by SKLB703 treatment in LL2 cells. In addition, SKLB703 inhibited LL/2 tumor growth significantly and induced apoptosis *in vivo*. Taken together, our results suggested that autophagy was a pathway-specific mechanism of SKLB703, and SKLB703 has high anticancer efficacy both *in vitro* and *in vivo*, and warrant further investigation toward possible clinical application in patients.

A47 A novel proapoptotic peptide ligand of cell-surface GRP78 potently suppresses tumor growth in mice. Mo Chen, Shruthi Venugopal and Ruowen Ge. Department of Biological Sciences, National University of Singapore, Singapore.

Isthmin (ISM) is a novel 60 kDa extracellular proapoptotic ligand of the cell-surface glucose regulated protein 78 kDa (GRP78). GRP78 is an endoplasmic reticulum stress responsive chaperone protein whose overexpression widely correlates with cancer aggressiveness and chemoresistance. Overexpression of GRP78 leads to its translocation to the cell-surface where it serves as a receptor for multiple extracellular ligands. Systemic administration of recombinant ISM not only suppressed subcutaneous melanoma and breast cancer growth in mice, but also potently suppressed metastasis of these cancers.
In this work, we report the further development of a high-affinity cyclic peptide ligand of cell-surface GRP78 based on ISM protein. This peptide (ISM-Pep1) selectively induces apoptosis of cultured tumor cells and endothelial cells that harbor high amount of cell-surface GRP78. When systemically administered, ISM-Pep1 preferentially targets GRP78 in tumor and potently suppressed subcutaneous melanoma and breast cancer growth in mice through apoptosis induction. In contrast, ISM-Pep1 does not target cells in normal tissues and has no observable effect on mice in general. Thus, ISM-Pep1 has the potential to be developed into a GRP78 targeted anticancer therapeutics.

A49 δ-Tocotrienol induces bladder cancer cell growth arrest, apoptosis and enhances chemosensitization to Gemcitabine through inhibition of STAT3 pathway. Yangyan Cui[1], Changxiao Ye[1, 2, 3], Wei Zhao[1], Minghui Li[1, 2, 3], Junlong Zhuang[1, 2, 3], Hongqian Guo[2, 3], Jun Yan[1]. [1]Model Animal Research Center, MOE Key Laboratory Model Animal for Disease Study, Nanjing University, Nanjing, Jiangsu, China, [2]Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China, [3]Nanjing Urology Research Center, Nanjing, Jiangsu, China.

Background: Vitamin E intake has been inversely related to bladder cancer risk among older individuals or heavy smokers from multiple epidemiologic studies. Both tocopherols (TP) and tocotrienols (T3) belong to the vitamin E family and each composed of four isomers: α-, β-, δ- and γ. The main difference between TP and T3 is the structure of their side chains with farnesyl for T3 and saturated phytyl for TP. Compared to TPs, which is commonly found in the leaves and seeds of most plants, T3 is less abundant and mainly found in palm oil and rice bran. Two clinical

trials on α-TP, the Women Health Study (WHS) trial and the Selenium Vitamin E and Prostate Cancer Chemoprevention Trial (SELECT), were carried out to investigate their cancer prevention properties. However, these two trials showed minimal effect of α-TP against lung, breast and colon cancer in women and prostate cancer in men. Due to their potential application as non-toxic dietary anti-cancer agents, different T3 isomers have evoked more research attention recently. Among them, δ-T3 showed strong potency against various cancers including pancreatic, colorectal and breast cancer. In the present study, we aimed to explore whether δ-T3 possesses anticancer activity against human bladder cancer cells.
Methods: Two human bladder cancer cell lines T24 and 5637 were treated with different concentration (50, 100, 150, 200 μM) of the vitamin-E isomers for 24-, 48- and 72-h, followed by MTT assay and colony formation assay for cell viability. Flow cytometry analyses by PI and Annexin V were carried to examine the change of cell cycle phase and apoptotic rate under various concentration of δ-T3. Moreover, Western blotting assay, quantitative RT-PCR, cellular fractionation, luciferase assay were exploited to analyze the change of STAT3 signal pathway and its downstream target, which is affected by δ-T3 in bladder cancer cell lines.
Results: δ-T3 inhibited proliferation and survival of cancer cells, through activation of caspase-3 and cleavage of PARP. Subsequently, δ-T3 also suppressed the expression of various proteins that mediate cell survival (Bcl-2, Bcl-xL,), induction of cell cycle inhibitors (p21, p27) and pro-apoptotic protein (Bax). Notably, δ-T3 inhibited ETK activation, induced SHP-1 expression and suppressed their downstream STAT3 signaling pathways. Lastly, we found that δ-T3 as low as 50 μM sensitized Gemcitabine-induced cytotoxic effects, in part, through suppression of STAT3 signaling pathway.
Conclusion: We demonstrated that δ-T3 is a potent agent against human bladder cancer cells. Mechanistically, through inhibiting ETK activation and inducing SHP-1 expression, δ-T3 suppresses STAT3 pathways. Finally, since it enhances Gemcitabine-induced cancer cell apoptosis, δ-T3 could be explored as a chemo-sensitizer in bladder cancer therapy. Overall, these observations suggest that δ-T3 can interfere with multiple signaling cascades involved in tumorigenesis and may be a promising therapeutic candidate for bladder cancer prevention and treatment.

A50 Targeted therapies in PDAC using genetically engineered mouse models of endogenous PDAC. A. Gupta[1], E. Kaliders[1], Rickmer Braren[2], R. M. Schmid[1], J. T. Siveke[1].
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Development of strategies for early tumor detection and evaluation of new therapies in preclinical models is one of the main goals of current cancer research. Pancreatic Ductal Adenocarcinoma (PDAC) is a lethal disease, with no effective treatment plan. Single agent gemcitabine and gemcitabine/erlotinib have long been the only approved therapeutic regimens, however their success was modest at best. Over the past decade much work has been done in developing effective targeted therapies for targeted intervention.
In this project we targeted various different signalling pathways known to play a significant role in PDAC progression alone and simultaneously in our endogenous PDAC mouse model in order to find the right combination for this exceedingly heterogeneous cancer. On one hand we

focused on the EGFR and the MAPK axis to inhibit cell proliferation and on the other we tried to modulate the PDAC stroma with the help of a JAK/STAT inhibitor.
Using a *Cre/loxP* approach, we generated a GEMM with pancreas specific activation of oncogenic Kras and concomitant deletion of p53 (*Ptf1a*$^{+/Cre}$, *Kras*$^{+/LSL-G12D}$, *p53*$^{loxP/loxP}$; *CKP*). These animals showed a 100% tumor incidence, detected with MRI and verified by histology. With such an endogenous GEMM for PDAC the disease progression can be very well mimicked, representing an excellent platform for preclinical studies. These mice are typically predisposed to develop lethal PDAC within 8 weeks of age. Combined with various imaging modalities this model provides us with a unique opportunity to assess the *in vivo* efficacy of various targeted inhibitors coupled with chemotherapeutic agents such as gemcitabine and Capecitabine. Tumor progression was tracked weekly by tumor volume measurements via non-invasive T2-weighted magnetic resonance imaging (MRI) on a clinical 3T MRI device.
Targeting the EGFR axis we saw a significant benefit in survival of *CKP* mice with a median survival advantage of 30 days after start of treatment. Furthermore, a dramatic decrease of the tumor load was observed already after 2 week of treatment. Similar results were seen when we co-targeted the MAPK axis using a MEK inhibitor along with a JAK/STAT inhibitor. Although no significant benefit was observed when both the EGFR and the MAPK axis's were targeted simultaneously.
In summary, besides numerous disappointments with targeted therapies in PDAC, these compounds bare the potential for a better treatment for pancreatic cancer. Although the molecular pathways in PDAC are well known but our preclinical findings demonstrate that much work needs to be done in the pre-clinical phase for the various targeted combinations and that we still need better-targeted and more individualized therapies for pancreatic cancer.

A51 A Sapogenin from *Gynostemma pentaphyllum* reverse the multidrug resistance via inhibiting ABCB1 activity. Xu Wang[1,2], Yan Ming[1,2], Qin Xu[1,2], Jianjun Zhang[1,2], Zhang Ping[1,2], Lihong Hu[*,3], and Wantao Chen[*1,2]. [1]Department of Oral and Maxillofacial-Head Neck Oncology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China, [2]Shanghai Research Institute of Stomatology, Shanghai, PR China, [3]Department of Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, PR China.

Multi-drug resistance (MDR) of cancer is a major obstacle of physicians after their patients have received the chemotherapy. ATP-binding cassette transporter B1 (ABCB1) was reported to induce MDR by pumping chemotherapeutic agents outside the cells in an ATP-dependent way. Developing ABCB1 inhibitors is a serious requirement for the drug discovery and clinical application. Our ongoing researches focus on discovering chemosensitizers from meidicinal plants. In this paper, we report that SJ, a sapogenin from the Gynostemma pentaphyllum, could enhance the cytotoxic effect of two anti-cancer drugs, DOC and ADM. The SJ single treatment has had no significant toxic effects on any of the cell lines used in this study. In addition, SJ significantly stimulated the ATPase activity of ABCB1 and inhibited the intracellular accumulation of ABCB1 substrates. Furthermore, Western blot analysis indicated the incubation of cells with SJ did not alter ABCB1 mRNA and protein expression. Molecular docking has revealed that SJ showed the higher docking score than Verapamil. Overall, our results suggest

that SJ reverses ABCB1-mediated MDR by directly blocking the drug efflux function of ABCB1. These findings may be used to guide the design of present and future clinical trials with SJ, elucidating potential pharmacokinetic interactions.
Keywords: ABCB1, multi-drug resistance, carcinoma, natural product.

A52 Targeting OncoR1, a GPCR for Hippo-YAP regulation and anti-cancer therapy. Zhi-Liang Chu. Arena Pharmaceuticals, Inc. San Diego, CA.

The Hippo-YAP pathway is critically involved in organ size control. Hippo functions as a tumor suppressor, a multi-subunit kinase complex that inhibits oncogene YAP via phosphorylation. YAP is a transcription co-activator. Together with TEAD, the unphosphorylated form of YAP drives the expression of genes important for cell proliferation, migration, and anti-apoptosis. The Hippo-YAP pathway is tightly regulated in normal cellular context, which is pivotal for maintaining tissue cellular homeostasis. Loss of function in Hippo or gain of function in YAP leads to cellular proliferation, transformation, and tumorigenesis. Recently, G-protein coupled receptors (GPCR), particularly those coupled to G12/13, were identified as major regulators of the Hippo-YAP pathway, thus offering a unique opportunity to develop small molecule GPCR modulators as novel targeted therapy for cancer. Here we showed the validation of one such GPCR, termed OncoR1, and our initial effort to develop small molecule OncoR1 inhibitors.
qPCR analysis revealed that OncoR1 expression is significantly elevated in tumors relative to adjacent non-involved normal tissues in multiple tumor types, including colorectal and pancreatic adenocarcinomas. Using a G13-Gs chimera and cAMP assay, we found that OncoR1 is strongly coupled to G12/13 protein. Importantly, OncoR1 overexpression in HEK293 cells activates YAP and increases TEAD-directed luciferase reporter activity in an OncoR1 DNA input-dependent manner. To search for OncoR1 inhibitors, we screened a GPCR-focused small molecule library, and identified inverse agonist hits. Efforts in chemistry SAR around the screening hits led to the generation of tool compounds with high potency of inverse agonism, and selectivity on OncoR1 in vitro. Importantly, the tool compounds robustly inhibited OncoR1 mediated G-protein signaling, YAP activation, and TEAD reporter activity. We are currently evaluating the tool compounds in OncoR1-expressing tumor xenografts in mice for their YAP and tumor growth inhibition activities. Taken together, our efforts in OncoR1 should provide important insights in validating GPCR modulators for YAP oncogene inhibition in tumor cells, and their potential use as novel targeted anti-cancer agents.

A53 Cellular interfacial engineering for single cell modification and manipulation. Ben Wang[1, 2, *]. [1]Key Laboratory of Cancer Prevention and Intervention of Ministry of Education, The second affiliated hospital of Zhejiang University School of Medicine, Hangzhou, China, [2]Institute for Translational Medicine of Zhejiang University, Hangzhou, China.

Introduction: Cellular interfacial engineering here as a avenue of research covers two points, Firstly, it is with the purpose of exploring the molecular structure of cell from a biological standpoint and secondly it aims at discovering the characterizations and prosperities of

materials from the perspective of material science, such as surface topography and structure of ECM, particles size and shape, and so on.
Methods: Biomimetic mineralization and surface chemistry are developed. SEM, TEM and AFM are used and cell viabilities are also evaluated in the study.
Results: We develop an approach for cellular shellization, which provide a strategy to prolong the lifetime of cells, and also a scaffold for chemical or biological modification of cells. Furthermore, cell-materials interfaces are considered together for cell capture, such as circulating tumour cells sorting [1].
Conclusions: Cellular interfacial engineering give the prospective for single cell manipulation, which could find their applications in translational medicine, such as cell sorting for cancer diagnostics, cell delivery or cell-mimetic particles for therapies of invasion diseases.
Acknowledgement: This study is supported by the Fundamental Research Funds for the Central Universities, National Science Foundations of China (No. 91127003 and 81072231) and the Chinese Postdoctoral Science Foundations (No. 2011M500136 and 2013T60600).

A54 Histone H3K4me2/3 reader Pygo2 is the therapeutic target in beta-catenin-driven cancer. Bin Xiang, Jiao Yue, Jing Jiang, Yanyan Yu, Wei Yi, Shaolian Zhou, Hailong Zhang, Yun Zhang, En Li, Peter Atadja. China Novartis Institutes for BioMedical Research, Pudong, Shanghai, China.

Nuclear beta-catenin functions as co-activator for transcription factor and regulates gene transcription. Elevated nuclear beta-catenin activity appears to be a driver signal in many types of cancer. The genetic studies and proof-of-concept experiments point to beta-catenin as a prime target for cancer therapy. In the past decades, there have been many efforts on targeting beta-catenin nuclear activity including inhibiting its upstream signaling components and its interaction with transcription factor. However, targeting the upstream signal components show limited effect in beta-catenin-driven cancer and targeting the interaction between beta-catenin and transcription factor is less feasible due to the large interface, which highlight the need of another therapeutic approach to target nuclear beta-catenin transcription activity in cancer. Accumulating evidences suggested that the histone reader Pygo2 plays a crucial role in the formation of beta-catenin transcription regulatory complex at target gene promoter. Pygo2 is PHD-containing protein and recognize di- and tri-methylated lysine 4 of histone H3 (H3K4me2/3), a chromatin mark associated with active transcription. Pygo2 and its associate B9L are required for efficient beta-catenin/TCF target gene transcription in Wnt-dependent tissue and human cancer cells.
In this study, we evaluated Pygo2's role in controlling cell proliferation in beta-catenin-driven cancer. Our data suggested that Pygo2 regulates cancer cell growth in both cancer cell lines and patient-derived xenograft mouse models in beta-catenin-dependent manner. Nuclear beta-catenin level serves as prediction marker but not the genetic mutation of beta-catenin and other signal component in Wnt signal pathway. Furthermore, both Pygo2 and beta-catenin regulate cell cycle progression in a similar manner in the sensitive cell lines. The mechanism study indicated that Pygo2 forms complex with beta-catenin and controls beta-catenin genome-wide localization. Taken together, our study indicates that Pygo2 is a therapeutic target in beta-catenin-driven cancer.

A55 Dopamine inhibits cancer stem cells growth and enhances the response of sunitinib in preclinical treatment of drug-resistant breast cancer. Siyuan Wang, Zhenzhen Mou, Jian Li, Xiwei Ji, Yuanheng Ma, Wei Lu, Tianyan Zhou*. Department of Pharmaceutics, School of Pharmaceutical sciences, Peking University, Beijing, China.

Background: Increasing evidence suggests that chemotherapy resistance may be explained by the theory of cancer stem cells (CSCs). One of the major concerns in developing anti-CSCs therapies was how to avoid the indiscriminate cytotoxicity that often affects human pluripotent stem cells (hPSCs). In 2012, dopamine receptor (DR) was identified as a promising biomarker which may distinguish CSCs from hPSCs. This study focused on determining whether dopamine, an agonist of DR, possessed anti-CSCs properties, investigating the underlying mechanism, and further evaluating the improvement in the efficacy of sunitinib with concomitant of dopamine in preclinical studies of drug-resisitant breast cancer.
Methods and Findings: MCF-7/Adr cells and breast cancer stem cells (BCSCs) were used for *in vitro* evaluation, as well as for the development of the xenograft animal model. The flow cytometry results demonstrated that dopamine significantly down-regulated the expression of CSCs markers (CD44/CD24) in both MCF-7/Adr cells and BCSCs, remarkably induced the apoptosis of BCSCs, and SRB assay indicated that dopamine greatly synergized the anti-cancer effect of sunitinib *in vitro*. Although dopamine alone hardly changed the growth rate of tumor, it inhibited the Wnt signaling pathway and activated the apoptotic associated signals in the tumor cytosol of MCF-7/Adr xenograft. Compared with sunitinib alone, when used in combination, dopamine strikingly increased the inhibitory ratio in both of the xenograft models, and the specific antagonist of D1DR (D1 dopamine receptor), SCH23390, completely reversed the combined effects. In addition, the results of flow cytometry and confocal immunofluorescence showed that dopamine increased the expression of D1DR both *in vitro* and *in vivo*, respectively, and dopamine augmented the cAMP levels in MCF-7/Adr cells in a dose-dependent manner.
Conclusions: In essence, dopamine was identified as an anti-CSCs agent in the treatment of drug-resistant breast cancer, and D1DR was responsible for the enhanced efficacy. More importantly, when used in combination with a VEGFR-TKI (Vascular Endothelial Growth Factor Receptor- Tyrosine Kinase Inhibitor), dopamine was able to enhance chemotherapeutic response, overcome drug resistance, as well as prolong progression-free survival. This promising combination therapy is likely to fulfill significantly improved response in the clinical treatment of breast cancer.

A56 Induction of resistances to the targeted therapies in patient derived xenograft models. Henry Q.X. Li[1,2*], Mengmeng Yang[1], Andrew McKenzie[1], Jiangyun Deng, Jie Cai[1], Sheng Guo[1], Martin Page, Rajendra.kumari[1], Jean Pierre Wery[1]. [1]Crown Bioscience, Inc., Santa Clara, CA, [2]State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

Oncogene activating mutations have been found to be oncogenic drivers in many different cancers, such as EGFR L858R mutation, EML4-ALK fusion (1, 2), CCDC6-RET or NCOA4-RET fusions (3), RSPO2,3 fusions, and c-met gene amplifications (4) in NSCLC (2, 5), CRC, gastric adenocarcinoma, etc. These oncogenes have also been demonstrated to be excellent drug

targets for a number of approved target therapies. However, like any other cancer therapy so far, the treatments always led to the development of resistances to these therapies, rendering them ineffective in the end. Understanding the mechanisms causing these resistances can potentially facilitate overcoming them.

We have recently established large collection of patient derived xenografts (PDXs), including NSCLC (6), CRC, and many other diseases. Among them is NSCLC-ADC LU6422 containing EGFR-L858R mutation, NSCLC-ADC LU1656 containing EML4-ALK fusion, NSCLC-LCC LU1901 containing c-met gene amplification, CRC CR1520 containing NCOA4-RET fusion and CRC CR2518 containing CCDC6-RET fusion. They all responded well to the corresponding targeting agents under treatments: LU6422 to Tarceva, LU1656 to crizotinib, LU1901 to crizotinib or other c-met inhibitors, CR1520 and CR2518 to Ponatinib as expected. However, these treatments eventually all led to resistances as seen in the clinic. We are interested in investigating the mechanisms of these induced resistances since it may reflect the resistances that might occur in patients in the clinic under the same treatments. We are currently profiling these resistant models and investigating the pharmacodynamics effects, and comparing them to those of their parental. The findings from these analyses will be presented at the meeting.

A57 Developing a gene signature for NSCLC patient stratification for cisplatin treatment.

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NSCLC has high incidence and also leading mortality among all malignances in different demographic populations. Chemotherapy is the main treatment option, among which cisplatin is one of the first line treatments per current guidance. All chemotherapy has undesirable side effect, and not all patients respond to these treatments. This causes unnecessary suffers for the unlikely responders, both physically and economically, and also might delay/prevent them from receiving other effective treatments. However, there is no scientific knowledge and medical guidance to select suitable patients for cisplatin treatment.

Patient derived xenografts (PDXs, HuPrime®) mimic patient tumors from which they are derived, and are believed to respond to therapy predicative of how patient would. We have recently established a large collection of NSCLC-PDX (1) and many of them have been extensively genomic-profiled, including expression and sequencing. In order to develop a molecular signature (HuSignature™) predictive of response to cisplatin among NSCLC patients, we subjected a cohort of randomly selected NSCLC-PDX to a mouse clinical trial (MCT) with efficacy readout $\Delta T/\Delta C$ (relative tumor volume change between the treatment group and the control group). The trial results demonstrated that each model responded to the treatment differently as anticipated. We then used a set of statistical methods that we developed to select signature genes per correlation between their expression and $\Delta T/\Delta C$, as well as application of certain defined filters. A 9-gene signature was constructed, and, for each PDX, a signature score was calculated, which is strongly correlated with corresponding $\Delta T/\Delta C$. We found that some of these genes directly interact with each other and some were potential prognostic and diagnostic

biomarkers based on existing research. Using a published clinical cohort of 17 lung cancer patients (2), we validated the predictive power of this 9-gene signature. We found that patients with lower signature score have longer overall survival (OS, p-value=0.0071). We believe signatures like this or of a more refined version could be potentially useful for patient stratification of cisplatin treatment, so that patients can receive best benefit, while minimizing suffers, from cisplatin treatment.

A58 Patient derived AML xenografts for drug evaluation. Jinping Liu[1], Xiaoyu (Annie) An[1], Na Wang[2], Di Wang[2], Liang Huang[2], Ran Wu[1], Jie Cai[1], Jean-Pierre Wery[1], and Henry Q.X. Li[1,3]. [1]Crown Bioscience, Inc., CA, [2]Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; [3]State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

We have recently successfully engrafted leukemic cells from bone marrow of two AML patients into immunocompromised NOD/SCID mice, AM7577 and AM8096. They are from patients of different subtypes: M5 (AM7577) and M2 (AM8096). The two diseases have very distinct genotypes and phenotypes. AM7577 also harbors many of the common AML genotypes of mutations for IDH2-R140Q, FLT3-ITD, DNMT3A R882H and NPM1. It displays typical aggressive M5-AML disease: starts at bone marrow and gradually expand to peripherals (spleen – massively enlarged spleen, lymphonode and peripheral blood). It causes full blown leukemia with severe symptoms (body weight loss, hunched, inactivity, labored breathing, ruffled coat, etc.) and eventual death with 100% mortality. In contrast to AM7577, AM8096 does not harbor any of these mutations. Although causing similarly aggressive disease symptoms and 100% mortality in the end, the disease of AM8090 mainly resides in bone, and only spread to peripherals at terminal stage at lower levels and with only lightly enlarged spleen. The leukemic cell morphology of AM8090 is also less differentiated as compared to AM7577, all together as expected for M2 diseases. For both models, the leukemic cells can serially be passed in mice with 100% take-rate and cause consistent disease (even with < 1e4 cells). This also creates a renewable and potentially unlimited source of leukemia cells. The leukemic cells in mice are identical to those of the original patient leukemic cells ($CD45^+$, $CD33^+$, $CD13^+$, $CD123^+$, and $CD19^-$). We are currently performing RNAseq of AM8096 and AM7577 in order to explore the underlying molecular mechanisms that drive both diseases. Meanwhile, we are also investigating the drug response to standard of care (SOC, AraC) and FTL3 inhibitor, and found both respond well to these treatments in term blood leukemia burden and symptoms despite different genotypes. In addition, we revealed complete difference in relapse, or in the maintenance of the remission, after the drug withdraws between the two treatments, which implicate the potential roles of bone marrow leukemia initiation cells (LICs). We the two AML models could serve as useful experimental models to investigate the diverse leukemogenesis and evaluate new treatments for AML.

A61 Preparation, characterization, pharmacokinetics, bio-distribution, anti-tumor efficacy and safety of Lx2-32c-containing liposome. Jianqiao Zhang, Guangyao Lv, Hongbo Wang*, Fenghua Fu. Key Laboratory of Molecular Pharmacology and Drug Evaluation (Ministry of Education of China), School of Pharmacy, Yantai University, Yantai, China.

Lx2-32c is a novel taxane that has been demonstrated to have robust antitumor activity against different types of tumors including several paclitaxel-resistant neoplasms. Since the delivery vehicles for taxane, which include cremophor EL, are all associated with severe toxic effects, liposome-based Lx2-32c has been developed. In the present study, the pharmacokinetics, biodistribution, antitumor efficacy and safety characteristics of liposome-based Lx2-32c were explored and compared with those of cremophor-based Lx2-32c. The results showed that liposome-based Lx2-32c displayed similar antitumor effects to cremophor-based Lx2-32c, but with significantly lower bone marrow toxicity and cardiotoxicity, especially with regard to the low ratio of hypersensitivity reaction. In comparing these two delivery modalities, targeting was superior using the Lx2-32c liposome formulation; it achieved significantly higher uptake in tumor than in bone marrow and heart. Our data thus suggested that the Lx2-32c liposome was a novel alternative formulation with comparable antitumor efficacy and a superior safety profiles to cremophor-based Lx2-32c, which might be related to the improved pharmacokinetic and biodistribution characteristics. In conclusion, the Lx2-32c liposome could be a promising alternative formulation for further development.

A62 Inhibition of PI3K/mTOR pathway exhibits antitumor activity in a patient-derived xenograft model of HER2-negative, PTEN-deficient gastric cancer. Qingyang Gu[1], Zhixiang Zhang[1], Weiwei Wen, Bin Zhang[1], Hongye Sun[1], Qiang Xu[1], Qin Luo[1], Huiping Guan[1], Weiqi Sheng[2], Douglas Fang[1]. [1]WuXi AppTec Co., Ltd., Shanghai, China; [2]Department of Pathology, Shanghai Cancer Center, Fudan University, Shanghai, China.

PTEN mutations and deficiencies are prevalent in many types of human cancers, including gastric cancer. Thus, targeting the deregulated PI3K/PTEN-AKT signaling pathway has emerged as one of the tenets in anticancer drug development. In the efforts to establish and characterize patient-derived xenograft (PDX) tumor models of gastric cancer, we identified a HER2-negative, PTEN-deficient PDX model, implicating for the targeted therapy of PI3K pathway inhibition. Consequently, we demonstrated that the model was exquisitely sensitive to the antitumor effects of PF-04691502, a dual PI3K/mTOR inhibitor. Pharmacodynamic studies revealed the inhibition of the key components of PI3K/mTOR pathway after the treatment. Collectively, our results assist in the identification of a subpopulation of gastric cancer patients likely to benefit from therapy with a dual PI3K/mTOR inhibitor.

A65 The unique mechanism of action of VAL-083 may provide a new treatment option for chemo-resistant non-small cell lung cancer. Anne Steino[1], Jeffrey A. Bacha[1], William J. Garner[1], Sarath Kanekal[1], Zahid H. Siddik[2], Nancy Dos Santos[3], Shun Lu[4], Dennis M. Brown[1] [1]DelMar Pharmaceuticals, Inc., Vancouver, Canada and California, USA; [2]The University of Texas MD Anderson Cancer Center, Houston, TX, USA, [3]BC Cancer Agency, Vancouver, BC, Canada, [4]Shanghai Jiao-Tong University, Shanghai China. *Corresponding /Sponsoring Author: Dr. Dennis M. Brown, Chief Scientific Officer.

Lung cancer is the most common cancer and leading cause of cancer death in China. The median overall survival time for patients with stage IV non-small cell lung cancer (NSCLC) is 4 months, while 1- and 5-year survival is less than 16% and 2%, respectively. Metastatic NSCLC is usually treated with either Tyrosine Kinase Inhibitors (TKIs) (e.g. gefitinib) or platinum-based regimens (e.g. cisplatin). TKIs have resulted in vastly improved outcomes for patients with EGFR

mutations; however, TKI resistance has emerged as a significant unmet medical need, and long-term prognosis with platinum-based therapies is poor. Additionally, the incidence of brain metastases is high in patients with NSCLC with a poor prognosis. Dianhydrogalactitol (VAL-083) is a structurally unique bi-functional alkylating agent mediating interstrand DNA crosslinks targeting N^7 of guanine, thus differing in mechanism from TKIs and cisplatin. VAL-083 further crosses the blood-brain barrier and accumulates in tumor tissue. VAL-083 has proven activity against NSCLC in preclinical and clinical trials, suggesting VAL-083 may be a therapeutic option for drug-resistant NSCLC and NSCLC patients with brain metastasis. VAL-083 is approved for treatment of lung cancer in China; however, specific questions regarding the efficacy of VAL-083 in comparison to cisplatin and in TKI-resistant NSCLC have to our knowledge not been addressed before. When tested side-by-side in a standard syngeneic mouse fibrosarcoma model (RIF-1 cell-line in C3H mice), VAL-083 demonstrated superiority to cisplatin in tumor growth delay. For mice treated with a single IP injection of VAL-083 (10 mg/kg) tumor growth was delayed by 5.6 days compared to control, versus 1.5 days for mice treated with single dose cisplatin (4 mg/kg). Combination treatment of VAL-083 and cisplatin produced a more than additive effect by delaying growth 8.7 days. Previous clinical studies showing VAL-083 activity in NSCLC combined with the new data on synergy with cisplatin, makes VAL-083 a promising alternative for NSCLC with secondary brain tumors as well as chemo-resistant NSCLC. In a 2nd study we evaluated the activity of VAL-083 in *in vivo* models of drug-resistant NSCLC in comparison to cisplatin. Rag2 mice bearing subcutaneous human lung adenocarcinoma xenograft tumors of either TKI-sensitive (A549) or TKI-resistant (H1975) origin were treated. The NSCLC cell lines, A549 and H1975, were used for xenograft tumor models in female Rag2 mice. VAL-083 was given *i.p*. 3 times/week for 3 weeks, and the *in vivo* efficacy of VAL-083 in controlling tumor growth compared to cisplatin (5 mg/kg). Saline was used as control treatment. Disease progression was evaluated by tumor volume, clinical observations and body weight measurements. Blood samples were analyzed for CBC/differential analyses to assess myelosuppression or other changes in blood chemistry.

A549: Tumour growth delay of 26 days was observed in animals treated with 3 mg/kg VAL-083 compared to controls, versus a 4-day delay for mice treated with cisplatin. Mean tumor volume on day 68 was significantly reduced in animals treated with 3 mg/kg VAL-083 compared to controls (p=0.001).

H1975: Treatment was stopped after 6 doses of VAL-083 in the 4 mg/kg group due to significant body weight loss and the mice quickly recovered. Median survival time in mice treated with 4 mg/kg VAL-083 was 41 days compared to 31 days for all other treatment and control groups. Mean tumor volume on day 31 was significantly reduced in animals treated with 4 mg/kg VAL-083 compared to control (p=0.04).

Taken together, the results suggest that VAL-083 is superior to cisplatin in both TKI-sensitive and resistant tumor models, has synergistic effect in combination with cisplatin, and suggest clinical potential in TKI-resistant NSCLC. This data provides direction to clinical research aimed at influencing practice patterns under VAL-083's current label and support expanded global development.

A69 Glyco-profiling of cancer biomarkers by a modified magnetic bead capture approach.

Nicolai N. Maolanon[1,2,3], Romain Gineste[3,4], Chunmei Cao[1,3], Xin Hu[1,3], Joy Burchell[5], Jiong Wu[1,3],

Ola Blixt[2,3]*, Zhimin Shao[1,3]*. [1] Department of Breast Surgery, Fudan University Shanghai Cancer Center, China, [2] Department of Chemistry, University of Copenhagen, Denmark, [3] IMMUNOCAN Program, FP7-INCOLAB, [4] Transgene Biopharmaceuticals Technology (Shanghai) Co. Ltd., China, [5] Breast Cancer Biology, King's College London, England.

We sought to develop a method for detection of the cancer related carbohydrate antigens, Neu5Ac-α2,6-GalNAc-β1-Ser/Thr (STn) and GalNAc-β1-Ser/Thr (Tn), on CA15-3 (MUC1) and other biomarkers. Aberrant glycosylation are observed in all cancers and the full potential of these aberrations have not fully been exploited in prognostics, prediction and diagnostics. Most procedures focused on glycomics in cancer involve chromatography (such as HPLC methods) and mass spectrometry for analysis and requires highly specialized lab facilities as well as staff. We recently demonstrated improved differential diagnosis of ovarian cancer patients by microarray glycoprofiling of CA125 (MUC16) (Chen *et al*. 2013). To further develop this assay into more user friendly assays, this work describes the magnetic bead based method, Luminex®, to measure the presence of the cancer related carbohydrate targets, STn- and Tn-antigen in serum or plasma samples. Luminex® is designed for measuring concentrations of circulating biomarkers in a multi-plexed fashion. However, by modifying the protocol by incorporating an enzymatic cleavage step and using the lectin, *Vicia villosa* lectin, for detection, the presence of STn- and Tn-antigens on individual biomarkers can be revealed. The method was successfully developed and although preliminary results from breast cancer stage I, II and III (80 patient sera) did not exhibit elevated MUC1 and thus detectable STn/Tn-antigens, the detection of these are expected from upcoming breast-, gastric- and lung cancer stage IV samples.
Keywords: Breast cancer, mucin, lectin, multi-plex, glycoprofiling.
Reference: K. Chen, A. Gentry-Maharaj, M. Burnell, C. Steentoft, L. Marcos-Silva, U. Mandel, I. Jacobs, A. Dawnay, U. Menon & O. Blixt. *J. Proteome Res*. 2013, 12, 1408-1418.

A71 The advantages of acoustic liquid handling for drug sensitivity screening. Charline Hsieh[1], Carl Jarman[1], Krister Wennerberg[2], Bhagwan Yadav[2], Tea Pemovska[2], Tero Aittokallio[2], Bonnie Edwards[1]. Labcyte Inc, California, USA.

Recently identified associations between variants of cancer genes and drug resistance have increased the value for comprehensive drug sensitivity screening in combination with molecular profiling of cancer cells. This in turn, has led to a demand for improvements to screening throughput and efficiency. Echo® Liquid Handlers use acoustic energy to provide high throughput, non-contact, liquid handling for a range of applications. Non-contact transfer avoids the risk of cross-contamination, eliminates tip costs, and facilitates the progression to high density assay formats. Echo Liquid handlers precisely and accurately transfer 2.5 nL droplets of sample and reagent, which enables the miniaturization of biochemical screens. This poster discusses the implementation of miniaturized drug sensitivity screening, at the Institute of Molecular Medicine in Finland (FIMM), with assay-ready plates produced by the Echo liquid handler.

A72 Demonstration of safety and efficacy of a 4th generation chimeric antigen receptor-modified T cells for the treatment of relapsed or refractory B cell lymphomas. Zhi-Tao Ying,

Hao-Hsiang Guo, Yu-Qin Song, Xiao-Pei Wang, Wen Zheng, Yan Xie, Ning-Jing Lin, Mei-Feng Tu, Ling-Yan Ping, Wei-Ping Liu, Chen Zhang, Hui-Ying Huang, Lung-Ji Chang, Jun Zhu. Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Lymphoma, Peking University Cancer Hospital & Institute, #America Yuva Inc. Beijing, China and *Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL, USA.

Background: Despite currently available therapies, most relapsed or refractory B cell lymphoma patients cannot be cured. Expression of CD19 is found in almost all B cell lymphomas. T cells genetically modified with chimeric antigen receptors (CARs) targeting CD19 have shown unprecedented therapeutic efficacy in B cell leukemia patients. This study reports the safety and efficacy of a 4^{th} generation CAR T cell treatment for the management of relapsed and refractory B cell lymphomas.

Methods: Lymphoma patients had relapsed or progressive disease and received at least 2 previous treatments were recruited. T cells were transduced with lentiviral CAR vectors containing anti-CD19-scFv and T cell signaling domains including CD28/CD137/CD27 and CD3zeta. For safety improvement, the CAR was fused with an apoptosis-inducing gene, FKBP-caspase 9 (iCasp9), to establish a 4^{th} generation safety-improved CAR (4S-CAR). All patients received conditioning chemotherapy using Gemcitabine+Oxaliplatin, Fludarabine+Cyclophosphamide or Fludarabine before infusion, and 10^6-10^7/kg of CAR T cells.

Results: We have enrolled 5 patients, 4 men and 1 woman, with a median age of 34 (22-37). Before 4S-CAR T cell therapy, all patients had received a median of 4 previous therapies (2-6), and had progressive or relapsed disease at the time of T cell infusion. Three patients were diagnosed with highly aggressive Burkitt's lymphoma and heavily treated; at the time of inclusion, all had high tumor burden with life expectancy less than 1 month. There were no infusion-related toxicities. Median follow-up as of June 30, 2014 was 4 months (1.5-6) for all patients. After 4S-CAR T therapy, 3 patients achieved CR, 1 SD and 1 PD. The median progression free survival time for the 3 CR patients was 3.0 months (2.5-3.8). All responding patients developed a delayed cytokine release syndrome (CRS) illustrated by ELISA for IL-6 and IFN-γ, which were concurrent with peak CAR T cell expansion as illustrated by qPCR for the integrated CAR gene. The CRS was manifested by fever, variable degrees of nausea, anorexia, transient hypotension and hypoxia. None of the patients required intervention for the CRS. One patient developed tumor lysis syndrome and was managed with fluid resuscitation and supportive care. Nevertheless, the CAR T cells disappeared after 2 months for the 3 CR patients followed by relapses. Two of the 3 relapsed patients received tumor biopsy and IHC indicated CD19 positive again, coincident with the disappearance of CAR T cells.

Conclusions: The 4S-CAR T cells induced variable responses in heavily-treated, relapsed or refractory late-stage B cell lymphoma patients; three of the five patients achieved CR yet with limited duration. Expansion of patient cohort is needed and the mechanisms behind the short *in vivo* survival of the 4S-CAR require further investigation.

Patient	Age	Sex	Malignancy	Number of prior unique regimens	Conditioning regimen	Number of CAR T cells infused	Response	PFS (months)

						(×10^6kg)		
1	37	F	Burkitt's	3	Gemcitabine 1g/m^2 x1d, Oxaliplatin 100mg/m^2 x1d	2.5	CR	2.5
2	34	M	Burkitt's	4	Fludarabine 25mg/m^2 x3d	6.2	CR	3.8
3	24	M	DLBCL	4	Fludarabine 25mg/m^2 x3d, Cyclophosphamide 250mg/m^2 x3d	3.1	SD	1.5
4	35	M	FL	6	Fludarabine 25mg/m^2 x3d, Cyclophosphamide 250mg/m^2 x3d	2.0	CR	3.0
5	24	M	Burkitt's	4	Fludarabine 25mg/m^2 x3d, Cyclophosphamide 250mg/m^2 x3d	6.0	PD	NA

A73 Targeted PIK3CA therapy for metastatic cervix cancer: A phase I clinical experience.

Ming-Mou Hou[1], Xiaochun Liu[1], Jennifer Wheler[1], Aung Naing[1], David Hong[1], Apostolia Tsimberidou[1], Karen Lu[1], Razelle Kurzrock[2], Funda Meric-Bernstam[1], Siqing Fu[1]. [1]MD Anderson Cancer Center, Taoyuuan, Taiwan; [2]UC San Diego Moores Cancer Center, San Diego, CA, USA.

Background: PIK3CA/PTEN pathway signaling is frequently aberrant in patients with metastatic or recurrent cervical carcinoma; however, the clinical benefits of matched therapy targeting this pathway have not yet been established.
Methods: We analyzed the outcomes of patients with metastatic or recurrent cervical carcinoma who had a *PIK3CA* mutation or *PTEN* loss and/or mutation and received treatment in one or more phase I therapeutic clinical trials at a designated phase I clinic between January 2006 and June 2013. All data were obtained from patients' electronic medical records.
Results: All 55 consecutive patients had received at least one systemic therapy prior to starting a phase I trial therapy. Patients with adenocarcinoma were older (median age, 51 years), had fewer *PIK3CA* mutations (14%), and survived significantly longer (median overall survival, 14.2 months) than those with squamous cell carcinoma (median age, 42 years; 48% with *PIK3CA* mutations; and median overall survival, 7.2 months; p=0.034, 0.016, and 0.001, respectively). Matched therapy targeting the aberrant PI3K pathway led to higher rates of stable disease for 6 months or longer, partial responses, and complete remission (53%) and significantly longer progression-free survival (median, 6.0 months) than non-matched therapy (11% and 1.5 months; p=0.08 and 0.026; respectively). Patients with *PIK3CA* mutations had significant longer progression-free survival (median, 9.4 months) than those without *PIK3CA* mutations (median, 4.2 months; p=0.019).

Conclusions: Matched therapy targeting the aberrant PI3K pathway provided meaningful clinical benefits. Thus, further evaluation of treatments targeting the aberrant PI3K signaling is warranted in this cohort of patients.

A74 First-line combination of gemcitabine, oxaliplatin, and L-Asparaginase (GELOX) followed by radiation therapy for patients with stage IE/IIE extranodal natural killer/T-cell lymphoma: An updated analysis with long-term follow-up. Liang Wang, Zhong-jun Xia. Sun Yat-Sen University Cancer Center, Guangdong, China.

Background: Extranodal natural killer/T-cell lymphoma (ENKTL), an Epstein-Barr virus associated lymphoma, is relatively common in China. Due to high expression of multidrug-resistant (MDR) gene, ENKTL is resistant to anthracyclines-based chemotherapy. Nowdays, several studies have incorporated asparaginase into the chemotherapy regimens and got impressive short-term results. However, no long-term efficacy and safety data of asparaginase-based treatment have ever been reported due to relatively short follow-up time.
Methods: As previously reported, we have conducted a phase II clinical trial evaluating the efficacy and safety of combination of gemcitabine, asparaginase and oxaliplatin (GELOX) followed by radiation in the treatment of localized ENKTL since 2008. At the end of treatment, the overall response rate (ORR) was 96.3%, with complete remission (CR) rate of 74.1%, and the toxicities were well tolerated. In the first analysis, the median follow-up time was only 27 months, and the 2-year overall survival (OS) rate and progression-free survival (PFS) rate were both 86.0%.
Results: As of July 6th 2014, the median follow-up time was 63.15 months. The 5-year OS and PFS rate was 83.0% and 74.0%, respectively. Recurrence within the RT field was observed in three patients, and the planning target-volume control rate at 5 years was 88.9%. One patient with confirmed lung invasion that did not respond to autologus stem cell transplantation (ASCT) was successfully salvaged by lenalidomide monotherapy, and the EBV-DNA load reflected the disease progression and treatment response. No clinically significant late toxicities were found during follow-up visits.
Conclusions**:** In conclusion, this updated analysis confirmed the long-term benefit of GELOX regimen followed by RT with good safety profiles, and we recommended this strategy to be one of the most suitable options for early stage ENKTL.

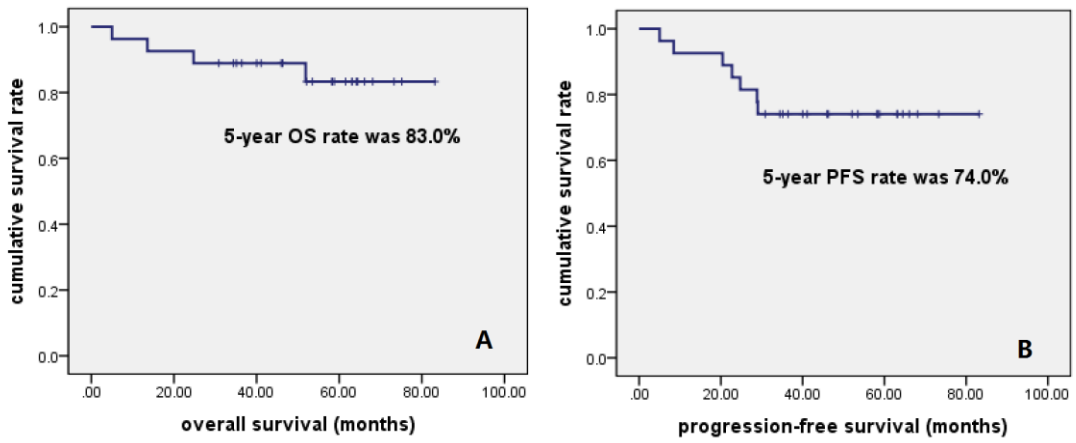

Figure 1: (A) Overall survival and **(B)** Progression-free survival of 27 patients treated with GELOX regimen followed by RT.

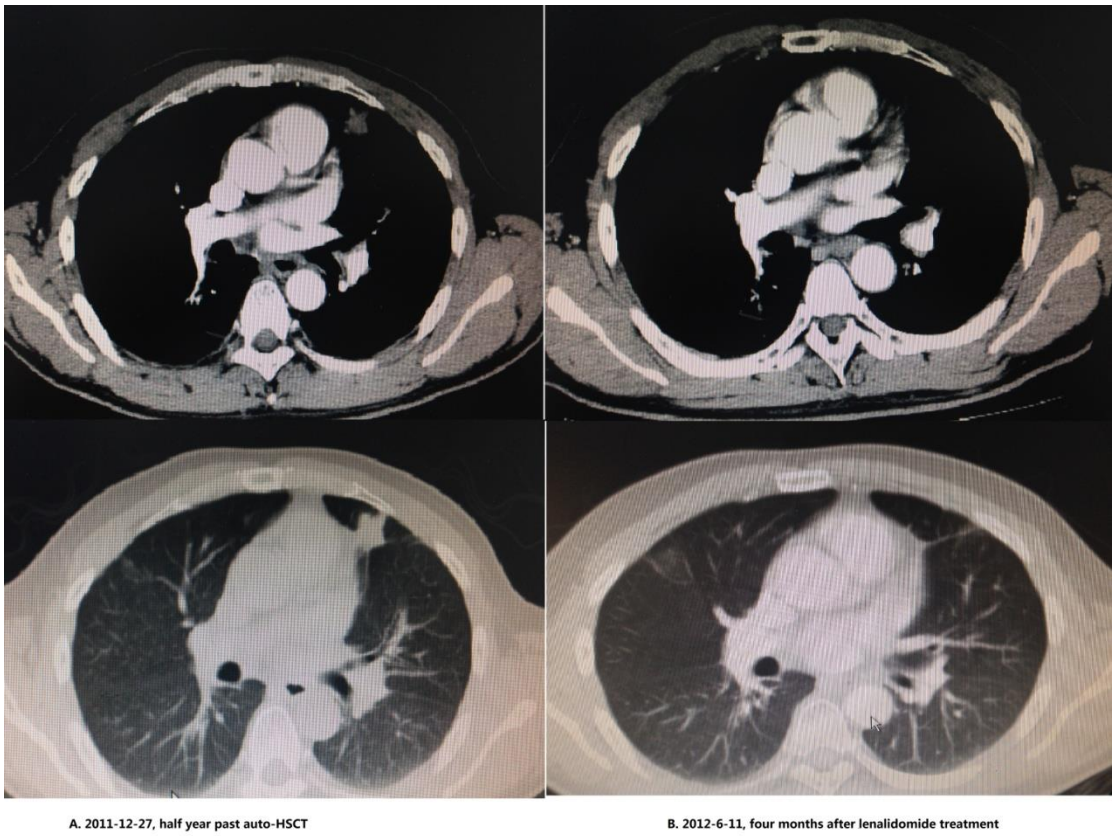

Figure 2: (A) The CT scan performed at half year post-ASCT revealed a 25*21mm size of solid nodule in the left lung and indicated disease progression; **(B)** The CT scan performed at four months after lenalidomide treatment revealed complete remission of the left lung lesion.

A75 Double cytotoxic drugs and hapten-enhanced therapeutic effect in advanced stages of pancreatic cancer by ultra-minimum incision personalized intratumoral chemoimmunotherapy (UMIPIC-2) compared with single cytotoxic drugs. Yuanfei Lu[1], Peng Jing[3], Han Wei[1], Feng Gao[2], Jian Liu[2], Guoliang Liu[1], Changjiang Guan[2], Qiang Fu[1], Zhenlu Ma[2], Baofa Yu[1,2,3]. [1]Jinan Baofa Cancer hospital, Jinan, China, [2]TaiMei Baofa Cancer Hospital, Dongping, China, [3]Beijing Baofa Cancer Hospital, Beijing, China.

Aims*:* UMIPIC is a new option for cancer treatment integrating local chemotherapeutic effect with systematic antitumor immunity by intratumoral drug delivery. We have explored the single cytotoxic combined with the hapten (immunomodulator) and the oxidant (coagulate tumor mass) previously(UMIPIC-1). In this study, we object to evaluate the clinical effectiveness of double cytotoxic drugs enhanced chemoimmunotherapy in the treatment of advanced pancreatic cancer by ultra-minimum incision personalized intratumoral chemoimmunotherapy (UMIPIC-2) compared with the single cytotoxic drugs

Method*:* 41 pancreatic **c**ancer patients with locally advanced and/or metastatic disease were included in the study, 30 of them (experimental group) were treated with UMIPIC therapy including two cytotoxic drugs (Cytosine Arabinoside: Ara-C and Bleomycin: BLM) plus an oxidant

and hapten, and 11 cases (control group) were treated with the single cytotoxic drug (Ara-C) intratumoral plus the combination of the oxidant and the hapten same as the UMPIC-therapy. Patient responses were assessed with CT scan at 4-6 weeks after treatment, and all of patients were followed until their deaths.
Results*:* Median overall survival (OS) was 15.5 months in experimental group and 3 months in control group *(P<0.01)*. The 6-month survival rate was 76.67% (experimental group) vs. 18.18% (control group) *(P<0.01)* and 1-year survival rate of 56.67% (experimental group) vs. 9.09% (control group) *(P<0.01)*.
Conclusion*:* UMIPIC-2 for pancreatic cancer is a non-invasive and potentially effective therapy, and double cytotoxic drugs applied in the UMPIC demonstrates a significant advantage in prolonging the survival time.

A78 Combination of high resolution melting methods to analyze human papillomavirus infection. Ta-Hsien Lee, Ching-Ping Tseng, Jiantai Timothy Qiu. Graduate institute of biomedical science, College of medicine, Chang-Gung University, Taoyuan, Taiwan, ROC.

Human papillomavirus (HPV), a small and nonenveloped double-stranded DNA virus, is established as the key etiological factor in cervical neoplasms. The recognition of the central role of HPV DNA screening in the etiology of virtually all cervical cancers has dramatically changed the perspectives of diagnoses and prevention of this neoplasia. Recent years, clinical outcome indicated that the correction of multiple HPV infection in early stage of cervical cancer progression is a important factor except those high-risk HPV genotypes. However, there is still no consistent evidence to determine the competition or synergy of multiple HPV infection. Lack of a cost-effective, accurate and a well and broad convinced approach to support this issue is an important reason. Therefore, a simple and available approach for the differentiation of multiple HPV genotypes is under consideration, which can contribute to patient management. Currently, we develop a High-Resolution Melting system (HRM) based HPV diagnostic system. And we further combined traditional multiplex PCR model with multiple unlabeled probes in asymmetric HRM system. So far, we designed 3 genotype–specific amplicon (HPV type 18, 39, 59) all located in HPV-L1 region, and each region is individual and isolated to the other 2-regions. Our preliminary determined the technical limitation is similar to our previous research in conventional PCR test, 10 DNA copies. And we further applied two unlabeled probes, MP-18P1 and MP-59P1 in single reaction with the addition multiple HPV DNA (include HPV18 and 59) to overcome the un-distinguish mixed major peak under derivative plot. Eventually, two probe-induced peaks expressed at designed temperatures to provide the genotype-specific signal.
Conclusion: Our research attempt to develop a simple and accuracy approach to differentiate multiple HPV infection. Basically, we just prove the possibility of this concept, the multiple HPV genotype can be determined in single amplification reaction in real-time PCR based method.

A79 Genome-wide association scans suggested modifier gene for the effect of heterocyclic amines on colorectal cancer risk. Hansong Wang[1], Terrilea Burnett[1], Suminori Kono[2], Christopher A. Haiman[3], Motoki Iwasaki[4], Lynne R. Wilkens[1], Laurence N. Kolonel[1], Brian E. Henderson[3], Temitope O. Keku[5], Robert S. Sandler[5], Lisa B. Signorello[6], William J. Blot[7], Sonja I. Berndt[8], Mala Pande[9], Christopher I. Amos[10], Dee W. West[11], Polly A. Newcomb[12], Robert W. Haile[13], Shoichiro Tsugane[4], Daniel O. Stram[3], Loïc Le Marchand[1,*].

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Colorectal cancer (CRC) is a leading cause of cancer morbidity and mortality in the world. Mainly explained by lifestyle/environmental risk factors (E), CRC is believed to be also attributable to genetic susceptibility (G) and/or interactions between genetic and environmental factors. The investigation of the interaction effects between G and E (G × E) on CRC risk will help to elucidate the biological basis of lifestyle causes of CRC, as well as generate new hypothesis for disease prevention and therapeutic. Although genome-wide association studies (GWAS) have identified more than 30 risk variants for CRC, few of them were confirmed to interact with environmental risk factors. In this study, we explored G × E effects on the risk of CRC by combining results from two large-scale GWAS in 2,627 cases and 3,797 controls of Japanese ancestry and 1,894 cases and 4,703 controls of African ancestry. Known lifestyle risk factors, namely, pack-years of smoking, red meat consumption, regular use of aspirin (Yes/No), average intake of ethanol, folate, calcium and heterocyclic amines were considered. Continuous E factors were dichotomized at medians within each participating study site. G × E effects were tested in case-control analysis using cross-product terms in logistic regression model with adjustment for age, sex, and the first four principal components, first separately in the two ethnic groups then combined through fixed-effect meta-analysis. The results suggested a potential genome-wide statistically significant interaction between intake of heterocyclic amines and a SNP in the olfactomedin 3 (*OLFM*3) region on chromosome 1 ($P = 2.0 \times 10^{-9}$ with between-study heterogeneity $I^2 = 0$). Further replication in larger populations is warranted.

A84 N-glycan cryptic sugar moieties as potential biomarkers of aggressive prostate cancer.

Denong Wang, Tumor Glycomics Laboratory, SRI International, Menlo Park, CA.

Although tumor-associated abnormal glycosylation has been recognized for decades, information regarding host recognition of the evolving tumor glycome remains elusive. We report here a carbohydrate microarray discovery and immunological validation of a number of potential glycan markers of prostate cancer. These carbohydrates are the precursors, cores and

internal sequences of *N*-glycans. They are usually masked by other sugar moieties and belong to a class of glyco-antigens that are normally "cryptic". However, viral expression of these carbohydrates may trigger host immune responses. For examples, HIV-1 and SARS-CoV display Man9 clusters and tri- or multi-antennary type II (Galβ1→4GlcNAc) chains (Tri/m-II), respectively; viral neutralizing antibodies often target these sugar moieties. We asked, therefore, whether prostate tumor expression of corresponding carbohydrates triggers antibody responses *in vivo*. Using carbohydrate microarrays, we detected IgG antibodies targeting the Man9- or Tri-/m-II-autoantigens in the sera of men with benign prostatic hyperplasia (BPH), as well as those with cancer. Importantly, these antibody activities were selectively increased in prostate cancer patients. We further verified these observations in a large-cohort of patients, including patients with 100% Gleason grade 3 cancer (N=84), with Gleason grades 4 and/or 5 cancer (N=204), and BPH controls (N=135). Radical prostatectomy Gleason grades and biochemical (PSA) recurrence served as key parameters for serum biomarker evaluation. Progress of this large-scale serological study will be discussed in this presentation.

A85 BiTE-hIgFc-based immunotherapy as a new therapeutic strategy in multiple myeloma.

Jianxuan Zou[1], Dan Chen[1], Yunhui Zong[1] , Sisi Ye[2], Jinle Tang[1], Huimin Meng[1] , Gangli An[1], Xingding Zhang[2], Lin Yang[1]. [1]The Cyrus Tang Hematology Center, Soochow University, Suzhou, Jiangsu, China, [2]Suzhou Cancer Immunotherapy and Diagnosis Engineering Center, Suzhou, Jiangsu, China.

Abstract Bispecific antibodies (bsAb) play an important role in immunotherapy. They have received intensive interest from pharmaceutical enterprises. The first antibody drug OKT3 (Muromonab-CD3) showed great performance in clinical treatment. We successfully developed a single-chain variable fragment (ScFv) combination of anti-CD3 ScFv and anti-CD138 ScFv with hIgG1 Fc (hIgFc) sequence. The novel bispecific T cell Engager (BiTE) with an additional hIgFc (BiTE-hIgFc, STL001) can target T cells, natural killer (NK) cells and multiple myeloma (MM) cells (RPMI-8226 or U266). In addition, BiTE-hIgFc (STL001) has nM level affinity to recombinant human CD138 (rCD138) and has shown more potent antitumor activity against RPMI-8226 cells than that of separated aCD3-ScFv-hIgFc and aCD138-ScFv-hIgFc *in vitro*.
Précis The world's first CD138/CD3 BiTE-hIgFc targeting T cells, natural killer cells and multiple myeloma cells simultaneously has nM level affinity to recombinant human CD138 (rCD138) and has shown potent antitumor activity against RPMI-8226 tumor cells.

A86 CTLA4 and CD28 single nucleotide polymorphisms correlate with the response of bladder cancer patients to BCG immunotherapy and gene transfection with the single chain variable fragment of the antibody to CTLA4 reduced bladder tumor growth in mice.

Mahendran, R. Lim YK, Tham, SM, Rahmat, JN, Sng, JH, Raman, LN, Ma, ZMW, Chiong, E, Chan, YH, Esuvaranathan, K.

Introduction: Though BCG is the gold standard for Non muscle invasive bladder cancer (NMIBC), there is a significant non responder rate. The reasons for this are not known though single nucleotide polymorphisms (SNPs) in some genes correlate with response to therapy. Bladder

cancer patients who were treated with anti-CTLA4 antibodies had increased $CD4^{+}ICOS^{hi}$ IFNγ expressing T cells over Treg cell numbers in the bladder and peripheral blood. CTLA4 competes with CD28 for binding to B7.1 and thus modulates T cell activation. CD28 splicing variants create a soluble CD28 (sCD28) isoform that may have reduced immunostimulatory effects. Thus it was possible that either or both CTLA4 and CD28 could modulate the response to BCG immune therapy.
To determine this we evaluated seven SNPs in the CTLA4 gene (Rs733618, Rs4553808, Rs5742909, Rs231775, Rs3087243, Rs7565213 and Rs960792) that regulate protein expression and function and one in CD28 (Rs3116494) that modulates the production of sCD28. The benefits of local CTLA4 suppression were evaluated in mice using the single chain variable fragment (scFv) of the antibody to CTLA4 in a murine orthotopic model of bladder cancer.
Methods: IRB approval was obtained for this project. SNPs in n=138 bladder cancer patients, who had previously been treated with BCG and for whom long term follow-up data was available, and n=146 healthy controls were analyzed. Genomic DNA was extracted and subjected to PCR followed by High Resolution Melt (HRM) analysis and sequence analyses. MB49PSA cells were implanted orthotopically in C57BL/6 female mice to establish bladder tumors. The tumors were treated intravesically with a plasmid carrying the scFv to the CTLA4 antibody twice a week for 3 weeks.
Results: There were no difference in the incidence of SNPs between healthy controls and patients. By Kaplan Meier analysis, GA at Rs4553808 (148 months) and CT at Rs5742909 (167 months) correlated with longer time to recurrence. By COX regression analysis, CT at Rs5742909; AA at Rs3087243; AA and AG Rs7565213 and TT at Rs960792 were significantly correlated with reduced risk of recurrence ($p<0.05$). While GA at Rs3087243 and TT at Rs7565213 correlated with a reduced risk of progression ($p<0.05$); GA at Rs231775 correlated with an increased risk of progression ($p<0.05$, HR 39.4, 95% CI 1.97-795.84). Increased survival was observed in patients with CT at Rs5742909 ($p<0.05$). TT at Rs3116494 **(**CD28) correlated with an increased risk of progression ($p<0.05$, HR 44.34, 95% CI 2.287-859.89) as well. Tumor growth was evaluated by measuring the weight of the bladders at the time of harvest.
Conclusion: Rs231775 GG is associated with significantly higher T cell activation. Here, Rs231775 GA was associated with an increased risk of disease progression but not AA which is expressed at a lower level in our population. These results indicate that CTLA4 blockade may be a beneficial co-therapy for some bladder cancer patients receiving BCG immunotherapy. A gene therapy based approach using a plasmid expressing the scFv to CTLA4 antibody, reduced tumor growth in the bladder when given intravesically. Thus local CTLA4 blockade could be easily combined with BCG immunotherapy.

A87 Myeloid-derived suppressor cells increase the expression of cancer-testis antigens MAGE-A4 in Lewis lung cancer murine model. Huiru Wang[1], Guilan Shi[2], Meifen Hu[3], Jie Geng[2], Aiyu Shao[2]. [1]Department of Immunology, Cancer Institute, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China, [2]Department of Immunology, Zibo Vocational Institute, Shandong, China, [3]No.2 Beijing Armed Police Hospital Cancer Biotherapy Center, Beijing, China.

OBJECTIVES: The cancer-testis (CT) family of antigens is expressed in a variety of malignant

neoplasms and is silent in normal tissues, except for the testis. Immune therapy targeting cancer/testis (CT) antigens improve the survival in several types of solid tumors. However the expression of CT antigens is related to poor survival in lung cancer. Myeloid derived suppressor cells (MDSC) are a heterogeneous population of immunosuppressive cells that are up-regulated in cancer. Little is known about the correlation between the expression of CT antigens and MDSC in Lewis lung cancer (LLC) murine model.
METHODS: The expression of CT antigens (melanoma-associated antigen (MAGE-A4) in tumor cells or tumor tissue was evaluated by using western blotting and the percentage of MDSCs (Gr-1+CD11b+) in blood was detected with flow cytometry assay. The suppressive capacity of MDSC and the effectiveness of MDSCs depletion were assessed in C57BL/6 tumor-bearing mice.
RESULTS: The depletion of MDSCs resulting in declined the MAGE-A4 expression, delayed tumor growth rate, prolonged median survival, and increased recruitment of T cells.
CONCLUSION: The expression of therapeutic target MAGE-A4 antigen has a negative relationship with MDSCs.

A88 Thymosin alpha1 enhanced cytotoxicity of iNKT cells against colon cancer via upregulating CD1d expression. Chao Ni[1], Ping Wu[1], Ting Zhang[2], Fuming Qiu[2], Jian Huang[1,2 *].
[1]Cancer Institute (Key Laboratory of Cancer Prevention & Intervention, National Ministry of Education, Provincial Key Laboratory of Molecular Biology in Medical Sciences), [2]Department of Oncology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China.

Aim: Thymosin-alpha1 (TA), used as an immune modulator in clinics, has limited studies about its effect on cancer cells. Here we aim to investigate the role of TA in the immunotherapy of iNKT cells against colon cancer.
Methods: Paired blood samples and fresh tumor specimens were collected from 9 colorectal cancer (CRC) patients; pure iNKTs were obtained by FACS of the concentrated leucocytes of healthy subjects. The cell proliferation was determined by CSFE labeling, cytotoxicity of iNKT cells against target cells was performed with LIVE/DEAD Viability/Cytotoxicity Kit, and blocking assays were applied to determine the mechanisms of iNKT cell-mediated toxicity. qRT-PCR and western-blot were also performed to determine the mechanism of TA worked on cancer cells.
Results: Result showed that with co-presence of cancer cells and TA (50μg), iNKT cells were greatly proliferated, and a synergistic effect was found between TA and αGalcer, which was widely acknowledged for specific activate iNKT cells. In consideration of the above results, we speculated that the indirect effect of TA upon iNKT cells was mainly via its impact on cancer cells. Rt-PCR and FCM revealed that TA could upregulate the expression of CD80, B7H2 and CD1d on CRC cells with time and dose dependent manner. Furthermore, the cytotoxicity assay showed the killing efficiency of iNKT cells could be potentiated by TA, and the blocking assay found iNKT cells mainly exerted cytotoxicity via CD1d-dependent recognition of cancer cells, but not CD80 or B7H2. Finally, we tried to illuminate the mechanism of TA affecting CRC cells. With specific blocking agent, we found TA-mediated CD1d and CD80 expression was dependent on activation of Erk/MAPK pathway.
Conclusion: Here we reported TA upregulate the expression of CD1d on CRC cells, especially cancer stem cells, which facilitated the mutual recognition between the cancer cells and activated iNKT cells, and resulted in its greater killing efficiency. Overall, our work increased

knowledge about TA's function and indicated a novel immunotherapeutic strategy of iNKT cells against colon cancer.

A89 Mixeno™ Mouse Models for in vivo evaluation of anti-human cancer immunotherapeutics. Juan Zhang, Junzhuan Qiu, Meng Qiao, Qian Shi. Crown Bioscience, Inc., China.

The past few years have witnessed a renaissance in the field of cancer immunotherapy, relating largely to the clinical advances associated with the development of immunomodulatory agents, e.g. monoclonal antibodies targeting the immune inhibitory pathways (CTLA-4 and PD-1/PD-L1). Often, the preclinical efficacy assessments are based on the evaluation of surrogate anti-mouse target antibodies using mouse syngenic tumor models. However, this strategy is limited due to the fact it can only be used to test surrogate molecules, rather than directly evaluate the therapeutic molecules that target human targets. Here we set out to validate mouse models that harbor human immune cells by engrafting the immuno-deficient mice with human PBMC (the Mixeno™ model), and use them for efficacy evaluation of the humanized anti-PD-1 antibody. PD-L1 high-expression human tumor cell lines are selected using Xenobase® and FACS analysis to develop the in vivo models. BMS-936558, a fully humanized anti-PD-1 IgG4 produced promising anti-tumor activity in the HCC827 lung cancer Mixeno™ model. Based on the preliminary result, the Mixeno™ models may be a useful tools in immunotherapeutic antibody development, and may greatly increase the clinical translatability of animal studies.

A90 Water Extract of deer bones activates macrophages and alleviates neutropenia. Han-Seok Choi, Soon Re Kim, Se Hyang Hong, Jin Mo Ku, Ji Hye Kim, Hye Sook Seo, Yong Cheol Shin, and Seong-Gyu Ko. Department of Preventive Medicine, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea.

Extracts from deer bones, called nok-gol in Korean, have long been used to invigorate Qi. While neutropenia is not well detected in normal physiological condition, it could be a cause of severe problems to develop diseases such as infectious and cancerous diseases. Thus, a prevention of neutropenia in normal physiology and pathophysiological states is important for maintaining Qi and preventing disease progress. In cell biological aspects, activated macrophages are known to prevent neutropenia. In this study, we demonstrate that water extract of deer bone (herein, NG) prevents neutropenia by activating macrophages. In mouse neutropenia model system in vivo where ICR mice were treated with cyclophosphamide to immunosuppress, an oral administration of NG altered the number of blood cells including lymphocytes, neutrophils, basophils, and eosinophils. This in vivo effect of NG was relevant to that of granulocyte colony stimulating factor (G-CSF) that was known to improve neutropenia. Our in vitro studies further showed that NG treatment increased intracellular reactive oxygen species (ROS) and promoted macrophagic differentiation of mouse monocytic Raw264.7 cells in a dose-dependent manner. In addition, NG enhanced nitric oxide (NO) synthesis and secretions of cytokines including IL-6 and TNF-α. Consistently, NG treatment induced phosphorylation of ERK, JNK, IKK, IκBα, and NF-κB in Raw264.7 cells. Thus, our data suggest that NG is helpful for alleviating neutropenia.

A91 Dynamic changes in cellular immunity of spleen in murine model of hepatocellular carcinoma. Jinna Li[1], Rui Zhou[2], Jun Li[2], Baohua Li[2], Jiangwei Li[2], Zongfang Li[2, 3] Shu Zhang[2, 3].
[1]Infection Control Administration, [2]National & Local Joint Engineering Research Center for Biodiagnosis and Biotherapy, [3]Department of Surgery, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China.

Background/Aims: As the largest secondary lymph organ of human body, spleen plays important role in tumor immunity, which has been neglect for a long time. We observed the dynamic changes in immune cells in spleen, the aim is to elucidate the role of spleen during the progression of tumor.
Methods: Orthotopic hepatocellular carcinoma was established in BALB/c mice, the spleens of normal mice and tumor-bearing mice were excised on day 7, day 14 and day 19, grinded and passed through 70 μm mesh. Red blood cells were lysed with ACK, 10^6 splenocytes were colloected for further staining. The splenocytes were stained with CD16/CD32 antibody for FcR blocking, then stained with Gr-1, CD11b, CD3, CD4, CD8a, CD49b, CD25 and foxp3 antibody, respectively. The percentages of MDSC, $CD3^+$ T cell, $CD3^+CD4^+$ T cell, $CD3^+CD8^+$ T cell, NK cell, Treg cell were analysed using flow cytometry.
Results: The percentage of MDSC was increasing during the progression of hepatocellular carcinoma (day 7: 4.83 ± 1.10%, day 14: 9.53± 5.48%, day 19: 9.93± 3.67% vs normal: 2.15± 1.24%). T cells including $CD3^+CD4^+$ T cell, $CD3^+CD8^+$ T cell and total $CD3^+$ T cell were decreased on day 14 (30.63± 2.34% vs 36.42± 4.35%, 14.48 ± 0.86% vs 17.28 ± 1.72%, 47.28± 2.80% vs 54.77± 6.19%, respectively). While the ratio of CD4/CD8 T cell was not changed during tumor progression. NK cells were decreased on day 14 (2.00 ±1.08%) and day 19 (1.40 ± 0.46%) compared to normal mice (4.55± 0.98%). Treg cells in spleen were elevated on day 14 (15.43 ±2.45%), but declined on day 19 (2.78 ±1.00%) compared to normal spleen (9.95± 1.39%).
Conclusions: MDSC and Treg cells, both have immune suppressive function, were increased in spleen from tumor-bearing mice, but T cells and NK cells were decreased. It is suggested the spleen play a negative role during the progression of orthotopic hepatocellular carcinoma.
Acknowledgements: The work was supported by the National Natural Science Foundation of China (81001309).

A92 Changes in peripheral blood immune cells in murine orthotopic liver tumor.
Baohua Li[1*], Shu Zhang[1, 2*], Jun Li[1], Rui Zhou[2], Chen Zhang[1], Fang Li[3], Zongfang Li[1, 2].
[1]National & Local Joint Engineering Research Center for Biodiagnosis and Biotherapy, [2]Department of Surgery, 3 Department of Anaesthesiology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. *Contribute equally to this work.

Background/Aims: Tumor immune editing includes immune elimination phase, immune equilibration and immune escape phase. To better understand the immune status of tumor-bearing host and to develop tumor targeted therapy, the orthotopic H22 transplanted liver tumor in BALB/c mice was established and the changes of immune cells in peripheral blood were observed.
Methods: H22 hepatoma cells (2×10^5, 20μl) were injected into the livers of BALB/c mice. On day 7, day 14 and day19, the peripheral blood were collected and stained using the following

fluorescent antibodies against CD11b and Gr-1 (MDSC), CD3, CD4 and CD8a (T cells), CD49b (NK, NKT), CD25 and foxp3 (Treg), then detected by flow cytometry.
Results: With the progression of liver tumor, the percentage of MDSC was markedly elevated (49.18 ± 10.50%, 67.67 ± 23.95%, 89.30 ± 5.10%) than that in the control group (31.77 ± 11.49%). The percentages of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells and NKT cells were all decreased on day 14 (26.33 ± 4.02%, 18.15 ± 3.73%, 6.38 ± 1.07%, 0.23 ± 0.18%) and day 19 (23.88 ± 2.59%, 15.35 ± 2.61%, 7.70 ± 2.24%, 0.10 ± 0.06%) compared to the control group (45.6 ± 9.94%, 31.68 ± 7.45%, 12.38 ± 3.78%, 0.80 ± 0.45%). While the ratio of CD4/CD8 T cell was not changed during tumor progression. NK cells were lowered early on day 7 and day 14 compared to the control group (8.43 ± 3.32%, 7.23 ± 2.34% vs 15.63 ± 7.08%). The percentage of Treg cells was not elevated during tumor progression, but decreased on day 19, perhaps because of cachexia due to lots of ascites.
Conclusions**:** On day 7 of tumor inoculation, the percentage of MDSC was elevated, while NK cells were decreased. On day 14 and 19, MDSC was continuously increased, while T cells including $CD3^+$ T, $CD4^+$ T, $CD8^+$ T and NKT cells were all decreased, indicating from the early stage of orthotopic liver tumor, an immune negative state dominated the tumor-bearing host, so a positive immunotherapy should be emphasized from the beginning of tumor.
Acknowledgements: The work was supported by the National Natural Science Foundation of China (81001309).

A93 Association of exhaled volatile organic compounds and risk factors of lung cancer: A study design. LQ Lin[1], YC Zou[1], L Lang[1], X Zhang[1], H Dong[1], P Wang[1], X Chen[1*]
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Background: Many studies have shown the concept of volatile organic compounds (VOCs) severed as biomarkers in early detection of lung cancer [1-2]. But few studies indicate the correlation between VOCs and prevention including risk factors of lung cancer. Our research aims to confirm the association of VOCs and risk factors of lung cancer.
Methods: The research is in its infancy, and the whole process is demonstrated as follows (Fig. 1). We jointed Sir Run Run Shaw hospital, and collected breath gas both of lung cancer patients and normal, as well as ambient air. Then each sample's VOCs were analyzed by gas chromatography/mass spectrometry (GC-MS) combined Thermal Desorber (TD). A program was written to deal with hundreds of VOCs of every sample. Each subject's data subtracted the background air and then were recorded into a form which included all subjects' subtracted VOCs' peak areas and retention times (RTs).
Meanwhile, we designed a questionnaire, and collected subjects' answers into a dataset. The survey items mainly included some risk factors, such as air quality in living place, smoking intension, exposure to heavy metal, dust, volatile solvent etc. Ultimately, the VOC form was associated with this dataset to form an eventual dataset. The association between VOCs and risk factors were analyzed with Statistic Package for Social Science (SPSS) software.

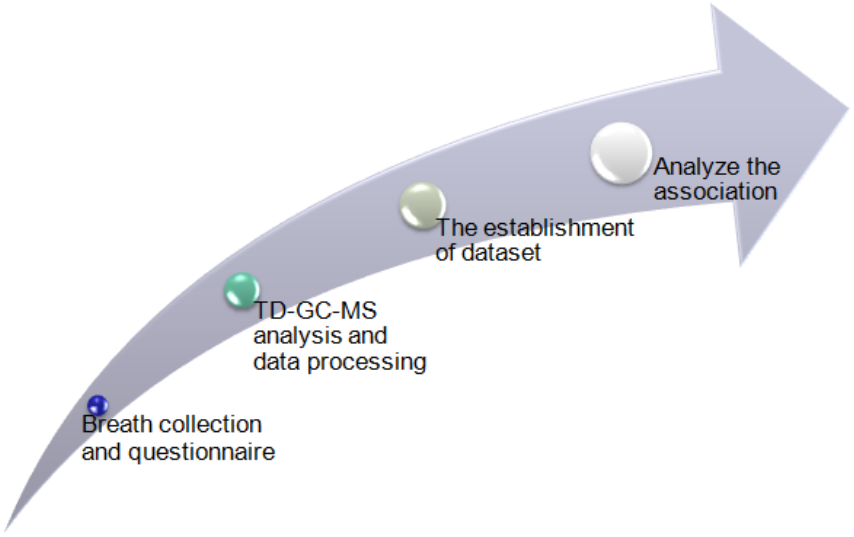

Fig.1. The whole research process.

Results: We have collected 71 normal subjects and 3 pulmonary nodule patients so far, Table 1 shows some Characteristics of 74 subjects recorded in the dataset.

Table 1 Some Characteristics of 74 subjects

Characteristics	**Study population (n=74)**
Gender (n, %)	
Male	56(75.7)
Female	18 (24.3)
Age (median, range)	49 (32–66)
Smoking status (n, %)	
Never smoker	26(35.1)
Former smoker	4 (5.4)
Current smoker	44 (59.5)
Mean smoking number per day (n, %) (aim to smoking and Stop smoking)	
0-20 cigarettes	30(63.8)
20-40 cigarettes	13 (27.7)
40-60 cigarettes	4 (8.5)
Exposure to smoking time (n, %) (aim to No smoking and Stop smoking)	
0-1 hour	21 (70)
1-4 hours	9 (30)
Air quality in living place (n, %) (subjective grade)*	
0-2	9(12.7)
3-5	29 (40.8)
6-8	23 (32.4)
9-10	10 (14.1)

*grade 0 means poor air quality, and grade 10 means well air quality

Judging from the preliminary research, people who smoke less than 20 cigarettes were more than people who smoke more than 20 cigarettes per day. And 40.8% people thought air quality in their living place was just OK, 32.4% people thought it was all right. Besides among the 74

subjects, thousands of VOCs had been found. And with the increase of sample number, the number of new increased VOCs, which were relative to those already existed in the form, presented downward trend (Fig. 2).

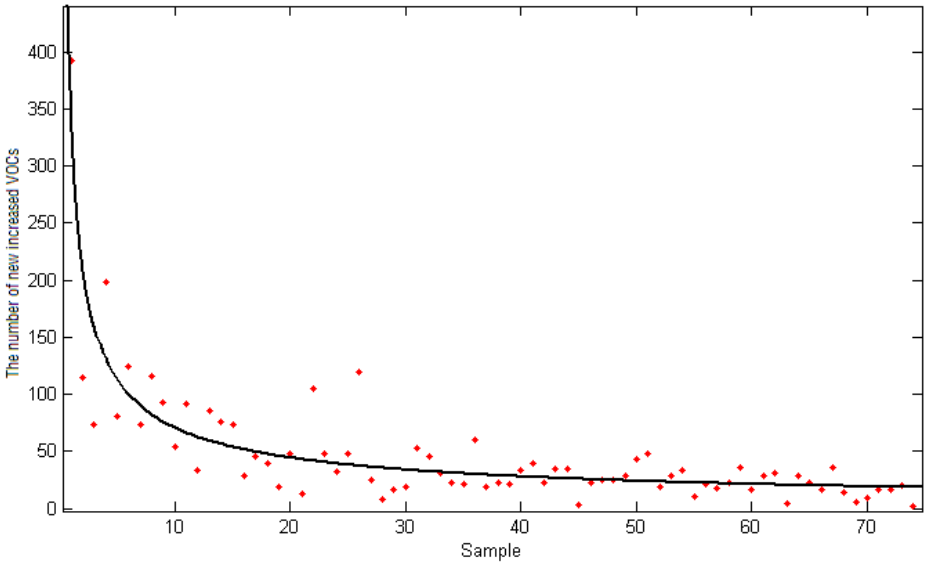

Fig.2. The number of increased VOCs' changing trend.

Conclusion: A stable trend of the number of increased VOCs occurred when the sample's amount reach 60. When it reaches a certain amount, we consider it can be able to cover nearly all the VOCs.

The association of VOCs and risk factors is not clear at present, which results from the limited sample size, especially the absence of lung cancer cohort. With the development of research, the dataset continued perfection gradually. For the further research, we may combine with a clinical dataset including lung cancer, histology type, stage, tumor size to optimize the research.

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A94 Anal cancer screening in an urban HIV clinic – Provider perceptions and practice.

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US surveillance data reveals a 125% increase in anal cancer incidence from 1975 to 2014[1]. Overall incidence is higher in women then men, however HIV positive men who have sex with men (MSM) have exceptionally high risk. Data from the Swiss Cohort revealed a standardized incidence ratio of 33.4 (95% confidence interval [CI] 10.5 – 78.6) comparing HIV positive MSM to HIV negatives [2]. Despite a steady increase in new cases there are no standardized screening guidelines even for high-risk groups. New York State Department of Health is one of the few organizations with guidelines in any form [3]. Discussions with our providers suggested that screening rates and practices varied significantly. We therefore surveyed our providers to get a

better understanding of the knowledge, perceptions and practice on screening for anal cancer using anal cytology.

Methods

Forty-seven medical staff providing care to HIV patients within three urban clinics were invited by email on 6/10/2013 to respond to an online survey on anal cancer screening. Providers were given 21 days to respond to this survey and had weekly reminders. On 6/30/2013 the data was downloaded and analyzed to determine the overall acceptability of anal cytology screening as well as to determine the perceptions of providers on the performance of anal pap smears. All data analysis was done using STATA version 10 software; chi-squared tests were done to test for associations between different categorical variables and student t-test was used to test for associations between different continuous variables. Analysis focused on determining the prior experience of providers in performing the procedure as well as to determine if provider characteristics had any correlation with their knowledge or perceptions with regards anal cytology tests.

Results: Of the 47 providers (26 males, 19 females) contacted, 26 (55.3%) responded and completed the online questionnaire (17 male (65.4%), 9 female (34.6%)). The response rate was 65.4% among males and 47.4% among female providers. On a 5-point scale for self-assessed knowledge on anal cytology screening providers scored on average 2.6 (95% CI 1.8 -3.3) out of 5, females scored higher than males 3.0 (95% CI 1.6 – 4.4) versus 2.3 (95% CI 1.4 – 3.2), respectively. When surveyed on which patient population to screen 52.2% of providers recommended anal cytology screening for all MSMs. However, providers $\leq$45 yrs of age were 10.6 times more likely to suggest screening for other subgroups such as those with a history of anogenital warts (57% compared with 11.1% of older providers). Males were 8 times more likely to recommend screening for HIV positive patients with anogenital warts compared to females (p-value =0.06). Only 2 out of the 23 patients who provided complete responses to the survey reported never performing an anal cytology test. Most providers(57.5%) rated themselves above 5 on a 10-point comfort scale at performing the procedure. Females rated their comfort level higher than males with mean rating 7.1 (95% CI 4.7 – 9.5) compared to 3.6 (95% CI 1.5 – 5.7). (p-value = 0.03.)

34.8% of providers reported receiving requests from patients to be screened. Younger providers and females were 2.6 – 2.7 times more likely to receive a screening request compared to older providers and males but this difference was not statistically significant (p-value =0.3). When surveyed on how best to address routine screening for patients, 56.5% of providers stated they would prefer to refer their patients to another provider to screen(67% males and 37.5% females). 61% of providers knew about High Resolution Anoscopy (HRA) and were aware it was used to in the evaluation of abnormal anal cytological changes. Almost all providers surveyed 95.6%, believed our practice needed a formal screening program for appropriate patients.

Conclusion

Our study revealed that 52.7% of providers in our survey had performed anal cytology screening for some of their patients. There was however no agreement among providers concerning which population to screen. Although most providers favored routine screening and self reported knowledge of the procedure was fairly high, the majority of providers would prefer another provider to perform the screening test on their patients.

These results underscore the need to establish more systematic guidelines for anal cancer screening in HIV infected patients. Large HIV clinics should evaluate the knowledge and attitude of their providers toward anal cytology screening and establish pathways for screening that best fits their needs, preferences and resources.

B01 Chemically modified synthetic microRNA-205 inhibits the growth of melanoma cells *in vitro* and *in vivo.* Yukihiro Akao. Gifu University, Aichiken, Japan.

We recently reported that microRNA (miR)-205-5p is downregulated and acts as a tumor suppressor in human melanoma cells. Previously, for clinical application, we added aromatic benzene-pyridine (BP-type) analogs to the 3'-overhang region of the RNA-strand and changed the sequences of the passenger strand in the miR-143 duplex. Here, we demonstrated the anti-tumor effect *in vitro* and *in vivo* of miR-205 that was also chemically modified by BP and had altered passenger sequence. *In vitro* experiments, transfection with the synthetic miR-205 (miR-205BP/S3) significantly inhibited the growth of human melanoma A2058 and Mewo cells. Exogenous miR-205BP/S3 suppressed the protein expression levels of *E2F1* and *VEGF*, which are validated targets of miR-205-5p, and *BCL2*, a transcribed molecule of E2F1, as did Pre-miR-205-5p. Based on the results of a luciferase activity assay, miR-205BP/S3 directly targeted *E2F1*, as did Pre-miR-205-5p. However, miR-205BP/S3 was much more resistant to RNase than Pre-miR-205-5p in fetal bovine serum and to RNase in mice xenografted with human melanoma tissues. Additionally, the intratumoral injection of miR-205BP/S3 exhibited a significant anti-tumor effect compared with the case of control miRNA or Pre-miR-205-5p in human melanoma A2058 cell-xenografted mice. These findings indicate that miR-205BP/S3 is a possible promising therapeutic modality for melanoma.

B04 Generation of *in vitro* organoid cultures derived from patients with advanced prostate cancer. Dong Gao[1], Ian Vela[1,2], Andrea Sboner[7,8,9], Phillip Iaquinta[1], Wouter Karthaus[5], Catherine Dowling[1,2], Anuradha Gopalan[4], Jackline Wanjala[1], Myriam Kossai[7], Eva Undvall[1], John Wongvipat[1], Stephen B. Solomon[6], Himisha Beltran[7], Juan Miguel Mosquera[7], Ping Chi[1,3], Brett Carver[1,2], Mark Rubin[7,8], Howard I. Scher[3], Hans Clevers[5], Charles Sawyers[1,9], Yu Chen[1,3]. [1]Human Oncology and Pathogenesis Program (HOPP), [2]Department of Surgery, [3]Department of Medicine, [4]Department of Pathology, [6]Department of Medical Imaging, Memorial Sloan-Kettering Cancer Center, New York, NY, [7]Department of Medicine, Institute for Precision Medicine, [8]Department of Pathology and Laboratory Medicine, [9]Institute for Computational Biomedicine, Weill Cornell Medical College and New York-Presbyterian Hospital, New York, NY, [10]Howard Hughes Medical Institute, Chevy Chase, MD, USA, [5]Hubrecht Institute, Utrecht, The Netherlands.

Introduction and Objectives: The inability to propagate patient-derived prostate cancer cells *in vitro* is a major impediment in the mechanistic understanding of tumorigenesis and therapeutic response. In order to generate accurate *in vitro* models that represent the diversity of *in situ* prostate cancer, we have developed a three-dimensional "organoid" system to culture metastasis samples and integrated it into our precision medicine workflow of attaining and characterizing pre-treatment biopsies.
Results: We plated prostate cancer metastasis samples, freshly collected by tissue biopsy and confirmed by pathology review to have >10% tumor cell content, into prostate organoid culture

and established organoid lines from six patients that have been continuously propagated for > 6 months. A seventh organoid line was established from circulating tumor cells (CTCs) of a CRPC patient with a high CTC count (>100 cells per 8 mL of blood) which, to our knowledge, represents the first CTC-derived cancer cell line from any solid malignancy. The clinical characteristics and prognostic variables of the seven patients span the spectrum of advanced prostate cancer.
In the initial 32 samples, 6 continuous organoid cultures (~18%) were established from distinct sites. Tumor content of the biopsy represents a major determinant of organoid growth. Once established, organoids propagate indefinitely with different kinetics (~48 hr to 1 week doubling time), and can be cryopreserved. Histological analysis shows that the organoids in vitro and in vivo recapitulate the structure of the *in situ* cancer.
These lines harbor copy number signatures of primary prostate cancer, including SPOP mutation, PTEN loss, TMPRSS2-ERG interstitial deletion, as well as alterations commonly found in CRPC including TP53, PIK3R1, FOXA1 and several chromatin modifier mutations. Further, the organoid lines recapitulate the phenotypic diversity of CRPC, including AR-dependent adenocarcinoma, AR-negative adenocarcinoma, neuroendocrine carcinoma, and squamous differentiation. Importantly, these lines are amenable to drug testing in vitro and in vivo.
Conclusion: This novel tissue culture technique enables the development of new cell lines derived from metastatic deposits. This advance will facilitate research by availing new and varied cell lines, which will hopefully be more closely aligned to the spectrum of behavior of the clinical disease in comparison to the limited and problematic cell line models currently available.
Funding: Prostate Cancer Foundation, MSKCC SPORE (P50 CA092629), Research and Therapeutics Program in Prostate Cancer, Geoffrey Beene Cancer Research Center.

B05 A model of tumor heterogeneity reveals vascular mimicry as a driver of metastasis in breast cancer. Elvin Wagenblast[1], Simon Knott[1], Mar Soto[1], Sara Gutiérrez-Ángel[1], Annika L Gable[1], Chuck J Harrell[2], Charles M Perou[2], John E Wilkinson[2], Gregory J Hannon[1]. [1]Cold Spring Harbor Laboratory, NY, US, [2]University of North Carolina at Chapel Hill, NC, USA.

Metastasis requires that primary tumor cells evolve the capacity to intravasate into the lymphatic or cardiovascular systems, extravasate into a target organ, and colonize secondary sites that are physiologically distinct from that of the primary. We have developed a mouse model to probe the role of breast tumor heterogeneity in multiple stages of disease, from primary tumor growth to the establishment of metastases, using molecular barcodes and next-generation sequencing technologies. We found that distinct clones display specialization, for example dominating the primary tumor, contributing to metastatic populations, or showing tropism for entering the lymphatic or vasculature systems. We correlated these stable properties to distinct gene expression profiles. Those clones that efficiently entered the vasculature expressed two secreted proteins that were necessary and sufficient to program these cells for vascular mimicry. Vascular mimicry is a recently described phenomenon wherein highly aggressive tumor cells form channels that emanate from the tumor to the vasculature to distribute blood to hypoxic regions of the tumor. We show that vascular mimicry drives the ability of some breast tumor cells to contribute to distant metastases while simultaneously satisfying a critical need of the primary tumor to be fed by the vasculature. Enforced expression

of these proteins in human breast cancer cell lines also enabled vascular mimicry, and both proteins were overexpressed preferentially in human patients that had metastatic relapse. Thus, these two secreted proteins, and the phenotype they promote, may be broadly relevant as drivers of metastatic progression in human cancer.

B07 Single-cell transcriptome analysis reveals cellular and molecular basis of intra-tumoral heterogeneity in hepatocellular carcinoma. Xin Wang[1,2,3], Yang Liu[2,3] , Xuerui Yang[1,2,3].
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In many cancers including hepatocellular carcinoma (HCC), intra-tumoral cell populations display remarkable heterogeneity, which is believed to be both cause and consequence of tumor development and progression. A comprehensive view on intra-tumoral heterogeneity therefore should provide valuable information of important cancer-related events such as tumor development, metastasis, drug sensitivities, etc. Traditional profiling techniques generate snapshots of molecular abundance averaged on thousands to millions of cells, therefore falling short of capturing the heterogeneous nature of tumors.
In the present study, we performed extensive single-cell transcriptome profiling, combined with a series of computational data analysis and experimental research, to systematically study the intra-tumoral heterogeneity of HCC at the single-cell resolution. Specifically, we first obtained transcriptome profiles of >200 single-cells isolated from a HCC tumor. These single-cells were then clustered into transcriptionally distinct sub-populations based on their gene expression profiles. Phenotypic differences among these sub-populations were established by *in vitro* assays of cell proliferation, migration, invasion, and *in vivo* tumorigenesis tests. Different but cooperative roles of tumor cell sub-populations in tumor development were proposed and experimentally validated, revealing cellular basis of tumorigenesis at the single-cell resolution. On the other hand, by taking advantages of the large gene expression dynamic ranges resulted from intra-tumoral heterogeneity, we reconstructed a patient/tumor-specific gene transcription regulatory network with these single-cell gene expression profiles. This “personalized” regulatory network therefore captures gene transcriptional regulatory machineries that are inferred from and for an individual HCC tumor. Interrogation of the network and further experimental studies identified transcription factor master regulators that drive the transcriptomic differences between single-cell sub-populations and thereby their tumor-related phenotypic differences. Taken together, our extensive interrogation of single-cell transcriptome profiles generated a comprehensive map of intra-tumoral heterogeneity, shedding lights on cellular and molecular basis of tumorigenesis. The data, methodology, and mechanistic information are also valuable resource for further research on patient-specific tumorigenesis mechanisms in HCC, and potentially for the development of personalized medicine.

B08 HIF-2α, a novel target of thioredoxin, promotes metastasis of hepatacellular carcinoma. Manqing Cao, Ti Zhang. Tianjin Medical University Cancer Institute & Hospital, Tianjin, China.

1. An introductory sentence indicating the purposes of the study

Hypoxia is an important factor involved in the progression of solid tumors and has been associated with various indicators of tumor metabolism, angiogenesis and metastasis. The hypoxia-inducible factors (HIFs) are identified as key molecular players when exposed to hypoxia. HIF-2α primarily responses to chronic hypoxia which is contrary to HIF-1α mainly reacted to acute hypoxia. However, we found HIF-2α was stabilized under conditions of normoxia in two hepatacellular carcinoma (HCC) cell lines MHCC97H and MHCC97L which have a relatively high metastasis potential. In these two cell lines, HIF-2α is active under non-hypoxic conditions, creating a pseudo-hypoxic phenotype with clear influence on tumor behavior. Our results show that thioredoxin (TXN) stabilizes HIF-2α to induce hypoxia-responsive genes under normoxic conditions in HCC cells. Thus we hypothesized that abnormal expression of HIF-2α might be a result of high level of TXN which means TXN could analog pseudo-hypoxic environment to stimulating expression of HIF-2α under normoxia. In recent years, HIF-2α regulation has gained significant prominence in tumor biology. High levels of HIF-2α have been related to advanced stage and/or poor patient outcome in several tumor forms. Among these are non-small cell lung cancer, neuroblastoma, renal cell carcinoma, and colorectal cancer, but the contribution of HIF-2α to the HCC has not come to an agreement. Here we sought to explore the regulation of HIF-2α and its role in the metastasis of HCC.
2. A brief description of pertinent experimental procedures
1) Immunohistochemistry was employed on the tissue microarray paraffin sections of surgically removed samples from HCC patients with clinic pathological data. The correlations between the expression of HIF-2α/TXN and prognosis of patients were investigated.
2) Small RNA interference and lentivirus transfection targeting TXN, HIF-2α or nontargeting control were used to regulate TXN and HIF-2α expression in HCC cells.
3) The levels of protein knockdown and overexpression were confirmed by western blotting, mRNA levels were identified by real-time PCR.
4) Transwell assay was used to determine invasive and migration ability of tumor cells.
3. A summary of the new, unpublished data
Our findings suggest that high HIF-2α and TXN expression are both correlated with poor prognosis of HCC. TXN is able to target HIF-2α for stabilization during normoxia in HCC cells. HIF-2α and its downstream target gene CUB domain–containing protein 1 (CDCP1) and frataxin are all subjected to the government of TXN. Our data support a role for HIF-2α as a TXN target gene involved in the regulation of cancer metastasis and provide evidence that HIF-2α may be a critical factor promoting tumor metastasis. More broadly, the mechanism of HIF-2α in promoting HCC may through its unique target gene CDCP1 that has been identified participating in the renal cell carcinoma metastasis through interacting with PKCδ.
4. A statement of the conclusions. Authors must accept sole responsibility for the statements in their abstract
In conclusion, our studies demonstrate that TXN and HIF-2α are both overexpressed in HCC and correlated with poor prognosis. HIF-2α is a novel target of TXN and act as a critical factor promoting tumor metastasis in HCC. These findings have important implications for cancer therapy making the HIF-2α an important target for improving treatment of malignant diseases.

B09 Deregulation of SIPA1 in tumor progression: Insights from epigenetic and tumor microenvironment studies. Wei Wang, Yilei Zhang, Ang Lu, Li Su. Key Laboratory of Molecular

Biophysics of the Ministry of Education, School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China.

SIPA1 (signal-induced proliferation-associated protein 1) is a RapGAP that can increase the hydrolysis of Rap1GTP to Rap1GDP. Accumulated evidences have shown that deregulated SIPA1 level contributes to tumorigenesis and metastasis. For example, SIPA1 could promote tumor invasion and metastasis in prostate, melanoma and breast cancer cells. It could accelerate cell proliferation in colorectal cancer. Our previous study identified that nuclear SIPA1 could activate integrin β1 promoter and promote breast cancer cell invasion and execute these functions independent of Rap1. However, how is SIPA1 deregulated in these tumors? Deciphering the underlying mechanisms will be of great help for controlling SIPA1 properly and subsequent therapeutic purpose in clinic.

Epigenetic modifications, such as DNA methylation and histone modifications are crucial for packaging and interpreting the genome in the presence of diverse physiological stimuli. Rap1GAP, another important member of RapGAPs, has been found down-regulated in many malignant tumors such as melanoma and thyroid cancer, which due to its promoter hypermethylation. Is there hypermethylation on SIPA1 promoter? Bioinformatics analysis indicated that a candidate sequence starting from -147bp to +218bp within *SIPA1* gene was the most inclined to be methylated. We initially used methylation-specific PCR for SIPA1 to detect the methylation status of the *SIPA1* promoter (from -111bp to +174bp) in eleven different tumor cell lines. *SIPA1* promoter methylation was found in eight of the tumor cell lines (SK-BR-3, MCF7, MDA-MB-361, SW480, Caco2, LNCaP, U87, U251), who consistently had decreased SIPA1 expression compared with the other three cell lines (MDA-MB-231, HCT116, PC3), as measured earlier by Western blot. Next, we will determine whether the demethylating agent 5-aza is effective in demethylating the *SIPA1* promoter in certain tumor cell lines, as well as the regulating mechanism involved.

Tumor cells trigger serial signaling pathways through critical receptors in response to extracellular stimuli such as hormone, growth factor and chemokine distributed widely in tumor microenvironment, which of course regulates gene expression if necessary. We investigated *SIPA1* gene promoter activity in HEK293 and MDA-MB-361 cells, and found that the relative luciferase reporter activity decreased in HEK293 but increased in MDA-MB-361 cells upon stimulating with serum. These results suggest that different response and regulation networks exist in the two cells. By immunofluorescent staining analysis of SIPA1 in63 breast cancer specimens, we found that SIPA1 expression was reversely correlated with positive estrogen receptor α (ERα) status. Next, we assessed the expression level of SIPA1 and ERα in different breast cancer cell lines. The results showed that SIPA1 level is higher in ERα-negative MDA-MB-231 than that in ERα-positive MCF7 and MDA-MB-361 cells. Transient overexpressing ERα in MDA-MB-231 cells decreased SIPA1 level in both dose and time dependent manners. This result was further confirmed by MDA-MB-231 cell line stably expressing ERα protein. The detailed mechanism by which ERα regulates SIPA1 expression is still under investigation.

Taken together, we believe that the deep understanding why SIPA1 is deregulated in tumors may support its serving as a novel therapeutic target in cancer treatment.

B10 Activation of p53-miR-192-5p/215-XIAP signaling by curcumin provokes apoptosis in lung cancer. Ming-Xiang Ye[1, 2, 3], Jiän Zhang[2, 3], Jian Zhang[1]. [1] Department of Pulmonary Medicine, Xijing Hospital, [2] Department of Biochemistry and Molecular Biology, [3] State Key Laboratory of Cancer Biology (CBSKL), Fourth Military Medical University, Xi'an, China.

Curcumin is a highly pleiotropic agent especially for cancer prevention and treatment in past decades. Previous studies have highlighted curcumin acts as an epigenetic agent on miRNAs. In the present study, we confirmed cytotoxic effects of curcumin on H460 and A427 lung cancer cells *in vitro* and *in vivo*. miRNAs microarray and qPCR indicated miR-192-5p and miR-215 were the most upregulated and responsive miRNAs upon curcumin treatment. Functional studies showed miR-192-5p/215 were potential tumor suppressors in lung cancer, and antagonizing miR-192-5p/215 expressions abrogated curcumin's cytotoxicity. Curcumin also upregulated miR-192-5p/215 expressions in A549 cells (p53 wild type) but not in H1299 cells (p53 null). By using the tetracycline inducible expression system (Tet-on system), it was noted conditional knockdown of p53 significantly attenuated curcumin-induced miR-192-5p/215 upregulation in the p53 wild-type H460, A427 and A549 cells. Conversely, ectopic expression of exogenous p53 in the p53 null H1299 cells enabled miR-192-5p/215 response, suggesting curcumin and miR-192-5p/215 converge at p53. Finally, X-linked inhibitor of apoptosis (XIAP) is proved to be a novel target of miR-192-5p/215. Upregulation of p53-responsive miR-192-5p/215 suppressed XIAP expressions, thereby promoted caspase-3 activation, PARP fragmentation and provoked apoptosis. Taken together, this study highlights the proapoptotic effects of curcumin depend on miR-192-5p/215 induction and p53-miR-192-5p/215-XIAP axis is an important therapeutic target for lung cancer.

B11 Identification of metastasis-associated proteins in head and neck cancer by comparative tissue proteomics. Kai-Ping Chang[1]; Lang-Ming Chi[2]. [1]Departments of Otolaryngology-Head & Neck Surgery, [2]Molecular Medicine Research Center, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan.

Purpose: Cervical lymph node metastasis represents the major poor prognosticator for head and neck cancers. Here, we aimed to identify novel markers that are potentially useful for the prediction of cervical metastasis of head and neck cancers.
Experimental design: An iTRAQ-based quantitative proteomic approach was employed to identify proteins that are differentially expressed between microdissected primary and metastatic head and neck cancers. The selected candidates were examined in tissue sections via immunohistochemistry, and their roles in head and neck cancers cell function (proliferation, migration and invasion) investigated using RNA interference.
Results: We identified 67 differentially expressed proteins in nodal metastases, including PRDX4 and P4HA2. Immunohistochemical analysis revealed significantly higher levels of PRDX4 and P4HA2 in tumor cells than adjacent non-tumor epithelia ($P < 0.0001$ and $P < 0.0001$, respectively), and even higher expression in the 31 metastatic tumors of lymph nodes, compared to the corresponding primary tumors ($P = 0.060$ and $P < 0.0001$, respectively). Overexpression of PRDX4 and P4HA2 was significantly associated with positive pN status ($P = 0.048$ and $P = 0.041$, respectively). PRDX4 overexpression was a significant prognostic factor for

disease-specific survival in both univariate and multivariate analyses (*P* = 0.034 and *P* = 0.032, respectively). Additionally, cell migration and invasiveness were attenuated in OECM-1 cells upon *in vitro* knockdown of PRDX4 and P4HA2 with specific interfering RNA.
Conclusions: We have identified two novel metastasis-related prognostic markers for head and neck cancers.

B12 Downregulation of the KLF4 transcription factor inhibits cell proliferation and migration and causes cell death on canine mammary gland tumor cells. Yung-Tien Tien[1], Mei-Hsien Chang[1], Pei-Yi Chu[2], Chen-Si Lin[1], Chen-Hsuan Liu, Albert Taiching Liao[3].
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Canine mammary gland tumor (cMGT) is the most common tumor in female dogs and over 50% of cMGT were diagnosed as malignant. Although the high incidence and malignancy of cMGT are recognized, there are still no useful adjuvant treatments to elongate patient survival time after surgical removal. Krüppel-like factor 4 (KLF4) is one of transcription factor in KLF families and is related to the regulation of cell bio-functions including proliferation, differentiation, migration and apoptosis. It was firstly characterized its tumor suppressor activity in various human cancers, such as colorectal cancer, bladder cancer, gastric cancers, prostate cancers, lung cancers, and Hodgkin lymphoma. However, some studies identified KLF4 might have oncogenic activity in other tissue such as breast cancer. In this study, we firstly identify 844bp sequences of canine Klf4 mRNA. Canine KLF4 exhibits high homology with other species (human, mouse and rat), especially human KLF4. The sequences of the last 106 amino acids (c-terminal domain) among these 4 species are the same. Moreover, the sequence of the last 131 amino acids of the c-terminal domain of canine and human KLF4 are identical. Immunohistochemical examination revealed diverse levels of KLF4 expression in various normal canine tissues including intestine, skin epithelium, thyroid, parathyroid, cardiac muscle, kidney, and mammary gland. Overexpression of KLF4 mRNA and protein was identified in three cMGT cells, CMT-1, MPG and CF41.Mg. Madin-Darby canine kidney (MDCK) cells also express KLF4, whereas A72 (canine fibroblast) cells do not express KLF4. Administration of KLF4 inhibitor, Kenpaullone, downregulated the expression of KLF4 mRNA and protein. The expression of P53, which is downstream molecule of KLF4, was also reduced by Kenpaullone. Downregulation of KLF4 expression inhibited cell proliferation on MDCK cells. However, it did not cause cell death. Downregulation of KLF4 expression by Kenpaullone also inhibited cell proliferation, migration and colony formation in soft agar and, more important, caused cell death on cMGT cells. In conclusions, overexpression of KLF4 is important for proliferation, migration, growth in soft agar and survival of cMGT cells and may contribute to their tumorigenesis.

B13 HGAL/GCET2 is highly sensitive for detecting germinal center B-cells derivation lymphomas. Hongyang Pan[1, 2], Xinxia Zhang[1], Yuekai Zhang[1], Fan Ren[1], Aihua Li[3], Taiying Chen[3], Ren Zhou[2]. [1]Abcam (Hangzhou) Biotechnology Co., LTD., Hangzhou, China, [2]Institute of pathology and Forensic Medicine, Department of pathology and pathophysiology, Judicial

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of adult B-cell lymphomas, and well known to be highly heterogeneous both histologically and clinically. Through gene expression profiling, DLBCLs can be divided into at least three subgroups: germinal center B-cell like (GCB) DLBCLs, activated B-cell like DLBCLs, and primary mediastinal large B-cell lymphomas. Today, although many DLBCL algorithms are in use, it is still impossible to distinguish GCB from non-GCB cases with high certainty.
HGAL/GCET2, which is a B-cell specific marker, expressed in normal germinal center B-cells and in lymphomas of GCB derivation, including DLBCL, follicular lymphoma (FL), and Burkitt lymphoma. Using RabMAb® technology, we developed a rabbit monoclonal antibody, clone EP316, with the specificity to HGAL/GCET2. The specificity of EP316 was confirmed by ELISA, immunohistochemistry, immunocytochemistry and flow cytometry. Sixty-eight (68) cases of DLBCLs were analyzed with the panel of markers included in Hans and Choi algorithms. HGAL/GCET2 was added to the two algorithms to test whether the sensitivity of detecting germinal center B-cell cases could be improved. The immunohistochemistry results showed that the addition of HGAL/GCET2 significantly increased the detection of GCB DLBCLs. The rates of GCB DLBCLs in Hans and Choi algorithms were 25% (17/68) and 28% (19/68), respectively. Interestingly, with the inclusion of HGAL/GCET2, the proportions of GCB DLBCLs increased to 53% (36/68) and 50% (34/68), respectively. In the same study, we detected five FLs and three Burkitt lymphomas, and they were both 100% positive for HGAL/GCET2. However, in FLs, the positive rates of CD10 and BCL6 were only 40% (2/5) and 20% (1/5), respectively. Furthermore, our results showed that the C-MYC high expression (>40%), which was associated with inferior survival, was detected in 16.7% (7/42) of HGAL/GCET2 negative DLBCLs and only 3.8% (1/26) of HGAL/GCET2 positive DLBCLs.
Based on results from this study, we conclude that 1) rabbit monoclonal antibody EP316 is a highly specific and sensitive tool for detecting HGAL/GCET2 protein in formalin-fixed and paraffin-embedded tissues; 2) inclusion of HGAL/GCET2 to Hans and Choi algorithms can improve the certainty of distinguishing GCB from non-GCB cases; and 3) HGAL/GCET2 is a sensitive marker for GCB derivation lymphomas and its expression may be associated with better prognosis.

B15 Loss of N-myc downstream-regulated gene 2 is involved in tumor progression via regulating epithelial-mesencymal transition in gallbladder carcinoma. Hyo Jin Lee[1], Dong Gwang Lee[2], Sang-Hyun Lee[2], Hyewon Ryu[1], Jeong-Ki Min[2], Jin-Man Kim[1]. [1]Chungnam National University, Korea; [2]Korea Research Institute of Bioscience and Biotechnology, Korea.

Background: N-myc downstream-regulated gene 2 (NDRG2) is a candidate tumor suppressor gene because it induces apoptosis in several cancer cells, and its transcription is down-regulated or absent in cancer cell lines and some human cancers. Additionally, the protein expression of NDRG2 in several tumors including gallbladder cancer was down-regulated compared to non-neoplastic tissues, suggesting that it plays an important role in tumorigenesis. However, the exact role of NDRG2 in gallbladder carcinoma has not been investigated.

Materials and Methods: NDRG2 expression and its clinical implications as well as biological role of NDRG2 in gallbladder carcinoma were evaluated. The expression of NDRG2 protein was studied by immunostaining in 72 patients who underwent cholecystectomy for gallbladder carcinoma. Biological function was assessed by proliferation assay, anchorage-independent growth assay, chemotactic and trans-endothelial cell migration assays, and invasion assay in gallbladder carcinoma cell lines, in which NDRG2 was genetically knocked-down or over-expressed, coupled with an investigation of the effects of NDRG2 expression on the epithelial-mesenchymal transition.
Results: NDRG2 was differentially expressed in patients with gallbladder carcinoma. A loss of NDRG2 tended to show higher grade, lymphatic invasion and distant metastasis. Furthermore, NDRG2-negative tumors were significantly associated with perineural invasion. About survival, multivariate analyses indicated that a loss of NDRG2 was an independent poor prognostic factor for cancer-specific survival (Hazard ratio, 6.621; 95% confidence interval, 1.852-23.673; *P* = 0.004). NDRG2 expression inhibited the proliferation, anchorage-independent growth, chemotactic and trans-endothelial migration, and invasion of gallbladder cancer cells. NDRG2 expression also modulated the expression of epithelial-mesenchymal transition-related genes such as Slug and downstream signaling in gallbladder carcinoma cells.
Conclusions: These results indicate that NDRG2 was lost in some patients with gallbladder carcinoma, and its loss was involved in the tumor progression. *In vitro* experiments demonstrated that NDRG2 expression suppressed the aggressive behavior of cancer cells via inhibiting the epithelial-mesenchymal transition and cellular signaling linked to tumor progression, collectively suggesting that it could be a useful biomarker in patient with gallbladder carcinoma and a therapeutic target.
Emerging risk-stratified approaches to the management of diffuse large B-cell lymphoma (DLBCL) will critically depend on real time cell-of-origin subtyping. Gold-standard microarray gene expression classification methods are unsuitable for routine use and immunohistochemistry algorithms lack precision; migration assays are therefore under evaluation. Here, we investigated the potential of QuantiGene Plex, a branched DNA signal amplification assay, as a low-cost option with a simple automatable workflow and high detection sensitivity directly from formalin-fixed paraffin-embedded (FFPE) tissue.
We previously identified 21 genes differentially expressed between ABC/GCB cell-of-origin subtypes in 40 diagnostic FFPE samples. The resultant 37-probeset transcriptional signature was trained using naïve Bayes classification in a publically available DLBCL dataset. The signature achieved mean AUC values >0.9 upon independent validation in fresh-frozen and FFPE samples. We then tested ability to recapitulate microarray results, including cell-of-origin classification, using immunohistochemistry, qRT-PCR or Quantigene Plex as migration assays.
Compared to qRT-PCR, QuantiGene Plex was superior in terms of proportion of validated targets (85.7% vs 47.4%), positive correlation with array data ($p<0.0001$) and cell-of-origin classification accuracy (92.1% vs 78.9%). These results suggest that QuantiGene Plex is promising for cell-of-origin subtyping of DLBCL, and supports the case for large-scale comparison with alternative approaches currently under development.

B16 Overexpression of Rab25 contributes to CSC stemness and EMT occurrence of nasopharyngeal carcinoma and highly related to NPC radioresistance. Lu Zhang[1], Xinghui

Yang[1], Nianhua Ding[1], Zhi Li[1], Jiang He[1], Yuanzhen Qiu[2], Lun-Quan Sun[1]. [1]Center for Molecular Medicine, Xiangya Hospital, Central South University; [2]Department of ENT, Xiangya Hospital, Changsha, China.

Nasopharyngeal carcinoma (NPC) differs from other head and neck cancers by its unique characteristics including epidemiology, pathological types and therapeutic managements. Radiotherapy is the primary therapeutic modality. However, about 20% of NPC patients develop local recurrence after radiotherapy, and radioresistance is a major cause of treatment failure in many cases. Rab25, a member of the Rab11 superfamily of small GTPases, controls the recycling of proteins from endosomes to plasma membrane. Rab25 is closely related to the progression of many cancers, particularly in tumor migration and metastasis by regulating cell polarity and inducing epithelial-mesenchymal transition (EMT). In this study, we focused on whether Rab25 contributed to the radioresistance of NPC (in vitro and in vivo) and the possible underlying mechanisms for development of radioresistance. We previously established a radioresistant NPC cell line CR. Compared with the parental cell line (CNE2), CR cell was resistant to irradiation at high dose and exhibited a series of EMT-like characters, such as the cadherin switch from E-cadherin to N-cadherin and resistance to anoikis. We found that Rab25 was highly expressed in CR cells and its expression was closely related to the EMT-like phenotypes. Knockdown of Rab25 by stable transfection of shRNA in CR cell reversed the expression of EMT-like biomarkers (E-cadherin, N-cadherin, and Vimentin) and increased the sensitivity of cell to anoikis. In addition, cell radiosensitivity was leveled up when Rab25 down-regulated. In order to validate the relationship among Rab25 expression, EMT occurrence, and radioresistance in vivo, we developed a radioresistant tumor model in nude mice and examined the EMT molecular markers by immunohistochemistry. We found that E-cadherin re-localized from cell membrane to cytoplasm and N-cadherin expressed highly in cytoplasm in tissues from radioresistant tumor. Rab25 was found in 2 out of 4 radioresistant tumors and its expression was not detected in four tumors initiated from CNE2 cells. On the other hand, overexpression of Rab25 in CNE2 cell raised the expression level of mesenchymal markers (N-cadherin and Vimentin). FACS analysis indicated that cells overexpressed Rab25 showed a stronger aldehyde dehydrogenase (ALDH) activity and higher expression of stem cell markers including ABCG2, CD24, CD29, and CD49b. Western blot analysis showed that Wnt/β-Catenin signaling pathway was activated by overexpression of Rab25 in CNE2 cell. These data indicated that the NPC cells may acquire radioresistance through up-regulation of Rab25, which facilitates the Wnt secretion or recycling, thus leads to an increase of CSC stemness. *This work is supported by Ministry of Science and Technology, China (2013CB967203).*

B17 LMP1-mediated repression of PERK contributes to high level of cellular ROS that drives NPC stem cell proliferation and self-renewal. Jiang He, Yiting Lin, Danming Ou, Liyu Liu, Zhi, Li, Lu Zhang, Lun-Quan Sun. Center for Molecular Medicine, Xiangya Hospital, Central South University, Changsha, China.

Latent membrane protein 1 （LMP1）, which is an Epstein–Barr virus (EBV)-encoded oncoprotein, induces nuclear factor-kappa B (NF-□B) signaling by mimicking the tumor necrosis factor receptor (TNFR). LMP1 signals primarily from intracellular compartments in a ligand-

independent manner. Here, we demonstrated that LMP1 could induce ROS generation in nasopharyngeal carcinoma cells (NPC). To explore the underlining mechanism, we found that PERK (PKR-like endoplasmic reticulum kinase) as a novel LMP1-interacting molecule, could be suppressed by LMP1.
Previous studies identified the Nrf2 transcription factor as a PERK substrate. Nrf2, a member of p45 NF-E2–related proteins (p45 NF-E2, Nrf1, Nrf2, and Nrf3), is the key transcription factor responsible for transcription of antioxidant genes. Studies using Nrf2-deficient mice and microarray-based assays suggested that Nrf2 modulated transcription of almost 200 genes whose protein products function as antioxidants, phase II detoxification enzymes, proteosomes, heat-shock proteins, and glutathione-synthesis enzymes. In unstressed cells, Nrf2 is maintained in the cytoplasm via association with Keap1. PERK-dependent phosphorylation triggers dissociation of Nrf2/Keap1 complexes and inhibits re-association of Nrf2/Keap1 complexes in vitro. Activation of PERK via agents that trigger the unfolded protein response is both necessary and sufficient for dissociation of cytoplasmic Nrf2/Keap1 and subsequent Nrf2 nuclear import. We showed that CNE1 and SUNE1 cells expressing LMP1 down-regulated Nrf2 target genes compared with that of CNE1 and SUNE1 cells transfected with control vector. The previous reports indicated p42/p44 MAPK consists of two serine/threonine protein kinases that modulate the activity of Nrf2, but some studies demonstrated that p42/p44 MAPK is not involved in regulating Nrf2 activity. To test whether the p42/p44 MAPK pathway is involved in LMP1-induced Nrf2 activity, we treated LMP1-transfected CNE1 and SUNE1 cells with PD98059, a MAP/ERK (MEK) inhibitor. The LMP1-induced Nrf2 activity detected by luciferase reporter activities was not affected by PD98059. Thus, p42/p44 MAPK signaling is not involved in regulation of LMP1-induced Nrf2 activity. Thus, we demonstrate that LMP1 inhibits PERK activity, in turn suppresses Nrf2 activity to increase cellular ROS level by a direct interaction with PERK without involvement of MAPK pathway. To explore whether cellular ROS level affects stemness of cancer stem cells (CSC), we examined the CSC sphere formation capacity and the stemness gene expression after elimination of ROS. Our results demonstrated that the numbers of CSC spheres decreased and stemness genes expression down-regulated after elimination of ROS. Together, the present data suggest a novel function of EBV oncoprotein LMP1 that drives NPC stem cell proliferation and self-renewal via elevating cellular ROS through LMP1-PERK-Nrf2 pathway. This work is supported by Ministry of Science and Technology, China (2013CB967203).

B18 Identification of resistance mechanisms in erlotinib-resistant subclones of the non-small cell lung cancer cell line HCC827 by exome sequencing. Kirstine Jacobsen, Nicolas Alcaraz, Rikke Lund, Henrik Ditzel. University of Southern Denmark, Denmark.

Background: Erlotinib (Tarceva®, Roche) has significantly changed the treatment of non-small cell lung cancer (NSCLC) as 70% of patients show significant tumor regression upon treatment (Santarpia et. al., 2013). However, all patients relapse due to development of acquired resistance, which in approximately half of the cases is due to a secondary mutation (T790M) in EGFR (Pao et al. 2005), and in 5-10% of cases is due to amplification of MET (Bean et al. 2007). Importantly, a significant fraction of resistant tumors are still unexplained (Lin et. al., 2012). Our aim was to identify novel resistance mutations in erlotinib-resistant subclones of the NSCLC cell line, HCC827.

Materials & Methods: We established 3 erlotinib-resistant subclones (resistant to 10, 20, 30 µM erlotinib, respectively). DNA libraries of each subclone and the parental HCC827 cell line were prepared in biological duplicates using the SeqCap EZ Human Exome Library v3.0 kit and whole-exome sequencing of these (100 bp paired-end) were performed on an Illumina HiSeq 2000 platform. Using a recently developed in-house analysis pipeline the sequencing data were analyzed. The analysis pipeline includes quality control using Trim-Galore, mapping and alignment using BWA, removal of PCR duplicates using Picard Tools, followed by single nucleotide polymorphism (SNP) calling using Strelka, SomaticSniper and VarScan2 together with insertion-deletion (INDEL) calling using Strelka and VarScan2.
Results: The resistant and sensitive clones exhibited a significant difference in viability over a time course of 25 days when treated with erlotinib. Importantly, the resistant clones did not acquire the T790M or other EGFR or KRAS mutations, potentiating the identification of novel resistance mechanisms in these clones. For the sensitive and the 3 resistant clones, an average 93% of the exome was sequenced to a depth of on average 25x (from 23x – 26x) of which on average 49% (from 46 – 51 %) of the exome was covered by at least 20x. During SNP and INDEL calling, we filtered all somatic mutations common between the sensitive and the resistant clones, and only analyzed mutations that were acquired by the resistant subclones compared to the parental cell line. A total of 9131 resistance-associated SNVs were identified of which 270 were identified by 2 callers and 100 were identified by all 3 callers utilized, on average in the 3 subclones. From the identified SNVs, 75, 96 and 87 were predicted to be non-synonymous in the 3 resistant subclones, respectively.Ten of these SNPs, distributed in 9 genes, were common to all 3 resistant subclones. On average 284 INDELs (from 275 – 299) were identified in the 3 resistant subclones, of which on average 6 (from 3 – 8) were found in exonic regions. However, no common INDELs were found.
Conclusion: We established 3 erlotinib-resistant NSCLC subclones, which did not harbor any of the common resistance mutations, potentiating the identification of novel resistance mechanisms. By exome sequencing we identified both novel and previously reported mutation SNVs and INDELs, including 10 novel non-synonymous SNVs common to the 3 resistant subclones. These SNVs might indicate a common molecular mechanism of erlotinib resistance in NSCLC and may form the basis for future medical intervention in the large number of patients with erlotinib-resistant NSCLC.

B19 The crosstalks between astrocytes and cancer cells in brain metastasis and chemoresistance. Qing Chen[1], Andrienne Boire[1,2], Manuel Valiente[1], Ruzeen Pawta[1], Xin Jin[1], Ke Xu[3] and Joan Massagué[1]. [1] Cancer Biology and Genetics Program, [2] Department of Neurology, [3] Molecular Cytology Core Facility, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Metastasis presents a major threat to the lives of cancer patients. Although the primary tumors can be surgically removed, the mortality rate of cancer remains high, mainly due to metastatic relapses. Brain metastasis is increasingly becoming a significant clinical problem and its incidence is rising. This is largely attributable to the fact that current therapies, which are effective in controlling extracranial metastasis that prolong patient survival, are ineffective in controlling metastatic disease of the brain. Thus, it is of our interest to study the biology of metastatic cancer cells in the brain microenvironment. Astrocytes, the unique brain stromal

cells, are activated at very early stage and accumulating around metastatic lesions. Our recent work has shown that astrocytes are the cellular source of the killing factor, soluble Fas ligand (sFasL), to the surrounding metastatic cells. Here, we focus on the protective effect of astrocytes on the invaded cancer cells, indicating the dual functions of astrocytes in the brain metastatic microenvironment. We develop in vitro co-culture, in vivo co-injection and brain metastasis assays to demonstrate the contribution of astrocytes in tumor growth and chemoresistance. Mechanistically, we identify protocadherin 7 (PCDH7), a member of cadherin family, as a mediator of gap junction formation between cancer cells and astrocytes. PCDH7 is highly expressed in the breast and lung carcinoma cells with high brain metastatic potential, as well as in astrocytes. At the molecular level, PCDH7 interacts with gap junction α1 (GJA1), the gap junction protein expressed in brain metastatic cancer cells to form intercellular channels with astrocytes. Finally, loss-of-function experiments indicate that PCDH7 and GJA1 protect cancer cells from environmental (sFasL) and chemotherapeutic stresses and facilitate brain metastasis. Overall, by mediating contact-dependent interactions, PCDH7 facilitate cancer-astrocyte gap junction communications to promote brain metastasis. This study also suggests PCDH7 and gap junctions as potential therapeutic targets of brain metastasis.

B20 The role of NFATc2 in regulating tumor initiating cell phenotype in non-small cell lung cancer. Zhi-Jie Xiao, Jing Liu, Vicky Pui-Chi Tin, Maria Pik Wong. Department of Pathology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR China.

Background: Targeting specific pathways responsible for Tumor Initiating Cells (TIC) regulation is postulated to be an important therapeutic strategy for more effective control of tumors. Nuclear Factors of Activated T-cells (NFATs), consisting of 4 homologues (NFATc1-c4), are calcium-dependent transcription factors that are activated upon dephosphorylation by the protein phosphatase calcineurin. NFAT is known to be involved in T cell activation and differentiation. Recently, the role of NFATc2 as a cancer regulator has been reported in breast, colon and pancreatic cancers. However, the role of NFATc2 in lung cancer, especially in TIC maintenance, remains unknown.
Objective: To study whether NFATc2 is involved in TIC phenotype regulation in non-small cell lung cancer (NSCLC).
Methods and results: The expression of NFATc2 in a panel of established and early passage patient derived NSCLC cell lines were first analyzed at the mRNA and protein levels, which showed NFATc2 was up-regulated in 92.6% (25 of 27) of cancer cells compared to normal bronchial cell line (BEAS-2B). To investigate the involvement of NFATc2 in TIC regulation, TICs were isolated as tumor spheres or by flow cytometry using $ALDH^{high}/CD44^{high}$ as markers. The expression of NFATc2 and downstream targets were significantly higher in tumor spheres and $ALDH^{high}/CD44^{high}$ cells compared to the corresponding monolayers and $ALDH^{low}/CD44^{low}$ cells. Correspondingly, luciferase reporter assay also showed higher NFAT activities in tumor spheres compared to monolayers. To study its functional importance, NFATc2 was stably knocked-down using lentiviral shRNA, which led to down-regulated expression of pluripotent genes (*SOX2, OCT4, NANOG*) and TIC markers (*ALDH1A1, CD133, CD166*) in both established (HCC827) and early passage patient derived (PTC02) NSCLC cell lines. Phenotypically, shNFATc2 inhibited formation of tumor spheres for 2 generations and suppressed cell migration. Further, NFATc2

knockdown cells showed reduced tumorigenic ability *in vitro* and *in vivo,* demonstrated by anchorage independent growth assay and mice xenografts compared to shRNA control. Besides, investigation of its role in drug resistance showed NFATc2 knockdown sensitized cancer cells to both short term paclitaxel and long term cisplatin treatments using the apoptosis assay and colony formation assay. In contrast to the effects of NFATc2 knockdown, over-expression promoted *in vitro* sphere formation, cell migration, tumorigenesis and drug resistance. To explore its clinical relevance, NFATc2 expression was analyzed in human resected lung cancers by quantitative PCR and immunohistochemsitry. Results showed that NFATc2 expression was significantly up-regulated in clinical NSCLC compared to corresponding normal lung (p =0.003), and higher NFAT expression predicted shorter disease free survival (p=0.036).
Conclusions: NFATc2 plays an important role in NSCLC tumorigenesis and TIC phenotypic regulation. Inhibition of this pathway is a potential approach for long term lung cancer control.

B21 TGFβ/Smad4-dependent EMT results in a lethal imbalance of master regulator transcription factors in pancreas cancer cell. Charles J. David, Yun-Han Huang, Joan Massague. Cancer Biology and Genetics, MSKCC, New York, NY, USA.

TGFβ is a powerful tumor suppressor in gastrointestinal cancers, with frequent mutations in *TGFBR2* and *Smad4* observed in gastric, colorectal, and pancreatic cancer. To investigate the molecular mechanisms of TGFβ tumor suppression in pancreas cancer, we have employed Smad4-null cells from a KrasG12D-driven mouse model of pancreatic ductal adenocarcinoma (PDAC). Re-expression of Smad4 in these cells depletes tumor-initiating capacity without affecting growth of established tumors. In vitro, Smad4-reintroduction sensitizes cells to TGFβ-induced EMT, followed by apoptosis which occurs after a lag time of approximately 36 hours. Importantly, blocking EMT by shRNA-mediated depletion of Snail rescues Smad4-restored PDAC cells from TGFβ-induced apoptosis in vitro, and restores the tumor-initiating capacity of these cells in vivo. Conversely, Snail overexpression prior to TGFβ treatment accelerates TGFβ/Smad4-dependent cell death. In addition to factors that drive EMT, an shRNA screen identified an additional Smad4-induced factor, Sox4, as a mediator of cell death in Smad4-restored PDAC cells. Interestingly, in Smad4-null PDAC cells, Sox4 is highly expressed, and is required for tumor growth. To identify potential modifiers of Sox4 activity that might contribute to the functional switch from growth/survival factor to pro-apoptotic factor, we identified other highly expressed transcription factors (TFs) using RNA-seq. Using this approach, we identified several known enforcers of epithelial phenotype that are among the most highly expressed TFs in PDAC cells. These putative epithelial master regulators are deeply downregulated in a Snail-dependent manner after 24 hours of TGFβ treatment in Smad4-restored cells. Overexpression of putative epithelial master regulators was sufficient to reverse the TGFβ-induced EMT and rescue the cell death phenotype. These results suggest that modulation of the cellular master regulator TF landscape underlies the protracted process of TGFβ-induced apoptosis.

B22 Novel function of STAT1 pathway as an oncogenic driver in serous papillary endometrial cancer. Budiman Kharma, Tsukasa Baba, Noriomi Matsumura, Hyun Sook Kang, Ryusuke Murakami, Ken Yamaguchi, Junzo Hamanishi, Masaki Mandai, Ikuo Konishi. Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Serous papillary endometrial cancers (SPEC) are highly progressive with poor prognosis, and its oncogenic profile is known different from endometrioid endometrial cancers. It, however, still remains unclear that which pathway promotes tumor progression. The IFN-γ transcription factor signal transducer and activator of transcription 1 (STAT1) has been considered as a tumor suppressor with transcription-dependent and transcription-independent mechanisms; however, recent studies exhibited STAT1 was associated with poor prognosis in some cancers but the mechanism was remained unclear, including serous papillary endometrial cancer (SPEC), which is highly progressive with poor prognosis.
In this study, our genome-wide analysis by microarray analysis revealed STAT1 pathway was significantly highly activated in SPEC, and its expression was confirmed significantly higher in SPECs tissue cancer by immunohistochemical staining ($p<0.001$) in Kyoto cohort as well as Vancouver cohort. Immunohistochemical staining also exhibited co-localization of ICAM-1 and PD-L1 at tumor frontier with CD8-T cells ($p<0.001$) as well as STAT1 expression in SPECs. Using a SPEC cell line, SPAC-1L, it was confirmed that IFN-γ induced STAT1 expression not only to promote cellular proliferation ($p<0.05$), adhesion ($p<0.0001$), and invasion ($p=0.0002$), but to induce expression of cMyc, ICAM-1 and PD-L1 ($p<0.05$). In contrast, suppression of STAT1 attenuated induction of these genes ($p<0.05$) and inhibited xenograft tumor growth on NOD-SCID mice ($p<0.0001$).
These results indicate that STAT1 pathway is specifically activated in SPECs to be associated with their aggressive features. Concerning immune-activity at tumor microenvironment, targeting STAT1 pathway with attenuation of tumor-immunity could be a potent candidate in the treatment of SPEC.

B23 Detection of PNPO as an ovarian tumor progression marker mediated by TGF-β1 signaling. Lingyun Zhang, Jimin Shi, Guoxiong Xu. Center Laboratory, Jinshan Hospital, Fudan University, Shanghai, China.

Pyridoxine 5'-phosphate oxidase (PNPO) is a conversion enzyme of pyridoxal phosphate, an active form of vitamin B6, and has been found to be expressed in several cancer cell lines. However, the expression of PNPO in ovarian cancer (OC), the most lethal gyneacological malignancy, has not been studied and whether its expression is mediated by transforming growth factor-β (TGF-β), a cytokine that regulates a variety of cellular functions in ovarian carcinogenesis, is unknown. The present study examined the expression of PNPO in human ovarian tumors, including benign, borderline and malignant tumors, by immunohistochemistry (IHC) and the effect of TGF-β1 on PNPO expression in OC cells by quantitative real-time PCR (qPCR) and Western blot analysis. IHC showed that PNPO was overexpressed in human ovarian surface epithelial tumors, including serous, mucinous and clear cell tumors. The immunoreactive staining of PNPO was strong in borderline and malignant tumors, weak in benign tumor and normal ovarian tissue. Statistical analyses showed that the expression of PNPO was significantly higher in malignant tumor than in benign tumor, indicating that PNPO may be involved in tumor progression. Subsequently, we investigated the influence of TGF-β signaling on the regulation of PNPO expression in two human serous ovarian cancer cells (SK-OV-3 and OVCAR-3). We found that these cells expressed PNPO and were responsible to TGF-β1 as showing an increase in

phospho-Smad2, a TGF-β1 signaling protein, detected by Western blot analyses after 24 hours stimulation. Furthermore, the downregulation of PNPO mRNA expression was observed in SK-OV-3 cells after 1 and 10 ng/ml TGF-β1 treatment for 24 hours detected by qPCR. The decrease of PNPO protein expression was dose-dependent both in SK-OV-3 and OVCAR-3 cells after TGF-β1 (0, 1 and 10 ng/ml) stimulation. Pre-treatment of SB-431542, a TGF-β type I receptor kinase inhibitor, abolished the inhibitory effect of TGF-β1 on PNPO expression. Taken together, PNPO was overexpressed in human ovarian malignant tumor. TGF-β1 significantly decreased the expression of PNPO at mRNA and protein levels which was blocked by its receptor inhibitor in OC cells, indicating that the expression of PNPO in ovarian cancer may be mediated by the TGF-β signaling pathway. *(This study was supported by grants National Natural Science Foundation of China, the Shanghai Committee of Science and Technology, and Shanghai Municipal Health Bureau).*

B24 Honokiol inhibits cancer cell stemness maintenance of bladder cancer through EZH2/miRNA-143 axis. Junlong Zhuang[1,2,3], Wei Zhao[1], Qing Zhang[1,2,3], Cunjie Chang[1], Changxiao Ye[1,2,3], Yangyan Cui[1], Hongqian Guo[2,3], and Jun Yan[1]. [1]MOE Key laboratory, Model Animal Research Center, Nanjing University, Nanjing, China, [2]Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, China, [3]Nanjing Urology Research Center, Nanjing, China.

Background: Urinary bladder cancer (UBC) is the most common urogenital malignant tumor, with high frequency of tumor recurrence. Cancer stem cells (CSCs), a small portion of cancer cells among tumor, is considered as one of the major reasons for tumor recurrence. Honokiol, a small-molecule polyphenol isolated from several species of the genus *Magnolia*, had been reported to have anti-tumor properties against leukemia, lung and colon cancer in vitro and in vivo. However, its anti-cancer mechanism has not yet been characterized in UBC cells. The aim of this study is to clarify the roles of Honokiol in bladder cancer and its underlying molecular mechanism in regulating stemness maintenance.
Methods: To evaluate the effects of Honokiol on UBC cells in vivo, we administrated Honokiol i.p. into the xenograft model of human UCB. The kinetics of tumor growth, tumor size and weight was analyzed. The immunohistochemical assessment of tumor proliferation and cancer stem cells function were also carried out. Western blotting and quantitative RT-PCR were performed to analyze CSC markers and miRNA expression levels. Chromatin immunoprecipitation (ChIP) assay was performed to assess the recruitment of EZH2 onto the miR-143 promoter.
Results: Honokiol inhibited tumor growth of human UBC cells T24 in nude mice significantly, without affecting body weight of mice, which suggests the biosafety of Honokiol in vivo. Immunohistochemical staining results demonstrated that Honokiol significantly reduced cell proliferation marker (Ki67) and CSC marker (CD44) in the experimental group, as compared with the control group. Western blotting assays further demonstrated that Honokiol treatment remarkably reduced protein levels of CD44, cleaved Notch1, Sox2, EZH2 and H3K27me3 level, with the concomitant induction of miR-143 by quantitative RT-PCR. We also found that Honokiol decreased self-renewal capacity of CSC-like cells by sphere formation and ALDH1a activity assay, associated with the reduced expression of CSC markers (CD44, cleaved Notch1, Sox2 and EZH2) in vitro. Since Honokiol inhibited EZH2 expression, the central epigenetic regulator of CSC

function both in vitro and in vivo, we knocked down EZH2 in both T24 and 5637 bladder cancer cells. Depletion of EZH2 reduced UBC cell proliferation, colonigenicity and CSC maintenance, with the induction of miR-143, whereas overexpression of miR-143 showed the same cytotoxic effects. Rescue assay by using miR-143 inhibitor showed that blocking of miR-143 induced by EZH2 depletion, reversed the loss of EZH2 induced cytotoxicity and CSC stemness. ChIP assay further corroborate that miR-143 is direct target of EZH2.
Conclusion: Our data suggested that Honokiol treatment may repress UCB through cancer stem cells function in vitro and in vivo. These findings increased interest in bringing Honokiol to the clinic as a novel chemotherapeutic agent to UCB.

B25 Parathyroid hormone-like hormone serves as a prognostic indicator in head and neck squamous cell carcinoma. Zhongjing Lv[1,2], Xiangbing Wu[1,2], Jianjun Zhang[1,2], Ming Yan[1,2], Wantao Chen[1,2*]. [1]Department of Oral and Maxillofacial-Head and Neck Oncology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. [2]Shanghai Key Laboratory of Stomatology and Shanghai Research Institute of Stomatology.

Background: In recent years, gene alteration has been the hotspot of research on malignant tumors. These genes not only play an important role in the pathogenesis of cancer but can also be used as biomarkers to predict the effect of treatment and clinical prognosis. In our previous study, parathyroid hormone-like hormone (PTHLH) was revealed to be up-regulated in head and neck squamous cell carcinoma (HNSCC) compared with paired adjacent normal tissue using an Affymetrix chip. However, the function and prognostic indicators of PTHLH in HNSCC remain obscure.
Methods: In this study, we first investigated the expression of PTHLH in 9 HNSCC cell lines and in 36 paired HNSCC specimens, including cancerous tissues and adjacent normal tissues, by reverse transcription-polymerase chain reaction (RT-PCR), real-time PCR and western blotting. The biological function of PTHLH was investigated using small interfering RNA (siRNA) in WSU-HN6, HN13 and CAL-27 HNSCC cells, and immunohistochemistry was used to estimate the prognostic value of PTHLH in patients with HNSCC.
Results: This study showed that the expression of PTHLH in 9 HNSCC cell lines was much higher than that in normal epithelial cells (p<0.0001), and we found higher expression of PTHLH in 36 HNSCC tissues than that in paired, adjacent normal tissues (p=0.0001). The results showed that the down-regulation of PTHLH by RNA interference could reduce cell proliferation and inhibit colony formation in HNSCC cell lines. The results also showed that the high expression of PTHLH was associated with poor pathological differentiation (p=0.0001) and poor prognosis (p=0.0003) in patients with HNSCC.
Conclusions*:* This study suggests that PTHLH is up-regulated in HNSCC specimens and cell lines. Therefore, PTHLH could play a role in the pathogenesis of HNSCC by affecting cell proliferation, and the protein levels of PTHLH might serve as a prognostic indicator for evaluating patients with HNSCC.

B26 Transcriptional control of PAX4-regulated miR-144/451 modulates metastasis by suppressing ADAMs expression. Jianjun Zhang, Xing Qin, Qiang Sun, Furong Xie, Qin Xu, Ming Yan, Zeguang Han, Wantao Chen*. Department of Oral and Maxillofacial-Head & Neck Oncology

and Faculty of Oral and Maxillofacial Surgery, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China.

Paired box gene 4 (PAX4) is a transcriptional modulator located on chromosome 7q32, and its expression is dysregulated in a variety of human cancers, suggesting that PAX4 may be important in multiple tumors as a driver gene. Here, we show that PAX4 promoted migration and invasion in human epithelial cancers by decreasing miR-144/451 expression levels. Accordingly, miR-144/451 suppressed the migratory and invasive phenotypes, even in PAX4-expressing cells. Mechanistically, miR-144/451 inhibited cancer cell metastasis by targeting the A disintegrin and metalloproteinase (ADAMs) protein family members ADAMTS5 and ADAM10, the dysregulation of which is associated with increased invasiveness and reduced patient survival in certain epithelial cancers, thereby indirectly affecting epithelial plasticity. This discovery suggests that a PAX4-miR-144/451-ADAMs axis regulates human epithelial cancer metastasis, thus opening up therapeutic possibilities for these cancer types.

B27 Tumor suppressive role of protein tyrosine phosphatase receptor delta under energy stresses. Dong Hu,[1*] Robert Banh[2], Richard Marcott [2], Ruiguang, Zhang [3], Jiahua Zhang[1], Hongli Liu [3], Benjamin Neel [2]. [1]Institute for Stem Cell Application and Research, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, [2]Ontario Cancer Institute, University Health Network, Toronto, Ontario, Canada [3]Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

Protein tyrosine phosphatase receptor delta (PTPRD) has been found to mutated, methylated or deleted in wide varieties of tumors such as neuroblastoma, lung, breast and colorectal cancers. To investigate whether PTPRD might function as a supper suppressor during cancer development, we generated PTPRD (pBABE) over-expressing stable cell lines models in H23, ABC-1 and H4 cell lines by retrovirus infection. We found that the expression level in this model is comparable to the endogenous expression of PTPRD in various positive cell lines or primary cells, indicating it may serve as a better model than the transient transfection models in which over-expressed protein is expressed numerous folds higher than physiological level. Using these models, we found that its tumor suppressive role is not evident under normoxia condition in either model. However, when we cultured the cells under hypoxia condition, the cell growth of cells expressing pBabe PTPRD wildtype are significantly inhibited when comparing to pBabe puro control group ($p<0.05$) in all the three cancer cell lines after 48 and 72hours of culture under 0.1% O2 condition. Meanwhile, we generated PTPRD knock-down model using a PTPRD positive cell line H460 by stably expression two different PTPRD shRNAs (pLK.O) using lentivirus infection strategy. We found that under hypoxia condition, the cell growth of H460 in both PTPRD knock-down groups are significantly higher than the shRNA control group ($p<0.05$) at 48 and 72 hours, but not under normoxia condition. Moreover, we generated a stable PTPRD knock down model using primary human lung cell line Beas2b and found that under starvation condition, when cells are incubated in HBSS instead of culture medium for 18 hours, there is significantly higher level of conversion of autophagic biomarker LC3-I to LC3-II in two different knock-down groups than in the shRNA control group, indicating a higher level of autophagy induction in the knock-down groups. In general, our results show that that under hypoxia

conditions, higher level of PTPRD confers suppressive roles on tumor survival, moreover, under nutrient starvation condition lower level of PTPRD may confer protective role on tumor survival through mechanisms like autophagy. The results indicate that PTPRD tends to exhibit tumor suppressive roles under energy stress conditions and in-depth mechanisms underlying the role involving hypoxia metabolism and autophay will be investigated.

B29 Secreted frizzled-related protein-2 is engaged in prostate cancer acquired resistance conferred by the tumor microenvironment damaged by genotoxic therapies. Fei Chen, Yue Dai, Yu Sun. Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.

Tumor microenvironments activated by first rounds of genotoxic therapies confer significant acquired resistance to subsequent anticancer treatments, and represent a major clinical barrier toward effective elimination of initiated and/or disseminated malignancies. However, the individual roles of extracellular factors released from stroma into the microenvironmental niches remain largely unexplored. Here, we show that secreted frizzle-related protein 2 (SFRP2), a modulator of Wnt signaling, is remarkably overexpressed by human primary prostate stromal cells as part of tissue non-autonomous responses to chemotherapy and radiation, classic genotoxic methodologies in modern clinical oncology. SFRP2 increased prostate cancer epithelial cell proliferation, migration, invasiveness, and protected against apoptosis induced by mitoxantrone or gamma radiation. Interestingly, SFRP2 is implicated in both canonical and non-canonical Wnt axes, by augmenting Wnt16B activities and wiring Wnt/Ca2+ pathway, the latter evidenced by an increase of NFATc3 in the nuclear fraction of SFRP2-treated cells. However, the canonical Wnt signaling activated by SFRP2 appeared a dominant driving force in contributing resistance to cancer cells. Such acquired resistance to anticancer treatments can be abrogated by Wnt16B antibody-mediated therapy, both *in vitro* and *in vivo*. Therefore, SFRP2 is an active and functional effector released from damaged tumor microenvironments; Wnt16B may be a novel, specific and favorable target for future therapeutic intervention of solid tumors including advanced prostate malignancy. Further, our study provides a realistic vista that synergistic chemoimmunotherapeutic regimen is a potent strategy for adjusting conventional anticancer agents to simultaneously target cancer cells and handle the tumor microenvironment, eventually promoting the overall efficacy of targeted therapeutics.

B31 Tumor suppressor protein DAB2IP participates in spindle assembly checkpoint and maintains chromosomal stability in prostate cancer cells. Lan Yu[1,*], Zeng-Fu Shang[1,*], Jer-Tsong Hsieh[3,4], Benjamin Chen[1,4] and Debabrata Saha[1,4]. Department of Radiation Oncology[1] and Urology[3], University of Texas Southwestern Medical Center, Dallas, TX, USA, [2]Department of Oncology, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan, [4]Simmons Comprehensive Cancer Center, Dallas, TX, USA.

DAB2IP has been reported as a novel tumor suppressor in prostate cancer (PCa), suppresses the PI3K-Akt pathway and enhance ASK1 activation leading to cell apoptosis. Decreased expression of DAB2IP is often detected in prostate cancer cells and linked with an increased risk of aggressive prostate cancer. This loss of DAB2IP is primarily due to altered epigenetic regulation

of its promoter. It is also reported that the loss of DAB2IP can lead to radiation resistance, increased DNA repair ability, and decreased apoptosis after radiation.
Recently, we discovered that DAB2IP plays a regulatory role in Spindle assembly checkpoint (SAC). SAC is a molecular device that prevents separation of the duplicated chromosomes until each chromosome is properly attached to the spindle microtubules through the formation of mitotic checkpoint complex (MCC) and inhibits the interaction between MCC and APC/C complex.
Here, we revealed that DAB2IP is cell-cycle regulator that localizes at mitotic apparatus during normal mitosis progression, including centrosome, kinetochore and midbody. We further demonstrated that DAB2IP participates in spindle checkpoint maintenance (SAC) and inhibits turnover of MCC-APC/C complex via blocking SAC dependent Cdc20 degradation. Moreover, direct interaction between DAB2IP and Cdc20, APC/C complex was demonstrated by co-immunoprecipiation. Consistent with this observation, we also found that the depletion of DAB2IP dramatically sacrifices nocodazole and paclitaxel –induced spindle checkpoint. Overexpressed DAB2IP in C4-2 prostate cancer cells can lead to metaphase arrest and stabilize the cyclinB1 protein. More importantly, we further noticed that DAB2IP can inhibit chromosomal instability (CIN), which is a major driving force of tumorgenesis. This is the first time we are reporting a novel function of DAB2IP on mitotic SAC regulation which is an essential pathway for maintaining chromosomal stability and demonstrate its tumor suppressive function.

B33 TAZ promotes cell growth and inhibits Celastrol-induced cell apoptosis in HEK293 cell.

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Hippo pathway is a highly conservative signaling pathway, which adjusts the organ size, maintains the dynamic balance of cell proliferation, apoptosis, and other functions. With the abnormal Hippo pathway, the cells will proliferate excessively or apoptosis insufficiently, and the organ will grow out of control, which eventually lead to the occurrence and the development of tumor. Moreover, TAZ/YAP, as the major downstream effectors in Hippo pathway, have been researched widely. Previous studies showed that the overexpression of YAP could promote cell proliferation and inhibit cell apoptosis. Hence, we assume that TAZ, which is 60% homologous with YAP, probably has the same function. To test this issue, we established a stable system of TAZ in the HEK293 cells.

To better evaluate the effect of TAZ on HEK293 cells, cell growth curve and colony formation assay were performed. HEK293/TAZ and HEK293/control cells were plated at a density of 50 cells/mm^2 in triplicate for 5 days. Cell numbers were counted every day. In addition, HEK293/TAZ and HEK293/control cells were seeded into six-well plates of 500 cells/well for two weeks. The result showed that HEK293/TAZ grew obviously faster than HEK293/control cells. Besides, HEK293/TAZ cells could form more colonies than HEK293/control cells. To further address the effect of TAZ *in vivo*, equal numbers ($1.5x10^6$)of HEK293/TAZ and HEK293/control cells were injected bilateral subcutaneously into nude mice. The mice were sacrificed 40 days

after injection. The weights of the tumors were measured. We found that the latency of tumor formation in nude mice injected with HEK293/TAZ cells was shorter than the tumor injected with HEK293/control cells. Furthermore, HEK293/TAZ cells could lead to a larger tumor size compared to the HEK293/control cells. Our data indicated that the overexpression of TAZ could significantly promote cell proliferation *in vitro* and *in vivo*.

To determine whether TAZ overexpression could inhibit cell apoptosis, HEK293/TAZ and HEK293/control cells were treated with Celastrol(0.75μM)for 24h . Western blotting result showed that the less PARP cleavage was definitely observed in HEK293/TAZ cells than HEK293/control cells. Moreover, TUNEL assays were performed. We found that 18.9% of HEK293/TAZ cells were TUNEL-positive, compared with 83.2% of HEK293/control cells after Celastrol treatment (0.75 μM, 24 h). Our data indicated that overexpression of TAZ could inhibit partially the Celastrol-induced cell apoptosis *in vitro*.

In summary, our finding demonstrated that TAZ as an oncogene plays an important role in the occurrence and the development of tumor, however, the mechanism and the function of TAZ needs to be further researched.

B34 Integrin α9 and WNT7A genes are hypermethylated and downregulated in nasopharyngeal carcinoma. Imran Nawaz, Zi-Ming Du, Ilya Ignatyev, Tatiana V Pavlova, Vladimir Kashuba, Khalid Moumand, Eugene R Zabarovsky, Ingemar Ernberg, Li-Fu Hu[1]*. Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden.

Epigenetic silencing of tumor suppressor genes (TSGs) by DNA promoter methylation is an early event in the multi-step process of carcinogenesis. Human chromosome 3 (Chr. 3) contains clusters of TSGs involved in many cancer types including nasopharyngeal carcinoma (NPC), the most common cancer in Southern China. Using Not1 microarray (NMA) on Chr. 3, ten candidate TSGs were found. Among them, Integrin alpha-9 (ITGA9) and Wingless-type Mouse mammary tumor virus integration site family, member 7A (WNT7A) were confirmed to be methylated by bisulfite sequencing. ITGA9 is a member of the integrin family, glycoproteins, which regulate cellular behavior by cell adhesion to the extra cellular matrix (ECM). WNT7A maintains epithelial differentiation. It has been demonstrated to be a TSG in lung cancer, and inhibits growth of the transformed cells in a subset of human Non-Small Cell Lung Cancer (NSCLC).
Bisulfite sequencing showed that CpG-rich regions in the promoter of ITGA9 and WNT7A were hypermethylated in NPC, compared to normal nasopharyngeal tissue. 5-aza-2’ deoxycytidine (5-aza-CdR) treatment restored the expression of ITGA9 and WNT7A in NPC cell lines CNE1 and TW03. Immunohistochemistry showed strong expression of ITGA9 and WNT7A in the membrane and cytoplasm of normal adjacent nasopharyngeal epithelium cells, while weak expression of ITGA9 and WNT7A was observed in NPC tumor cells. The average expression levels of ITGA9 and WNT7A in NPC tumors by Q-PCR were downregulated as 3 and 4.9 fold respectively, compared with their expression in normal epithelial tissues.
The ITGA9 methylation was detected by MSP in 58.8% (20/34) EBV positive cases of NPC with specificity 100% specificity (0/18 of non-cancerous controls) while detection rate for WNT7A

methylation was 68.8% (11/16) in EBV positive NPC with 79.8% (3 out of 18 in non-cancerous controls).
Taken together, it suggests that they might be TSGs in NPC. As both proteins execute significant functions related to the tumor cell biology, the potential of ITGA9 and WNT7A as therapeutic targets for NPC should be considered.

B35 Ectopic expression of JMJD3 inhibits hepatocellular tumor growth by regulating DACH1 expression. Zhangang Xiao[1] and Yangchao Chen[1, 2*]. [1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, [2]Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China.

Background JMJD3 is a jmjd domain containing histone demethylase which can remove methyl groups from lysine 27 of histone 3 (H3K27) to active histone methylated genes. Previous studies have demonstrated that JMJD3 played a crucial role in inflammation. The role of JMJD3 in caners is still unclear. Our study is to investigate the role of JMJD3 in hepatocellular carcinoma (HCC).
Methods The expressions of JMJD3 and its targets in HCC were measured by immunohistochemistry in tissue microarray. The effects of ectopic expression of JMJD3 in *in vitro* models were investigated using cell viability assay, invasion assay and chromatin immunoprecipitation (ChIP) assay. The *in vivo* anticancer activity of JMJD3 was evaluated in HCC xenografted nude mice model.
Results Our study indicated that JMJD3 was significantly down-regulated in HCC cell lines and tissues. Over-expression of JMJD3 inhibited HCC cell growth, transformation, invasion *in vitro* and suppressed tumor formation *in vivo*. We examined the potential downstream targets of JMJD3 in order to explore the mechanism underlying tumor suppressive roles of JMJD3 in HCC. We identified Dachshund homolog (DACH1) as one of JMJD3 downstream targets. It is shown that the expression level of DACH1 was positively correlated with that of JMJD3 in HCC. ChIP assay showed DACH1 promoter was in a higher H3K27 tri-methylation (H3K27me3) status in HCC cell lines compared to non-tumorigenic liver cells. Over-expression of JMJD3 decreased DACH1 H3K27me3 level, indicating that DACH1 was epigenetically regulated by JMJD3.

Conclusion We identified JMJD3 as a tumor suppressor in HCC for the first time. One target of JMJD3, DACH1 was found to be epigenetically regulated by JMJD3 in HCC.

Acknowledgement
This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#11100452), General Research Fund (CUHK462211 and CUHK462713), Research Grants Council, Hong Kong SAR Government and Direct Grant from CUHK to YC.

B36 Loss of polarity protein AF6 promotes pancreatic cancer metastasis through inducing of Snail expression. Yi Xu, Lixing Zhan. Institute for Nutritional Sciences, Shanghai, China.

Pancreatic cancer is a particularly lethal form of cancer with high potential to metastasize to distant organs. Understanding the molecular mechanisms of metastatic tumors is crucial for successful development of novel therapies for pancreatic cancer. Here, we show that the polarity protein AF6 was frequently down-regulated in pancreatic cancer. Notably, decreased expression of *AF6* was associated with poor outcomes in pancreatic cancer patients. We additionally demonstrated that knockdown of *AF6* expression markedly increased proliferation of pancreatic cancer cells, and showed that the elevated metastasis of cancer cells was associated with increased Snail protein expression. We further characterized that the induction of *Snail* gene expression in AF6-deficient cells was mediated primarily by Dvl2, and that this induction was effected by Dvl2 activation of the binding of transcriptional factor FOXE1 to the *Snail* promoter region. In summary, our data identify AF6 as a novel metastasis suppressor that functions through the Dvl2-FOXE1-Snail axis. Thus, the AF6 protein may serve as a novel target for suppressing pancreatic cancer cell metastasis.

B37 Silence of HIC1 in triple-negative breast cancer mediates progression through LCN2 misregulation. Guangcun Cheng[1], Xueqing Sun[1], Jinglong Wang[1], Gang Xiao[1], Xiumin Wang[1], Xuemei Fan[1], Lidong Zu[1], Mingang Hao[1], Qing Qu[2], Yan Mao[2], Yunjing Xue[1], Jianhua Wang[1]*. [1]Department of Biochemistry and Molecular Cell Biology, Shanghai Key Laboratory of Tumor Microenvironment and Inflammation, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2]Comprehensive Breast Health Center, Rui Jin Hospital, Shanghai Jiao tong University School of Medicine, Shanghai, China.

HIC1 (hypermethylated in cancer 1) is a tumor suppressor gene frequently deleted or epigenetically silenced in many human cancers. Emerging evidence suggests that restoring HIC1 expression is responsible for an improved prognosis in multiple cancers. However, the role and regulatory mechanism of HIC1 in the triple-negative breast cancer (TNBC) still remain unclear. Here we showed that HIC1 expression was only silenced in TNBC as compared with the other molecular subtypes. *In vitro* and *in vivo* experiments indicated that restoring HIC1 expression in TNBC cells had a marked effect on reducing cell migration, invasion and metastasis. In contrast, reduction of HIC1 expression by shRNAs in MCF-10A and HBL-100 cells greatly increased its invasion ability. Microarray analyses, luciferase reporter assays and chromatin immunoprecipitation experiments identified Lipocalin2 (LCN2), a small secreted glycoprotein, as immediately downstream target of HIC1. Moreover, restoring LCN2 expression in HIC1 infected cells could partially rescue HIC1-induced abolition of cell invasion *in vitro* and metastasis *in vivo*. Mechanistic analyses showed that autocrine secretion of LCN2 induced by loss of HIC1 could activate AKT pathway through the potential NGALR receptor, associated with TNBC progression. These findings suggest that HIC1-LCN2 axis may serve as the subtype-specific prognostic biomarker and provide an attractive therapeutic target for TNBC.

B39 Cooperation of epithelial-mesenchymal transition and cellular stress response in promoting metastasis. Yu-xiong Feng[1], Dexter X. Jin[1,2], Piyush B. Gupta[1-5]. [1]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA, USA, [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA, [3]Koch Institute for Integrative

Cancer Research, Cambridge, MA, USA, [4]Harvard Stem Cell Institute, Cambridge, MA, USA, [5]Broad Institute, Cambridge, MA 02142, USA.

Epithelial-to-mesenchymal transition (EMT) plays a central role in cancer progression. Through an EMT, cancer cells acquire a spectrum of malignant properties, including invasiveness, chemo-resistance and stem-like traits. With an aim to effectively target EMT-cancer cells, we previously performed a high throughput chemical and RNAi screen, and discovered that EMT-cancer cells are hypersensitive to perturbations of endoplasmic reticulum (ER) function, which represents a specific vulnerability of cancer cells that have undergone this cell-state transition. This increased sensitivity to ER stress of EMT-cancer cells is largely due to the increased synthesis and secretion of extracellular matrix (ECM) proteins. In line with this fundamental change, EMT-cancer cells constitutively activate the PERK-ATF4 branch of the unfolded protein response (UPR) pathway. Surprisingly, we found that the PERK pathway, in addition to mediating ER stress response, is required for acquisition of malignant features of the EMT-cancer cells. Mechanistically, the PERK-ATF4 signaling cooperates with the EMT transcription factors to induce a CREB family transcription factor, which is critical for maintaining the EMT-specific secretome. Inhibition of this factor markedly suppresses EMT-driven metastasis, while overexpressing this protein endows non-EMT cancer cells an increased metastatic capacity. In summary, we discovered a non-canonical role of the PERK-ATF4 stress response pathway in driving metastasis.

B40 RhoE/ROCK signaling modulates chemoresistance in HCC through IL6/JAK2/STAT3 pathways. W. Ma, K.M. Sze, L.K. Chan, J.M. Lee, V.C. Cheung, T.K.W. Lee, C.C.L. Wong, I.O.L. Ng. Department of Pathology, Li Ka Shing Faculty of Medicine and State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong, China.

Liver cancer (hepatocellular carcinoma, HCC) is a major malignancy worldwide and the second commonest fatal cancer in Southeast Asia and China including Hong Kong, due to the high prevalence of hepatitis B viral infection. HCC is highly chemoresistant, limiting treatment options to patients. There is an urgent need to delineate the underlying molecular mechanism of HCC chemoresistance so as to identify novel therapeutic targets for this aggressive cancer. Deregulation of Rho GTPase pathway is demonstrated to play important roles in HCC tumorigenesis. RhoE/Rnd3 belongs to the Rnd subfamily of the Rho GTPase which lacks the intrinsic GTPase activity. In our previous study, we have shown that RhoE is frequently downregulated in human HCCs and acts as a metastasis suppressor, whereas ROCK2 is upregulated in human HCCs. In this study, we aimed to investigate whether RhoE is also involved in the regulation of chemoresistance in HCC. Using short-hairpin RNA and a lentiviral approach, we knocked down RhoE in BEL-7402 and MHCC-97L HCC cells. RhoE knockdown cells displayed increased resistance to chemotherapeutic drugs, cisplatin and doxorubicin. Knockdown of RhoE also reduced cisplatin-induced apoptosis. On the other hand, addition of ROCK inhibitor, Y27632, sensitized the HCC cells to these two drugs. We further demonstrated that knockdown of RhoE enhanced chemoresistance in an *in vivo* subcutaneous injection model in nude mice; treatment with cisplatin significantly suppressed the tumor growth of the non-target control group while it had no effect in the RhoE knockdown group. In addition, treatment with ROCK inhibitor Y27632 sensitized HCC cells to cisplatin treatment *in vivo*; co-treatment of

cisplatin and Y27632 suppressed subcutaneous tumor growth of BEL-7402 to a greater extent than cisplatin alone.
The downstream molecular targets of Rho/ROCK that regulates cell survival and chemoresistance remain largely unknown. Therefore we also investigated the molecular pathway which Rho/ROCK signaling might act on in mediating its pro-survival effect. IL6/JAK/STAT3 pathway has been shown to be important in regulating HCC chemoresistance, and previous studies have also suggested that Rho/ROCK pathway may interfere with the IL6/JAK/STAT3 pathway. We tested whether RhoE/ROCK modulated chemoresistance through IL6/JAK/STAT3 in HCC. Addition of Y27632 suppressed IL6 mRNA expression and IL6 secretion in BEL-7402 and SMMC-7721 HCC cells. We also observed up-regulation of JAK2 and STAT3 phosphorylation levels in the transient RhoE knockdown BEL-7402 cells using Western blot analysis. On the other hand, knockdown of ROCK2 and addition of Y27632 suppressed the phosphorylation of both JAK2 and STAT3. Dual luciferase reporter assay showed that the transcription activity of STAT3 was reduced upon Y27632 treatment in BEL-7402 and SMMC-7721. Immunohistochemical staining of the phospho-STAT3 in the subcutaneous tumors from nude mice treated with and without cisplatin and Y27632 confirmed that treatment of Y27632 also repressed STAT3 phosphorylation *in vivo*.
To conclude, downregulation of RhoE in HCC enhanced chemoresistance both *in vitro* and *in vivo* via upregulating the activities of ROCK. We identified the IL6/JAK2/STAT3 pathway to be a novel target of ROCK in promoting cell survival. Targeting the Rho/ROCK pathway may be a potential therapeutic in enhancing HCC treatment.

B41 GPR56 inhibits melanoma progression by internalizing TG2 in extracellular matrix.

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A critical component in tumor microenvironment is extracellular matrix (ECM). ECM is assembled by large polypeptides in the extracellular space. It forms a scaffold to support tissue structure and development and also serves as a repertoire of growth factors to promote cell growth. ECM proteins also directly bind to and signal through adhesion receptors, such as integrins, to regulate cell survival, proliferation, and migration. Excessive accumulation and crosslinking of ECM is a hallmark of cancer and has been shown to promote cancer progression by activating integrins and Rho GTPases. Its removal should have therapeutic benefits for cancer patients, but this potential has not been actively pursued. We recently discovered that a novel adhesion receptor, GPR56, inhibits melanoma growth and metastasis via removing its ligand, TG2, from ECM. GPR56 belongs to the family of adhesion G protein-coupled receptors (GPCRs), a group of important but poorly understood receptors. It was one of the down-regulated genes in the highly metastatic derivatives compared with the poorly metastatic melanoma parental line. Over-expression of GPR56 led to inhibition on melanoma growth and metastasis, and its knockdowns led to their enhancement. Results from later studies suggested that this inhibitory effect of GPR56 might apply to both BI-sensitive and BI-resistant melanomas, and probably occurs during the expansion of micrometastases into overt macrometastases. Our earlier work also discovered that TG2, a crosslinking enzyme in the extracellular matrix (ECM),

binds to the N-terminus of GPR56, linking GPR56 with ECM remodeling during melanoma growth and metastasis. Indeed, studies using the immunodeficient *Tg2*$^{-/-}$ mice showed that TG2 itself promotes melanoma metastasis but GPR56 antagonizes it. Biochemical analyses were performed to understand this antagonism and showed that GPR56 removes TG2 from melanoma cell surface via receptor-mediated endocytosis, leading to impaired ECM deposition and adhesion. These results provided the first line of evidence that ECM removal may be feasible for cancer treatment, and GPR56 may serve as a target for this ECM-based therapy in treating metastatic melanoma.

B42 MET suppresses epithelial VEGFR2 via intracrine VEGF-induced ER-associated degradation. Tom T. Chen[1], Ellen Filvaroff[1*], Jing Peng[2], Scot Marsters[1], Adrian Jubb[3], Hartmut Koeppen[3], Mark Merchant[2], and Avi Ashkenazi[1**].

Hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) drive cancer through their respective receptors, MET and VEGFR2. VEGFR2 inhibits MET by promoting MET dephosphorylation; however, whether MET conversely regulates VEGFR2 remains unknown. Here we show that MET suppresses VEGFR2 by inducing its endoplasmic reticulum-associated degradation (ERAD), via intracine VEGF action. HGF-MET signaling in epithelial cancer cells promoted VEGF biosynthesis through PI3-kinase. In turn, VEGF-VEGFR2 association within the ER activated inositol-requiring enzyme 1α, driving ERAD-mediated VEGFR2 depletion. MET disruption upregulated VEGFR2, inducing compensatory tumor growth via VEGFR2 and MEK. However, concurrent disruption of MET and either VEGF or MEK circumvented this, enabling more profound tumor inhibition. Our findings uncover unique cross-regulation between MET and VEGFR2 with potentially important therapeutic implications.

B43 Deciphering the role of large chromosome deletions in acute myeloid leukemia. Chong Chen[1], Yu Liu[1], and Scott W. Lowe[1,2]. [1]Cancer Biology and Genetics Program, [2]HHMI, MSKCC, New York, NY, USA.

Recurring deletions of chromosome 7 and 7q [-7/del(7q)] occur in myelodysplastic syndromes and acute myeloid leukemia (AML) and are associated with poor prognosis. However, the role of these chromosome alterations remains mysterious in AML over last half century. Using RNAi and CRISPR/Cas9 approaches, we show that a ~50% reduction in gene dosage of the mixed lineage leukemia 3 (MLL3) gene, located on 7q36.1, cooperates with other events occurring in -7/del(7q) AML to promote leukemogenesis. Mll3 suppression impairs the differentiation of HSPC. Interestingly, restoring the expression of Mll3 releases the differentiation block and impairs the progression of established AML. Further, shRNA library screening identifies additional tumor suppressors in -7/del(7q), whose loss might cooperate with Mll3 deficiency to promote AML. Thus, our in vivo functional analysis identifies tumor suppressors in -7/del(7q) in AML. Our murine AML models recapitulate the pathology and drug response of human disease and therefore would provide a unique tool to understand the pathogenesis and therapeutic susceptibility of human AML with these chromosome alterations.

B44 **Inhibiting cMET in cancer: Small molecule inhibitor NVP-INC280 versus antibody MetMAB.** Huaixiang Hao[1], Angela Tam[1], Qing Sheng[1], Xiaojian Xu[2], Yao Yao[2], Alan Huang[1].

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Several mechanisms of cMET activation exist in cancer: HGF ligand overexpression (autocrine or paracrine), *MET* gene amplification and mutation such as exon14 skipping. There are two modalities of inhibiting cMET: ATP-competitive small molecule inhibitor such as Novartis' NVP-INC280 and antibodies targeting HGF binding sites on cMET such as MetMAB from Genentech and LY2875358 from Eli Lilly. Among all the c-MET inhibitors, MetMAB (a unique one arm antibody to avoid agonist activity) is clinically the most advanced (Phase III). A comparative study and differentiation strategy is needed for the clinical development of small molecule cMET inhibitor NVP-INC280. In this study, INC280 and internally produced MetMAB were tested side by side in two cell lines with distinct types of cMET activation: U-87MG (glioblastoma line, HGF high) and MKN-45 (gastric line, *MET* amplification). As measured by both pMET inhibition and growth inhibition, INC280 and MetMAB showed differential activities in those cell line models. Detailed results will be discussed in the poster.

B45 A meta-analysis of somatic copy number alterations in hepatocellular carcinoma. K. Hao[1,2], Z.Zhang[1,2], Y. Hoshida[3], D. Sia[3,4,5], M. Mahajan[1,2], B. Zhang[1,2], V. Mazzaferro[4], R. Pinyol[5], X. Sun[3], E.E. Schadt[1,2], J.M. Llovet[3,5,6]. [1]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, [2]Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY, [3]Mount Sinai Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, [4]Gastrointestinal Surgery and Liver Transplantation Unit, National Cancer Institute, Milan, Italy, [5]HCC Translational Research Laboratory, Barcelona-Clinic Liver Cancer Group, Institutd'Investigacions Biomediques August Pi I Sunyer (IDIBAPS), CIBERehd, Liver Unit, Hospital Clinic, University of Barcelona, Catalonia, Spain, [6]Institucio Catalana de Recerca i Estudis Avancats (ICREA), Catalonia, Spain.

Introduction: Hepatocellular carcinoma (HCC) is the second leading cause of cancer mortality worldwide. With the development of high-throughput technology, several studies have surveyed genome-wide somatic copy number alterations (sCNAs) and unveiled a few altered genes as potential drivers. However, a comprehensive sCNA landscape in HCC and driver alterations are yet to be identified. Here we report the largest HCC sCNA study to date, a meta-analysis of 594 resected HCCs tomor-normal pairs with different etiologies and ethnic background.
Methods: We generated or downloaded whole-genome SNP array and whole-genome mRNA transcription array data of 594 HCCs tumor and adjacent normal tissues in three published studies (Hong Kong, n=162; Korean, n=286; and European Commission Framework Program 7 (HEPTROMIC), n=146). The individual sCNA profiles were quantified using a segmentation-based approach. To compare the results from different studies, the sCNA signals were properly rescaled with the germline copy number variants (CNVs) information detected in adjacent normal tissues. We applied GISTIC2 to the combined dataset as well as to ethnicity- and etiology-specific datasets. We quantify the CNV-transcription correlation to further refine the candidate list of HCC driver genes. Genes within these regions were compared with existing HCC

driver genes and established oncogenes and tumor suppressor genes (TSGs) in other tumors. Lastly, we overlap the known/candidate HCC driver genes under sCNA influence to liver co-expression networks to reveal module and biological function categories disrupted by sCNA. Results: The three cohorts analyzed consisted mostly of East Asian and European patients with HBV (n=388, 65%) or HCV infection (n=89, 15%). Broad gains and losses of chromosome arms detected in our analysis were consistent with previous studies. Among the focal CNVs, we identified focal amplifications at 11q13.3 (FGF19, CCND1), 6p21.1 (VEGFA), 8q24 (MYC), 5p15.33 (TERT), and 7q31.2 (MET), and focal deletions at 9p21.3 (CDKN2A/B), 1p36 (ARID1A), 13q14.2 (RB1), and 10q23.31 (PTEN), which were equally distributed among HBV and HCV-infected patients. Interestingly, focal deletion at 16p13.3 (AXIN1) occurred more frequently in HBV than HCV-infected individuals (111/388, 29% vs 9/89, 10%; p<0.01). Similarly, focal deletion of the potential TSG PTPRS (19p13.3) were found more frequent in
HBV-related patients (79/388, 20% vs 8/89, 9%; p=0.03). Previously unknown focal CNVs were also unveiled, such as focal amplifications at 19p13.11 (JAK3, 5%), 19p13.12 (NOTCH3, 4%)

B56 Pharmacological evaluation of anti-tumor agent using imaging-based orthotopic tumor models. Wenjie Gao, Jie Xu, Jiaying Hu, Wenying Wang, Kai Zhou, Lixia Bao, Dongrong Hao, Yunbiao Yan, Guizhu Yang, Yong Cang, Henry Lu, Norman Zhang, Oncology Business Unit, WuxiAppTec Co., Ltd.

Since its emergence in the mid 90's, the in vivo bioluminescent imaging (BLI) technology has been widely accepted by academia and pharmaceutical industry as a platform for research and pre-clinical drug development. The non-invasiveness and high sensitivity nature of BLI enables tumor lesions being detectable far before a palpable stage is reached, allowing longitudinal tumor tracking from early developmental stages. BLI is particularly advantageous for monitoring systemic and orthotopic tumors that are inaccessible by caliper measurement. Current preclinical evaluation of anti-cancer agents relies heavily on subcutaneous xenograft models. However, ectopically developed tumors in subcutaneous tissue differ drastically in tumor stromata in aspects of cell contents and drug permeability, thus will often poorly predict pharmacological efficacy of the test agents. The notion that orthotopic models can better recapitulate clinical tumor development and metastasis, thus better predict the clinical outcome of anticancer drugs has gradually gained acceptance. To better serve the need of pre-clinical cancer drug development, we have established over twenty orthotopic and/or metastatic models using luciferase expressing tumor cell lines. In all the models, we monitored tumor growth with bioluminescent imaging and observed that increase of the bioluminescent signal was accompanied with animal death. In the U87-luc glioma model, we showed that temozolomide treatment significantly suppressed the bioluminescent signal and prolonged animal survival. In the PC3-luc orthotopic prostate cancer model, we demonstrated therapeutic efficacy of Docetaxel on primary tumor and metastases to a number of organs including liver and lymph node. In conclusion, our study showed that the imaging based orthotopic models offer real time tracking of tumor development and metastases that can ready serve the oncology preclinical drug discovery need with incomparable data quality over conventional subcutaneous xenograft models.

Summary**:** We have established over twenty orthotopic tumor models and demonstrated the utility of these models for tracking the growth of primary tumor as well as the tumor metastases. A positive correlation between tumor size and photon intensity was established which supports the use of bioluminescent signals as readout of tumor growth. Some of the models were further validated with standard-of-care compounds, demonstrating that the therapeutic efficacy on both primary tumor development and tumor metastases was accompanied by prolongation of animal survival. These models will have great value in testing novel cancer-therapeutic compounds in a more clinically relevant setting.

B59 JNJ-42756493, a potent pan-FGFR family inhibitor, showed significant efficacy in HCC standard xenograft and PDX models with FGF19 amplification/overexpression. Liang Xie[1], Yiping Rong[1], Na Cheng, Peng Wu[1], Jingyu Zhang[1], Ziliang Qian[1], Ke Li[1], Xiaoyun Wang[1], Zhui Chen[1], Jennifer Yang[1]. Shanghai Discovery Center, Janssen China Research & Development. Shanghai, China.

HCC is the third leading cause of cancer-related deaths worldwide. Sorafenib is the only approved target therapy compound for advance HCC patients. However, nonclinical and preliminary clinical data indicate that sorafenib-mediated inhibition of VEGF driven angiogenesis may be overcome by activation of the FGF-pathway. With a median TTP of 2.8 to 5.7 months and a considerable number of subjects with severe side-effects or intolerance to sorafenib, there is an urgent medical need for an effective and well tolerated treatment for HCC patients. JNJ-42756493 is an orally bioavailable, potent pan-FGFR family inhibitor with single digit nanomolar biochemical inhibitory IC50s againstFGFR1-4. It has demonstrated potent inhibition of cell proliferation in a variety of FGFR pathway activated cell lines including HCC. In HCC cell lines, the sensitivity to JNJ-42756493 correlates with the secreted FGF19 protein level in cell supernatants. In amplified or overexpression FGF19 cell lines, JNJ-42756493 shows submicromolar IC50s in cell proliferation assay and dose dependent inhibition of phosphorylated extracellular signal-regulated kinase (pERK). Furthermore, JNJ-42756493 shows significant tumor growth inhibition in HCC Hep3B xenograft mouse model and HCC PDX models with FGF19 amplification/overexpression at 50 mg/kg qd dosing. The downstream pharmacodynamics marker, pERK is also downregulated after treatment with JNJ42756493 in vivo. These data support the potential clinical utility of JNJ-42756493 for the therapeutic treatment of HCC patients harbouring FGF19 amplification/ overexpression.

B61 A transient inactivation mode of action for a caretaker tumor suppressor that is indispensible for cell proliferation. Zhiyuan Shen, Huimei Lu, Yiyuan Huang, Jingmei Liu. Rutgers Cancer Institute of New Jersey, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA.

Dysfunctions of caretaker tumor suppressors contribute to genomic instability, and tumor initiation and progression. However, many caretaker genes are also indispensible for cell proliferation, permanent or complete inactivation of these caretaker genes would prohibit tumor progression. Thus, it remains elusive on how defects of essential caretaker genes contribute to tumorigenesis. BCCIP was originally identified as a BRCA2 interacting protein and

play an accessory role in homologous recombination and resolution of stalled replication forks. Dysfunction of BCCIP causes various forms of genomic instability. We previously built a LoxP-Cre based conditional BCCIP knockdown mouse model. By conditional BCCIP knockdown and concomitant p53 deletion, we found that an incomplete down-regulation of BCCIP is sufficient to initiate medulloblastoma-genesis. These tumors bear a wide spectrum of chromosome rearrangements and alternations, consistent with the caretaker responsibilities of BCCIP on genomic integrity. Surprisingly, once the tumorigenesis is initiated due to stochastic mutagenesis that activated the autonomous Shh growth-stimulating pathway, the tumor cells actually selectively delete the BCCIP knockdown cassette to cancel the original BCCIP defect in order to continue the progression. We propose that this transient inactivation of BCCIP represents a typical mode for caretaker tumor-suppressors that are also indispensible for cell proliferation, and may contribute to epigenetically regulated tumor suppressors with essential roles for cell growth.

B62 A partial but not complete loss of BCCIP function contributes to mammary tumorigenesis in mice. Roberto Droz-Rosario, Huimei Lu, Zhiyuan Shen. Rutgers Cancer Institute of New Jersey, Department of Radiation Oncology, Robert Wood Johnson Medical School, Rutgers the State University of New Jersey, New Brunswick NJ, USA.

The BRCA2 interacting protein BCCIP has been characterized as an important player in DNA damage response and genomic stability maintenance. In a preliminary study with human breast tumor specimens, BCCIP levels were found to be down-regulated in approximately 33% of the cases, including 49% among Triple Negative Breast Cancer (TNBC) and 25% among non-TNBC. We hypothesize that BCCIP defects contribute to mammary tumorigenesis. To test our hypothesis, we developed two K14-Cre and LoxP based conditional transgenic mouse models to modulate BCCIP expression in the myoepithelial/basal cells of the mouse mammary epithelium. In the first model, a complete loss of BCCIP was achieved by homozygous deletion of BCCIP exon 5, designated $BCCIP^{Ex5\Delta/Ex5\Delta}$ *(or BCCIP-CKO).* However, none of the *BCCIP-CKO* animals developed mammary malignancies throughout their lifespan. In contrast, a high percentage of mice developed discrete benign mammary nodules starting at about 3-6 month of age, when the BCCIP expression is partially down-regulated by a conditional BCCIP knockdown approach. Interestingly, ~10% of these benign lesions formed at 6 months of age further progressed to malignant tumors after a long latency. These observations suggest that partial BCCIP deficiency can initiate the development of breast tumors to an early stage, but additional genetic event(s) is needed for the transition from a benign lesion to a malignant tumor. Next Generation Sequencing technology is being used to identify the tumor promoting genetic changes in BCCIP deficient benign lesions. Our data further indicate that partial BCCIP loss is sufficient to initiate mammary tumorigenesis but a complete loss of BCCIP may be an obstacle for breast cancer development.

B63 Hepatitis B virus X protein promotes hepatocellular carcinoma transformation through interleukin-6 activation of microRNA-21 expression. Chi Han Li[1], Sheungching Chow[1], Yangchao Chen[1,2]. [1] School of Biomedical Sciences, Faculty of Medicine, the Chinese University of Hong Kong, [2] Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China.

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and chronic hepatitis B virus (HBV) infection is the major risk factor of HCC. The virus encodes HBV X (HBx) protein that plays critical role in the development of HCC. Studies have revealed numerous HBx-altered genes and signaling pathways that heavily contribute to tumorigenesis of non-tumour hepatocytes. However, the role of HBx in regulating other critical gene regulators such as microRNAs is poorly understood, which impedes the exploration of a complete HBx-associated carcinogenic network. Besides, critical microRNAs that drive the transformation of non-tumour hepatocytes are yet to be identified. Here, we overexpressed C-terminal truncated HBx protein in a non-tumour hepatocyte cell line MIHA, and measured a panel of cancer-associated miRNAs. We observed that oncogenic miR-21 was upregulated upon ectopic expression of this viral protein variant. HBx-miR-21 pathway was prevalent in HCC cells as inhibition of HBx in Hep3B and PLC/PRF/5 cells significantly suppressed miR-21 expression. Subsequently, we showed that the upregulation of miR-21 was mediated by HBx-induced interleukin-6 pathway followed by activation of STAT3 transcriptional factor. The high dependency of miR-21 expression to HBx protein suggested a unique viral oncogenic pathway that could aberrantly affect a network of gene expression. Importantly, miR-21 was essential in the HBx-induced transformation of non-tumour hepatocytes. Inhibition of miR-21 effectively attenuated anchorage-independent colony formation and subcutaneous tumour growth of MIHA cells. Our study suggested that overexpression of miR-21 was critical to promote early carcinogenesis of hepatocytes upon HBV infection.

B65 SPC25 plays important role in the oncogenesis of neuroblastoma. Hui Zhao. School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China.

Neuroblastoma is the third most common childhood cancer after leukemia and brain cancers, and accounts for 8% to 10% of all childhood cancers and for approximately 15% of cancer deaths in children. Although treatment for children with high-risk metastatic neuroblastoma has improved significantly in the past 20 years, only 45% of these patients become long-term, disease-free survivors. Mechanism studies are still urgent needed to illustrate the oncogenesis of neuroblastoma. In an attempt to identify gene involved in oncogenesis of neuroblastoma, we found SPC25, one of the four subunits of NDC80 complex, which is the key microtubule-binding complex at kinetochores during mitosis, may play important roles in neuroblastoma. In this study, we showed that high expression of SPC25 is related with bad prognosis of neuroblastoma. The survival of neuroblastoma patients with high SPC25 expression is significantly lower than those with low SPC25 expression. MYCN amplification and ALK mutation are the two most often identified genetic lesions in neuroblastoma. SPC25 is significantly up-regulated in neuroblastoma with MYCN amplication. ALK mutation can also up-regulate SPC25 expression. All these results suggest an important role of NPC25 in the oncogenesis of neuroblastoma. SPC25 could be a useful diagnostic marker and treatment target of neuroblastoma. More details of SPC25 in neuroblastoma will be further investigated.

B66 EBV encoded RNAs (EBERs)-TLR3-TNF axis triggers inflammation and promotes nasopharyngeal carcinoma progression. Zhi Li, Yumei Duan, Shiyue Chen, Yan Chen, Lu Zhang,

Jiang He, Qiong Liao, Lifang Yang and Lun-Quan Sun. Center for Molecular Medicine, Xiangya Hospital, Central South University, Changsha, China.

EBERs, existing as the most abundant non-coding RNA transcribed by Epstein-Barr virus (EBV), are known for their anti-apoptosis activity, growth-promoting capacity and the potential to transform primary cells. However, the association between EBERs, inflammation and progression of EBV-associated solid tumors (e.g., nasopharyngeal carcinoma, NPC) remains to be elucidated. To explore the proinflammatory role of EBERs in vitro, we transfected EBV negative NPC HNE2 cells with in vitro transcribed EBERs, which significantly stimulated IL-1α, IL-6 and TNF transcription and the release into supernatant. To mimic the EBV latent infection status, we established a cell line with HNE2 which constitutively harbors an expression cassette with 10 copies of EBERs and observed that TNF was the sole cytokine up-regulated. Furthermore, knockdown of EBERs transcription with specific siRNA in EBV-positive cell line C666-1 led to a decline of TNF transcription. We then verified the involvement of TLR3, a dsRNA receptor for viral RNAs, in this process. TLR3 expression was up-regulated after stimulation with in vitro transcribed EBERs and the EBER expression plasmids. In accordance, the EBER- triggered TNF transcription, release and NFκB pathway activation were impaired after TLR3 was knockdown by shRNAs, compared with the controls. Moreover, treatment of C666-1 cells with a TLR3-dsRNA complex inhibitor considerably prevented the cells from TNF transcription and release into the culture supernatants.

The potent oncogenic EBV encoded protein LMP1 has been reported to induce inflammatory response in vitro via NFκB signaling pathway. Thus it's intriguing to explore whether EBERs and LMP1 could coordinate to manifest this cancer promoting inflammation. Surprisingly, treatment of C666-1 cells with in vitro transcribed EBERs vigorously stimulated LMP1 expression, while knockdown of EBERs' transcription decreased LMP1 transcription. To investigate the mechanisms underlying the EBER-mediated LMP1 transcription, we established reporter gene constructs inclusive of LMP1 promoter with or without mutation of predictive NFκB binding site. We found that EBERs stimulation increased the luciferase activity driven by the LMP1 promoter, while this increase was impaired tremendously in the cells transfected with the plasmid including LMP1 promoters with mutation of NFκB binding site. In addition, transfection of NFκB subunit P50 was able to up-regulate LMP1 translation. In a CHIP assay, we found a direct binding of P50 to the promoters of EBERs. In accordance, P50 transfection triggered EBERs transcription. Interestingly, co-transcription of EBERs and LMP1 synergistically increased TNF and IL-6 transcription, which validated our hypothesis that there exists an EBER-LMP1-NFκB inflammatory feedback loop in EBV positive NPC cells.

To examine the pro- cancer inflammation function of EBERS, we inoculated B16 cells with or without EBER expression into WT or TLR3-/- C57 mice and observed that EBERs could stimulate xenograft growth via TLR3 pathway and this process was accompanied by an infiltration of tumor parenchyma of monocytes and tumor-promoting macrophages. In support of these observations, incubation of monocyte cell line U937 with the supernatant of the EBER-stimulated HNE2 cells vigorously stimulate M2d (tumor associated macrophages) marker CXCL-8 expression. Moreover, the xenografts derived from the EBERs or TLR3 knockdown C666-1 cells grew much slower than their respective counterparts when inoculated into nude mice. Finally, in human NPC and non-NPC tissues from the patients we found a positive correlation between

expression of EBERs and TNF and proposed a prognostic significance of EBERs and TNF. Our work demonstrated a critical role of EBER-TLR3-TNF axis in inflammation-to-carcinogenesis transition. *This work is supported by National Nature Science Foundation, China (91129709).*

B67 Integrative identification of Epstein-Barr Virus-associated mutations and epigenetic alterations in gastric cancer. Qiaoyi Liang, Jun Yu. Institute of Digestive Disease and Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, Shenzhen Research Institute, The Chinese University of Hong Kong, Hong Kong, China.

Background & Aims: The mechanisms by which Epstein-Barr virus (EBV) contributes to development of gastric cancer are unclear. We investigated EBV-associated genomic and epigenomic variations in gastric cancer cells and tumors.
Methods**:** We performed whole-genome, transcriptome, and epigenome sequence analyses of a gastric adenocarcinoma cell line (AGS cells), before and after EBV infection. We then looked for identified alterations in gastric tumor samples, with (n=34) or without (n=100) EBV infection, collected from patients at the Prince of Wales Hospital, Chinese University of Hong Kong (from 1998 through 2004) or the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China (from 1999 through 2006).
Results: Transcriptome analysis showed that infected cells expressed 9 EBV genes previously detected in EBV-associated gastric tumors and 71 EBV genes not previously reported in gastric tumors. Ten viral genes that had not been previously reported in gastric cancer but were most highly expressed in EBV-infected cells were also expressed in primary EBV-positive gastric tumors. Whole-genome sequence analysis identified 45 EBV-associated non-synonymous mutations. These mutations, in genes such as *AKT2*, *CCNA1*, *MAP3K4*, and *TGFBR1*, were significantly associated with EBV-positive gastric tumors, compared with EBV-negative tumors. An activating mutation in *AKT2* was associated with reduced survival times of patients with EBV-positive gastric cancer (*P*=.006); this mutation was found to dysregulate MAPK signaling. Integrated epigenome and transcriptome analyses identified 216 genes transcriptionally downregulated by EBV-associated hypermethylation; methylation of *ACSS1*, *FAM3B*, *IHH*, and *TRABD* increased significantly in EBV-positive tumors. Overexpression of *IHH* and *TRABD* increased proliferation and colony formation of gastric cancer cells, whereas knockdown of these genes reduced these activities. We found 5 signaling pathways (axon guidance, focal adhesion formation, interactions among cytokines and receptors, MAPK signaling, and actin cytoskeleton regulation) to be commonly affected by EBV-associated genomic and epigenomic alterations.
Conclusion: Using genomic, transcriptome, and epigenomic comparisons of EBV infected vs non-infected gastric cancer cells and tumor samples, we identified alterations in genes, gene expression, and gene methylation that affect different signaling networks. These might be involved in EBV-associated gastric carcinogenesis.
ACKNOWLEDGEMENTS
This project was supported by research funds of a China 863 Program (2012AA02A203), a National Natural Science Foundation of China (81272304), a China 973 Program

(2010CB529305), Theme-based Research Scheme of the Hong Kong Research Grants Council (T12-403-11). The authors declare no conflicts of interest.

B68 Expression of human steroid sulfatase induces aerobic glycolysis in HeLa cells.

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Steroid sulfatase (STS) expression has been considered to play a pivotal role in estrogen-dependent cancers. STS is known as a target enzyme for suppressing estrogen-mediated carcinogenesis. STS is able to convert estrogen sulfate to estrone form, like dehydroepiandrosterone sulfate (DHEA-S) to its' active form dehydroepiandrosterone (DHEA). Aerobic glycolysis is a hallmark of cancer metabolism. Hypoxia inducing factor 1 subunit HIF1□ is recognized as an important regulator of aerobic glycolysis. To elucidate whether STS is able to regulate cancer metabolism, the effects on aerobic glycolysis were determined. STS overexpression significantly converted DHEA-S to DHEA. STS and the major product DHEA enhanced HIF1□ protein, mRNA, promoter activity in HeLa cells. Glycolytic enzymes such as hexokinase 2 (HK-2), phosphofructokinase isotype P (PFKP), M2 type pyruvate kinase (PKM2) and glucose transporter (GLUT) were increased by STS overexpression and DHEA treatment in protein and mRNA levels. When cells were treated with HIF1□ siRNA, all glycolytic enzymes induced by STS and DHEA were down-regulated. Lactic acid production was also increased by STS and DHEA. STX064, a STS specific inhibitor inhibited DHEA formation and lactic acid production in HeLa cells. In conclusion, STS and the major product DHEA may regulate aerobic glycolysis via HIF1□□induction.

B69 CYP1B1 induces cell migration and invasion via up-regulation of EMT-inducing factors.

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Human cytochrome P450 1B1 (CYP1B1) belongs to the CYP1 family and acts as a hydroxylase for 17□-estradiol. Due to the high expression level of CYP1B1 in the tumor tissues, it has been suggested that CYP1B1 may play a major role in carcinogenesis but the role has not been fully identified yet. In tumor progression, repressors for E-cadherin expression such as Snail, Zeb-1 or Zeb-2 are induced and epithelial-mesenchymal transition (EMT) occurs. To explore the role of CYP1B1 in cellular carcinogenesis, we investigated whether CYP1B1 induces cell migration and invasion. The cellular morphology of immortalized mammary epithelial cell line, MCF-10A cells were observed following induction of CYP1B1 by 7, 12-dimethylbenz[□]anthracene (DMBA), or over-expression by expression vector and the morphological changes from epithelial cell-like form to mesenchymal cell-like form were identified. Subsequently, induction of cell migration and invasion by CYP1B1 were investigated using wound healing assay and trans-well cell invasion assay and these promoting results were blocked by specific CYP1B1 inhibitor, tetramethoxystilbene (TMS), in both MCF-10A and MCF-7 cells. To clarify whether CYP1B1 promotes cell migration and invasion via induction of EMT, we investigated the expression level of EMT-inducing factors and marker proteins and it showed that CYP1B1 up-regulates EMT-inducing factors like Zeb-1/2, Snail1, and Twist1 and mesenchymal markers such as vimentin,

fibronectin, □-SMA, integrin □5, and N-cadherin in protein and mRNA levels. The epithelial marker proteins E-cadherin and □-catenin were down-regulated by CYP1B1. Using luciferase assay and confocal fluorescence microscopy, transcriptional suppression of E-cadherin by CYP1B1 was also identified. To investigate whether the induction of EMT is regulated by enzymatic activity of CYP1B1, cells were treated with 4-hydroxyestradiol (4-OHE2), a major product of CYP1B1, and it was clarified that 4-OHE2 mediates induction of EMT by CYP1B1. Treatment with 2-OHE2, a minor product of CYP1B1, was also able to induce EMT but the changes in expression levels of EMT-inducing factors were relatively low. Taken together, these data suggest that CYP1B1 up-regulates cell migration and invasion by induction of EMT-inducing factors. The enzyme activity of CYP1B1 may play an important role in inducing EMT because 4-OHE2 can regulate EMT-inducing factors.

B70 Epigenetic silencing of the zinc transporter ZIP8 in cadmium-resistant lung epithelial cells. Yang-Min Gao, Yan-Ming Xu, Andy T. Y. Lau*. Laboratory of Cancer Biology and Epigenetics, Department of Cell Biology and Genetics, Shantou University Medical College, Shantou, Guangdong, China.

Cadmium (Cd), a non-essential toxic metal, stealthily enters the cells by utilizing the essential metal importing pathways. The zinc transporters ZIP8, ZIP14, and divalent metal transporter 1 (DMT1) are now emerging as several important metal transporters involved in cellular Cd incorporation and their expressions have been shown to be down-regulated in several Cd-resistant cell lines. However, the involvement of these transporters during the development of Cd-resistance in lung cells is unclear. In this study, we therefore checked the expression of these metals transporters by RT-PCR and found that the expression of ZIP8 was stably abolished in Cd-resistant lung epithelial cells (Cd^R) as compared with control. Subsequent measurement of the cellular Cd content indicated that Cd^R exhibit decreased Cd accumulation, possibly due to the loss of ZIP8 expression. We investigate the possibility that epigenetic silencing of the *ZIP8* gene by DNA hypermethylation is involved in the down-regulation of ZIP8 expression. Cd^R showed a higher mRNA level of DNA methyltransferase 3b (DNMT3b) than parental cells. Treatment of Cd^R with 5-aza-deoxycytidine (5-aza-dC), an inhibitor of DNA methyltransferase, reverted ZIP8 expression and Cd-sensitive phenotype in these cells, indicating the critical role of ZIP8 for Cd import. Taken together, our results demonstrate that epigenetic silencing of the *ZIP8* gene is involved in the acquisition of resistance against Cd in lung cells, representing an adaptive survival mechanism that resists Cd-induced cytotoxicity.

B71 Solamargine; a potential promising anti-tumor agent? Exploring and comparing its potency with nitroso-solamargine and solanine. Noha Nagdy Farrag[1], Mohieldin Magdy Youssef[2,4], Mai F. Tolba[3] , Muhammad A. Alsherbiny[6], Marawan M. Shabana[6], Mostafa A. Abdel-kawy[6], Eman El-Ahwany[5], Suher Zada[2]. [1]Biotechnology Program, School of Sciences and Engineering (SSE), American University in Cairo, New Cairo, Egypt, [2]Department of Biology, School of Sciences and Engineering (SSE), American University in Cairo, New Cairo, Egypt, [3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, [4]Department of Pharmacology, College of Pharmacy, Egyptian Russian University, Badr City, Cairo, Egypt, [5]Department of Immunology, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt, [6]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Purpose: Solamargine; is a herbal glycoalkaloid derived from *Solanum seaforthianum*. This natural agent was previously reported to exhibit antitumor effect against several cancer cell lines such as; HT-29 (colon cancer), PC3 (prostate cancer), T47 (breast cancer), PLC and Hep3B (liver cancer). The main objective of this study is to characterize the potency of solamargine as a promising anticancer agent in HepG2 (liver cancer) and SCaBER (urinary bladder cancer) cell lines. The current study also aimed at comparing the potency of solamargine with the pure potent alkaloid; solanine (derived from solanum family of plants), and its nitroso derivative; nitroso-solamargine. The underlying mechanisms for their anticancer activity were also investigated with special emphasis on changes in cell cycle distribution and apoptosis signals.
Methods: Sulforhodamine B (SRB) cytotoxicity assay was conducted using both of the cell lines; HepG2 and SCaBER, in order to estimate the half maximal inhibitory concentration (IC_{50}) for each of the three compounds; Solanine, Solamargine and Nitroso-Solamargine. Cell cycle studies were then done using DNA-flow cytometry. Immunocytochemistry was used to assess the protein abundance of the cell cycle inducer cyclin D1. Gene expression of the proliferation and tumerogenesis marker, Ki67 was evaluated using real time PCR. Levels of active caspase-3, a key executioner of apoptosis, were assessed using ELISA, while gene expression of survivin, an important inhibitor of apoptosis was tested using real time PCR.
Results: Solamargine exhibited the most potent effect on HepG2 with IC50 of (2.346 uM), compared to that of solanine (2.569 uM) and that of nitroso-solamargine (3.27 uM). Surprisingly, assessing the IC50 on SCaBER cell line had intriguingly different results with the nitroso-solamargine exhibiting the most potent anticancer effect with IC50 of (5.9 uM), compared to that of Solamargine (8.9 uM) and that of solanine (6.5 uM).
Cell Cycle analysis using DNA-flow cytometry showed a significant increase in the accumulation of cells at the Pre-G phase corresponding to an increase in the percentage of dead cells at 72 h of exposure of HepG2 cells to the different treatments compared to control. Other recorded cell cycle alterations included reduction in the percentage of cells in both Go/G1 and S phases. Cyclin D1 was demonstrated to be significantly down regulated in the same groups, as shown by immunocytochemistry.
The three compounds have shown a clear down-regulation of the anti-apoptotic proliferation marker; survivin. nitroso-solamargine exhibited the highest down-regulation, as compared with solamargine and solanine. Ki 67 was also shown, to be down-regulated in the three compounds with nitroso-solamargine exhibiting the lowest expression compared to solamargine and solanine, at72 h of exposure.
Caspase-3 was found to be up-regulated at 72 h of treatment with nitroso-solamargine showing the highest elevation as measured by ELISA. Solanine and Solamargine exhibited almost close values of Caspase-3 over expression.
Conclusion: Solamargine a natural glycoalkaloid derived from *Solanum seaforthianum* showed promising potency to be utilized as a potential anticancer agent. This study has compared solamargine anticancer potency with that of solanine and nitroso-solamargine. Addition of the nitroso group seemed to increase potency of solamargine against urinary bladder cancer. It also appeared to enhance the antiproliferative and the pro-apoptotic activity of nitroso-solamargine as compared to solamargine and solanine, on HepG2 hepatocellular carcinoma cells. Further in vivo investigations are recommended in order to evaluate if there is a difference in their bioavailability that can interfere with the antitumor potency.

B72 FIH-1 functions as a tumor suppressor in human colorectal cancer by repressing HIF1α pathway. Tao Chen, Shi-Lun Cai, Li-Qing Yao, Yun-Shi Zhong. Zhongshan Hospital of Fudan University, Shanghai, China.

Colorectal cancer (CRC) is one of the most common cancers worldwide. The molecular mechanisms underlying CRC development involve a multistep process with the accumulation of both genetic and epigenetic changes. To deeply understand CRC tumorigenesis and progression, advances in identification of novel mechanisms and key factors are therefore in an urgent need. In this study, we analyzed the correlation of FIH-1 expression with clinicopathological features of CRC. The finding that FIH-1 was not only significantly decreased in tumor tissue as compared with the adjacent normal tissue but also was significantly correlated with tumor invading depth, lymph node involvement, and metastasis indicated the role of FIH-1 as a tumor suppressor in CRC development (Figure 1). To further support the above hypothesis, we performed both *in vitro* and *in vivo* experiments to identify the role of FIH-1 in CRC development: 1) FIH-1 was found to inhibit CRC cell proliferation, migration, invasion, and colony formation *in vitro* (Figure 2); 2) FIH-1 was also shown to repress LOVO xenograft tumor growth *in vivo*(Figure 3). In the present study, we found that FIH-1 was able to inhibit HIF1α mediated transcription of GLUT-1 and VEGF in human CRC cells (Figure 4). The above observation points to the possibility that loss or decreased expression of FIH-1 gene may lead to a constitutive activation of HIF1α and an alteration of HIF-1 targets such as GLUT-1 and VEGF. These findings highlight the critical role of FIH-1 in CRC and reveal that FIH-1 functions as a tumor suppressor in human CRC by repressing HIF1α pathway.

B73 LOC401317, a p53-regulated long non-coding RNA, inhibits cell proliferation and induces apoptosis in nasopharyngeal carcinoma cell line HNE2. Zhaojian Gong, Qian Yang, Shanshan, Zhang, Zhaoyang Zeng, Yong Li, Guiyuan Li, Wei Xiong*. Cancer Research Institute, Central South University, Hunan, China.

Recent studies have revealed that long non-coding RNAs (lncRNAs) participate in all steps of cancer initiation and progression by regulating protein coding genes at the epigenetic, transcriptional and post-transcriptional levels. LncRNAs are in turn regulated by other genes, forming a complex regulatory network. The regulation networks between the p53 tumor suppressor and lncRNAs in nasopharyngeal carcinoma (NPC) remain unclear. The aim of this study was to investigate the regulatory roles of the *TP53* gene in regulating lncRNA expression profiles and function of a *TP53*-regulated lncRNA, LOC401317, in NPC cell line HNE2. LncRNA expression profiling indicated that 133 lncRNAs were upregulated in a human NPC cell line HNE2 cells following *TP53* overexpression, while 1057 lncRNAs were downregulated. Among these aberrantly expressed lncRNAs, LOC401317 was the most significantly upregulated lncRNA. Further studies indicated that LOC401317 is directly regulated by p53 and that ectopic expression of LOC401317 inhibits HNE2 cell proliferation *in vitro* and *in vivo* by inducing cell cycle arrest and apoptosis. LOC401317 inhibited cell cycle progression by increasing p21 expression and decreasing cyclin D1 and cyclin E1 expression, and promoted apoptosis through

the induction of PARP and caspase-3 cleavage. Collectively, these results suggest that LOC401317 is directly regulated by p53 and exerts antitumor effects in HNE2 NPC cells.

B74 MiR-194 deregulation contributes to colorectal carcinogenesis via targeting AKT2 pathway. Hui-Jun Zhao [1], Lin-Lin Ren [1], Zhen-Hua Wang[1], Haoyan Chen, Jie Hong[1], Jing-Yuan Fang[1]. [1]State Key Laboratory for Oncogenes and Related Genes Division of Gastroenterology and Hepatology, Ren Ji Hospital, Shanghai Institute for Digestive Diseases, School of Medicine, Shanghai Jiao Tong University, Key Laboratory of Gastroenterology & Hepatology, Ministry of Health, Shanghai, China.

Recent studies have increasingly linked microRNAs to colorectal cancer (CRC). MiR-194 has been reported deregulated in different tumor types, whereas the function of miR-194 in CRC largely remains unexplored. Here we investigated the biological effects, mechanisms and clinical significance of miR-194. Functional assay revealed that overexpression of miR-194 impaired CRC cell viability and invasion *in vitro* and suppressed CRC xenograft tumor growth and metastasis *in vivo*. Conversely, block of miR-194 in $APC^{Min/+}$ mice promoted tumor growth. Furthermore, miR-194 reduced the expression of AKT2 both *in vitro* and *in vivo*. Clinically, the expression of miR-194 gradually decreased from 20 normal colorectal mucosa (N-N) cases through to 40 colorectal adenomas (CRA) cases and then to 40 CRC cases, and was negatively correlated with AKT2 and pAKT2 expression. Furthermore, Expression of miR-194 in stool samples was gradually decreased from 20 healthy cases, 20 CRA cases, then to 28 CRC cases. Low expression of miR-194 in CRC tissues was associated with large tumor size (*P*=0.006), lymph node metastasis (*P*=0.012) and shorter survival (HR =2.349, 95% CI = 1.242 to 4.442; *P*=0.009). In conclusion, our data indicated that miR-194 acted as a tumor suppressor in the colorectal carcinogenesis via targeting PDK1/AKT2/XIAP pathway, and could be a significant diagnostic and prognostic biomarker for CRC.

B75 Molecular characterization of tumor-associated macrophage polarization in colitis-associated tumorigenesis. Weina Zhang, Lechuang Chen, Hongxia Zhu, Ningzhi Xu.
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Macrophages are important components of the leukocyte infiltrates in various tumor stroma. Macrophages that infiltrate tumors are called tumor-associated macrophages(TAM).TAM show anti-(M1) or pro-tumorigenesis (M2) functions depending on the cytokine milieu of the tumor microenvironment. The aim of this study is to explore the molecular character of TAM in colitis-associated colorectal cancer (CAC). C57BL/6J mice were injected with azoxymethane (AOM,12.5mg/kg) and then subjected to repeated cycles of dextran sodium suflate(DSS, 2.5%). Mice were sacrificed and the colon was collected. We found that all AOM/DSS-induced mice (AD) developed adenocarcinomas, and the length of colon in AD group is significantly shorter than control group. By Immunofluorescence staining, we found that total macrophages(F4/80 positive cell) were accumulated in AD induced colerectal neoplasm tissue, the percentage of M2 (Arginase1 positive cell)increased， but M1(iNOS positve cell) was scarcely detected. Flow cytometry was used to quantitatively assess the change of macrophage populations in control and AD group, F4/80 and CD11b were used as total-macrophage markers,CD206 as a maker of

M2 macrophage.We found that high expression level of F4/80 and CD206 macrophage in AD group. Additionally, the protein and mRNA levels of Arginase1 were higher in AD group and M2-related cytokine IL-10 was significantly upregulated in the AD group. In conclusion, Macrophages accumulated in AOM/DSS induced colorectal cancer and TAM infiltraing the colorectal neoplasm of AOM/DSS mice were polarized to M2 phenotypes.

B76 Reverse relationship of tumor growth, metastasis and survival probability by thymosin beta-4 overexpression *in vivo.* Eun-Yi Moon, Jae-Wook Lee, Jin-Mi Oh, Jiyoung Lee. Department of Bioscience & Biotechnology, Sejong University, Seoul, Korea.

Thymosin beta-4 (Tβ4), actin-sequestering protein, functions as a cellular mediator in many tissues. Cell migration plays a key role in many physiological and pathological processes, including tumor metastasis, which usually lead to a decrease in survival probability. Here, we investigated how Tβ4 affect tumor growth and metastasis and survival probability using Tβ4-transgenic mice. Data show that Tβ4 overexpression increased not only tumor growth rate and metastasis but also survival probability as compared to those in wildtype mice. Tumor metastasis by tumor resection was higher in Tβ4-overexpressed mice with higher tumor growth rate as compared to that in wildtype control mice. Similar pattern of data was shown in male and female mice. When mice were injected with B16F10 melanoma, the level of various cytokines, IL-6, TNF-α, and IL-1β was lower in Tβ4-transgenic mouse serum than that in control group. In addition, the level of nitric oxide and cyclooxygenase-2 produced in Tβ4-transgenic mice was lower than that in control group. In contrast, myeloid-derived suppressor cells (MDSCs) were higher in Tβ4-transgenic mouse bone marrows and spleens than those in control group. Data suggest that inflammatory responses could be regulated by Tβ4 through the elevation of MDSC, which lead to the increase in survival probability.

This work was supported by grants from Mid-career Researcher Program (#2012-R1A2A2A01005449) and Nuclear R&D Program (#2013M2B2A9A03051296 and 2010-0018545) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST).

B77 PRAS40 promotes the tumorgenesis of hepatocellular carcinoma. Lin HUANG, Hongyong Fu, Lianying Guo. Dalian Medical University, Dalian, China.

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third leading cause of cancer-related death worldwide. Although treatment options have improved in the past 30 years, 5-year survival rate remains below 20%. To improve the therapeutics of HCC, great efforts are needed in understanding the mechanisms underlying HCC. PRAS40, a substrate of Akt, plays an important role in the development of cancer, such as melanoma and Ewing sarcoma family tumors. To clarify the role of PRAS40 in the development of HCC, we investigated the expression level of PRAS40 in 32 HCC samples, and found PRAS40 level is much higher in carcinoma than adjacent tissue. We also got similar results in HCC and normal liver cell lines. Further, the growth and motility of HCC cell lines were suppressed significantly by siRNA-mediated PRAS40

knockdown. These data suggested that PRAS40 is a crucial factor in the development of HCC, which might therefore represent a novel therapeutic target for HCC.

B78 Loss of ChREBP in mice delays the initiation and ameliorates the progression of diethylnitrosamine-induced liver cancer. Jian Meng, Ming Feng, Yemin Zhu, Dianqiang Yang, Yakui Li, Lifang Wu, Ping Zhang, Minle Li, Xuemei Tong. Dept. of Biochemistry and Molecular Cell Biology, Institute of Medical Science, Shanghai Jiao Tong University School of Medicine, Shanghai, P.R. China.

Carbohydrate-responsive element binding protein (ChREBP) is a glucose-responsive transcription factor regulating expression of several glycolytic and lipogenic enzyme genes in liver. We previously found that ChREBP was required for cell proliferation as well as high glycolytic and anabolic activities in liver cancer cells. In order to explore the role of ChREBP in regulating liver carcinogenesis, we induced liver cancer in ChREBP+/+, +/- and -/- male mice using the well-established hepatic carcinogen diethylnitrosamine (DEN) injection protocol followed by high-fat diet (HFD) feeding. Body weight of ChREBP-/- male mice was lower than that of age-matched wild type or ChREBP+/- male mice after DEN injection and HFD feeding. About 95% wild type or ChREBP+/- male mice developed liver cancer at 9-month old whereas the tumor incidence decreased to about 50% in age-matched ChREBP-/- male mice. Liver tumor number and size was significantly decreased in ChREBP-/- male mice compared with age-matched controls. BrdU staining of liver tumors showed that deletion of ChREBP suppressed cell proliferation. The level of AFP mRNA and protein was lower in ChREBP-/- livers compared with age-matched controls. Less triglyceride and more glycogen was synthesized in ChREBP-/- liver compared with age-matched controls. The level of IL6, STAT3 and IL-1b decreased in ChREBP-/- livers compared with age-matched controls. The mechanism by which loss of ChREBP delays the initiation and ameliorates the progression of DEN-induced liver cancer will be discussed.

B86 Prognostic significance of ^{18}F-FDG PET parameters and plasma EBV DNA load in patients with nasopharyngeal carcinoma. Kai-Ping Chang[1], Ngan-Ming Tsang[2], Tzu-Chen Yen[3]. [1]Department of Otolaryngology-Head & Neck Surgery, Chang Gung Memorial Hospital & Chang Gung University College of Medicine, Taipei, Taiwan, [2]Department of Radiation Oncology, Chang Gung Memorial Hospital & Chang Gung University College of Medicine, Taipei, Taiwan, [3] Department of Nuclear Medicine and Molecular Imaging Center, Chang Gung Memorial Hospital & Chang Gung University College of Medicine, Taipei, Taiwan.

Purpose: The measure of plasma Epstein-Barr virus (EBV) DNA is associated with tumor burden and prognosis in nasopharyngeal carcinoma (NPC). Herein we sought to examine the association of ^{18}F-FDG-PET functional parameters and EBV DNA load with the clinicopathological characteristics and clinical outcomes of NPC patients.
Methods: A total of 108 NPC patients who underwent ^{18}F-FDG-PET before treatment were included. We determined (i) total lesion glycolysis (TLG) of the primary tumor, the cervical nodes, and their combination; and (ii) the maximal standardized uptake value (SUVmax) of the primary tumor and cervical lymph nodes. EBV DNA was measured by real-time polymerase chain reaction.

Results: EBV DNA was significantly associated with tumor TLG, nodal TLG, total TLG, tumor SUVmax, and nodal SUVmax in the 108 examined patients (r=0.251, P=0.008; r=0.447, P<0.0001; r=0.589, P<0.0001; r=0.199, P=0.038; and r=0.462, P<0.0001, respectively). Total TLG values had the highest correlation with EBV DNA load and were significantly associated with tumor, nodal, and overall stages. However, tumor TLG greater than the median (> 65 g) was the only parameter significantly associated with overall, local recurrence-free, disease-free, and distant metastasis-free survivals (P = 0.033, = 0.014, < 0.001, and = 0.023, respectively). After allowance for potential confounders, tumor TLG retained its independent significance for overall and disease-free survival rates (P = 0.045 and 0.006, respectively).
Conclusions: Total TLG values are primarily associated with tumor burden and clinical stage, while tumor TLG is the best predictor of patient survival after treatment.

B88 Does radiation treatment of prostate cancer increase rectal cancer risk? The perfect storm. John W. Morgan, Brice Jabo, Holly Harlin, Mark Ghamsary, Mark E. Reeves, Liang Ji. Loma Linda University, Loma Linda, CA.

Background: Prostate cancer (PC) is the most common malignancy among men. Most PCs are localized at diagnosis and are candidates for radiation, prostatectomy (SURG), other treatments, or active surveillance. Data were from the three California SEER registries that encompass the statewide population.
Problem: Radiation treatment of localized PC exposes perirectal tissue to ionizing radiation that may increase rectal cancer hazards.
Methods: Localized invasive prostate cancers diagnosed from 1988-2005 were assessed for rectal cancer proportional hazards >5 years following diagnosis of localized PC that had been treated with any radiation (AnyRT), external beam radiation therapy (EBRT-Only), brachytherapy (BT-Only), EBRT+BT, or no radiation+no SURG (NoRT+NoSURG), *versus* SURG. Adjusted covariates included 4 categories each for age and race/ethnicity, socioeconomic status (SES) quintiles and diagnostic year.
Results: 309 rectal cancers were diagnosed among 64,673 PCs treated with AnyRT, while 312 of 71,693 men that had received SURG, were subsequently diagnosed with rectal cancer 5+ years following treatment. The age-, race/ethnicity-, SES- and year of diagnosis-adjusted rectal cancer proportional hazards ratio (HR) for AnyRT *versus* SURG was 1.47, with 95% confidence interval limits (95%CI) from 1.24-1.74. The HR for EBRT-Only *versus* SURG ($HR_{EBRT\text{-}Only/SURG}$=1.47; 95%CI=1.23-1.77) was nearly identical to AnyRT-Only *versus* SURG, with a weaker association for BT-Only *versus* SURG ($HR_{BT/SURG}$=1.25; 95%CI=0.86-1.81) and the strongest effect for EBRT+BT *versus* SURG ($HR_{EBRT+BT/SURG}$=1.62; 95%CI=1.10-2.40). The NoRT+NoSURG *versus* SURG rectal cancer HR was 1.15; 95%CI=0.93-1.43. A dose-gradient effect for EBRT+BT > EBRT > BT, versus SURG (p<0.001) was evident.
Conclusion/Discussion: Findings support hypotheses of slightly increased rectal cancer hazards among localized PC cases treated with AnyRT and EBRT modalities, *versus* SURG. Higher HR for, arguably, higher radiation exposure modalities (EBRT+BT > EBRT > BT), relative to SURG, with a null finding for NoRT+NoSURG *versus* SURG, suggest that the radiation effect may be direct.

B89 A novel immunotherapy approach with multiple tumor antigen activated autologous T cells in hepatocellular carcinoma. Yanyan Han[1,2], Ran Tao[1], Jing Huang[2], Yabing Guo[2], Dongyun Wu[1], Yujing Liao[2], Ping Chen[1], Jin Li[1], Xiangjun Zhou[1] and Jinlin Hou[2]. [1]SYZ Cell Therapy Co., Shenzhen, China; [2]Department of Infectious Diseases and Hepatology Unit, Nanfang Hospital, Southern Medical University, Guangzhou, China.

Background: Hepatocellular carcinoma (HCC) is one of the most common tumors in China, and frequently occurs in the patients with chronic hepatitis B virus (HBV) infection. Although liver resection, liver transplantation, transcatheter arterial chemoembolization (TACE) and other therapies may improve survival of patients, HCC is rarely cured and with high risk of recurrence and metastasis. Adoptive T cell transfer (ACT) is an emerging treatment for various cancers including HCC. However, the complex procedures to manipulate autologous tumor-infiltrating lymphocytes or tumor specific T cell clones largely limit the application of ACT in clinical practices. Here we present a novel strategy named “Smart T” to prepare multiple tumor antigens activated T lymphocytes containing HCC-specific CTLs ex vivo, with a promising outcome in a preliminary clinical applications with HCC patients. Patients and Methods: The monocytes from PBMCs of the patient were firstly differentiated into immature dendritic cells (iDCs) and then pulsed with multiple synthetic peptide antigens pool including tumor-associated antigens and HBV specific antigens. The semi-mature DCs were further stimulated by diverse toll-like recptor (TLR) ligands to differentiate into mature DCs (mDCs). The maintaining non-adherent T cells were co-cultured with mDCs for another 7-9 days. The resulting activated T cells were infused into patients every 1-2 months with 5-10x10^7cells/kg body weight for multiple cycles. Results: The resulting cells are mainly $CD3^+$T cells (96.65%±2.99%), among which 78.98%±5.88% are $CD8^+$ T cells co-expressing NKG2D (76.6%±7.44%) and Granzyme B (40.4%±12.9%). The activated T cells generated from HLA-$A2^+$ patient exhibited greater cytotoxic activity to the HLA-$A2^+$ HCC cell line HepG2 than to the HLA-$A2^-$ Huh-7 cells, suggesting a specific cytotoxicity by T cells. After 3 cycles of “Smart T” infusion, a significant increase was observed in the patients’ PBMCs for the frequency of $CD3^+$ (p=0.025), $CD8^+$ (p=0.023), $CD8^+NKG2D^+$ (p=0.043), $CD8^+CD107a^+$ (p=0.0082), especially effector T cells (p=0.0013) and central memory T cells (p=0.0038). A significant decrease of regulatory T cells (Treg) was observed (p=0.0015) as well. A specific proliferation of T cells stimulated by multiple tumor antigens pool was also detected (p=0.043) in the HCC patients’ PBMCs as compared to irrelevant antigen stimulation. Moreover, the IFNg ELISPOT assay demonstrated specific responses against individual peptides out of the multiple antigens pool, such as telomerase (3.22±0.86), survivin (2.22±0.86), carcinoembryonic antigen (CEA) (3.56±0.58), Alpha fetoprotein (AFP) (2.00±0.33), HBV core antigen (14.34±1.34) and HBV DNA polymerase (1.50±0.00) in the HCC patients` PBMCs. Clinical measurements show a better response (analyzed as CR: complete remission; PR: partial remission; SD: stable disease and PD: progressive disease) in patients (B stage, N=18) who received more than three infusions, as compared to patients (B stage, N=19) who received once or twice infusions (*p*=0.0093). No toxicity was observed. Conclusion: Our study, for the first time, demonstrates tumor antigens specific T cell responses can be robustly raised in HCC patients after Smart T treatment, and provides a safe treatment which may improve the immunologic function and clinical outcome of the HCC patients. On this basis and for the further research, a prospective, multi-center, randomized controlled clinical trial for early stage HCC

patients were initiated to observe the efficacy of multiple antigens specific cells therapy to low the recurrence and metastasis of HCC (ClinicalTrials.gov Identifier: NCT02026362).

B90 MiRNA expression analysis of pretreatment biopsies predicts the pathological response of esophageal squamous cell carcinomas to neoadjuvant chemoradiotherapy.

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Objective: Neoadjuvant chemoradiotherapy (neo-CRT) followed by surgery, which improves survival in comparison with surgery alone, has been recommended for the treatment of advanced-stage esophageal squamous cell carcinomas (ESCCs). However, the outcomes of neo-CRT were heterogeneous. No clinical parameters, such as TNM classification, tumor location or differentiation, could predict CRT response effectively. MiRNAs function in cancer cells by suppression of translation and promotion of mRNA decay to affect tumor cell biological behaviors, including chemo- and/or radio-sensitivity. In this study, we aim to identify miRNA markers useful for ESCC CRT-response's prediction.

Design: MiRNA expression analyses were performed on pre-treated cancer biopsies from 28 ESCCs who received neo-CRT and surgery. Surgical specimens were assessed for pathological response to CRT. The differentially expressed miRNAs identified by profiling were validated by real-time quantitative polymerase chain reaction (qPCR), and classifying models to predict neo-CRT response were built from qPCR data by using several class prediction algorithms, including logistic regression, k-nearest neighbor, regression trees, support vector machine with radial basis kernel (SVF-RBF), and linear and quadratic discriminant analyses approach. The predictive power of models were further assessed in a second set of 42 ESCCs. The differentially miRNAs' expression values and paired mRNA expression profiles of the same ESCC tissues were integrated for miRNA target prediction using MAGIA[2] by combing 6 miRNA target prediction algorithms.

Results: Eleven differentially expressed miRNAs with more than a 1.5-fold change between pathological complete responders (pCRs) and less than pCRs (<pCRs) were identified by miRNA profiling and validated by qPCR. A SVM-RBF prediction model based on 6 miRNAs' qPCR values (miR-144-3p, miR-194-5p, miR-424-5p, miR-584-5p, miR-29b-1-5p, and miR-139-5p) was generated and proved to be the best model, which provided an overall accuracy of 92.9% with sensitivity and specificity of 90.9% and 94.1% for pCR identification in the training set, and an overall accuracy of 88.1% with sensitivity and specificity of 73.3% and 96.3% in the validation set. By multivariate analysis, the subgroup determined by the SVM model was the only independent factor that significantly associated with neo-CRT response in both the training (P = 0.001) and validation (P < 0.001) sets, respectively. Thirty-two mRNAs were predicted to be downstream target of differentially expressed miRNAs, which could be clustered into two major groups functionally. Specifically, ESCO1, NBN and USP1 regulated by miR144-3p and miR-584-5p are involved in DNA repair, while miR424-5p-regulated RORA, ZNF117, CTNND1, and PDCD4, and miR-144-3p-regulated FOXF2, SPEN, and NRIP1 are participants in transcription regulation.

Conclusion: Our study demonstrated that the combination of 6 miRNAs by qPCR in our study provides possibility for ESCC CRT prediction, which will facilitate individualization of ESCC treatment. The identification of miRNA targets would lay foundation for future functional

studies. Further perspective validation in a large independent cohort with the same or different regimens as this study is warranted to fully assess the predictive power of this prediction model.

B91 Hapten-enhanced therapeutic effect in advanced stages of lung cancer by ultra-minimum incision personalized intratumoral chemoimmunotherapy (UMIPIC) therapy. Baofa Yu[1], Yuanfei Lu[2], Jian Liu[3], Peng Jing[1], Wei Han[2], Feng Gao[3], Ning Ru[3], Guanghui Cui[3], Chenglin Sun[2], Yebing Che[2]. [1]Beijing Baofa Cancer Hospital， Beijing, China; [2]Jinan Baofa Cancer hospital, Jinan, China; [3]TaiMei Baofa Cancer hospital, Beijing, China.

Aims: Lung cancer remains the leading cause of cancer-related deaths, accounting for about 14% (228,190 cases) in total cases and 27% (159,480 deaths) of all cancer deaths in 2013 in the United States. Routine clinical treatments include surgery, radiation therapy and chemotherapy. The 5-year survival rate for all stages combined, however, is only 16%. Currently, as a first-line treatment with chemotherapy, several agents clinically approved in targeted therapies for lung cancer have ongoing developments such as bevacizumab (Avastin) anderlotinib (Tarceva), as well as the second generation drugs afatinib (BIBW2992) and crizotinib (Xalkori). However, they still exhibit toxicities and limitations due to the differences in molecular and histological profiles of lung cancers. UMIPIC is a new option for cancer treatment, as it integrates local chemotherapeutic effect with systematic antitumor immunity by intratumoral drug delivery. We have applied UMIPIC in the treatment of advanced lung cancer with a compounded solution including three components, *i.e*. an oxidant, a cytotoxic drug (Cytosine Arabinoside: Ara-C) and hapten. To evaluate the therapeutic effects of hapten-enhanced chemoimmunotherapy in the treatment of advanced lung cancer by UMIPIC and to analyze the effect of this immune booster.
Materials and Methods: Ninety seven patients with advanced lung cancers were treated with UMIPIC or intratumoral chemotherapy (ITCT). UMIPIC was delivered intratumorally incombination with a proprietary therapeutic regimens composed of three components including an oxidant, a cytotoxic drug and hapten. ITCT applied the same procedures and regimens only without hapten. All data from two groups were reviewed and analyzed. Fifty-five patients were treated with UMIPIC and 42 with ITCT. Patient responses were assessed with CT scan at 4-6 weeks after treatment, and all of patients were followed until their deaths.
Results: Median overall survival (OS) was 11.23 months in UMIPIC (test) group and 5.62 months in ITCT (control) group (P<0.01). The 6-months and 1-year survival rate of the UMIPIC and ITCT groups were 76.36 % vs. 45.23 % (P<0.01) and 45.45 % vs. 23.81 % (P<0.05), respectively. UMIPIC group with two circles of treatments (19) had a significant survival benefit compared with ITCT (29) group with two circles of treatment; significant benefits in survival time were also found in UMIPIC (n=20) compared to ITCT (n=13) when both of which were performed without adjuvant treatment.
Conclusion: UMIPIC for lung cancer is a non-invasive and potentially effective therapy with a satisfying profile of high specificity and prolonged survival time. It offers a prospect of tailoring treatments much more precisely and could lead to a better response, especially in patients in advanced stages of inoperable or drug-resistant types of lung cancer. Surely, more effective control of the disease is needed for us to investigate the UMIPIC with two cytotoxic drugs with hapten under clinical study; it may reduce less injection and give more conveniences to *Good Practice*.

B92 Hapten-rnhanced therapeutic effects in advanced hepatocellular carcinoma by percutaneous intratumoral drug delivery. Baofa Yu[1], Yuanfei Lu[2], Jian Liu[3], Peng Jing[1], Wei Han2, Feng Gao[3], Ning Ru[3], Guanghui Cui[3], Chenglin Sun[2], Yebing Che[2], Zhenlu Ma[3].
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Background: Hepatocellular carcinoma (HCC) is an aggressive cancer. An estimated 30,640 new cases and 21,670 cancer deaths will occur in the US in 2013. The current treatments for advanced HCC, including TACE (TACE), adoptive immunotherapy, interferon therapy, percutaneous ethanol injection, radio-frequency ablation and a molecular target drug sorafenib, use either alone or combination, showed benefits on survival rates but still limited. Current therapeutic approaches of TACE (oil-water of drug emulsion) is often used in clinical practice of HCC therapy, it belongs to local therapy with a less side effect compared to systematic chemotherapy. The concept of percutaneous intratumoral drug delivery has been known for several decades as one of local therapies. Some successful examples have clearly shown the clinical feasibility of such treatment options, with significant reduction in both toxicity and tumor growth, but most research of intratumoral drug delivery with single drug had limited the clinical impact on the survival time, the combination of different drugs in a aqueous solution for intratumoral therapy is strongly needed in application of intratumoral drug delivery. In the last decade we had started the clinical research of therapeutic combination in a aqueous solution of single chemotherapeutic drugs and oxidant with hapten (as HECT) or without hapten (as ITCT) in advanced HCC patients using percutaneous intratumoral injection and the data was not reported previously since the combination with double chemotherapeutic drugs and oxidant with or without hapten as comparing study in HCC treatments is still undergoing in clinical research. Now the data using single drug and oxidant with or without hapten in the study of HCC treatment were collected and analyzed. The primary objective of this cohort study was to assess the feasibility, safety and efficacy of HECT versus injection of ITCT.
Purpose: To comparison therapeutic effects in treatment of advanced hepatocellular carcinomas (AHC) by Hapten-Enhanced Chemoimmunotherapy (HECT) with intratumoral Chemotherapy (ITCT) and to analyze the effect of hapten as an immune booster.
Materials and Methods: Patients with AHC were treated with HECT or ITCT, both had same therapeutic procedure, HECT had a proprietary regimen composed of components includes an oxidant, a cytotoxic drug and hapten; ITCT delivered the same drug excluded hapten. Of 339 patients, HECT patients (n=214) had 119 with response data and 214 with survival data, and ITCT patients (n=125) had 61 with response data and 125 with survival data. Tumor response was assessed with a CT scan at 6-8 weeks after initial treatment, survival rate was evaluated by follow up visits.
Results: The response rates (CR+PR+SD) were 78.68% and 81.52% in the HECT and ITCT group respectively with no significant difference, but the median overall survival was 7 months in HECT(Test) and 4 months ITCT (control), respectively (P<0.01). The 6-month and 1-year survival rates of HECT and ITCT were 58.88% vs 32.3% and 30.37% vs 13.6%, respectively significance (P<0.01). Single and multiple treatment in HECT significantly improved the survival time and efficacy compared with single and multiple treatment in ITCT.
Conclusion: Inflammatory response may involve anti-tumor immunity, the therapeutic coagulation mass as a inflammatory tissue with cytokine and chemokine release are attracting dendritic and other antigen-presenting cells to meet the antigens released from the dead tumor to drain lymph nodes, driving an adaptive acute immunity to further eradicate cancer cells at distant sites. This clinical study showed that HECT could induce more inflammatory response in local tumors, with no significant response effect of HECT and ITCT groups at the time of re-examined for evaluation of response rate, but for long term of follow-up it did show significantly increased survival time for the HECT group. We believe that HECT-induced inflammatory

response with hapten may play an important role in the tumor's autologous vaccine-like function, for patient with large tumor this immunological power may be weak to reach significant clinical benefit, so that multiple treatments are strongly needed.
Finally, we can concluded that Hapten had enhanced therapeutic effect with increased survival time in HECT patients compared to ITCT, while at re-examined the response rate was no difference due to inflammation by hapten. It suggests hapten plays an important role in immune modulation with chemoimmunoterhapy to improve patient survival time.

B93 Hapten-enhanced therapeutic effects in advanced pancreatic cancer. Baofa Yu[1]，Yuanfei Lu[2]，Jian Liu[3]，Peng Jing[1]，Wei Han[2]，Feng Gao[3]，Ning Ru[3]，Guanghui Cui[3]，Chenglin Sun[2]，Yebing Che[2], Zhenlu Ma[3]. [1]Beijing Baofa Cancer Hospital，Beijing, China,[2]Jinan Baofa Cancer hospital, Jinan, China, [3]TaiMei Baofa Cancer hospital, Beijing, China.

Background: Pancreatic cancer, with only a 6% five-year survival rate, and a median survival of 6-9 months, remains one of the most malignant and aggressive cancers. It is the 10th most commonly diagnosed cancer, the 4th leading cause of cancer death in the U.S. In 2013 approximately 45,220 people were diagnosed with this malignancy, with 38,460 attributed deaths worldwide during the period. The lack of progress in prevention, early detection, and diagnosis of this disease places most patients in an advanced stage at the time of diagnosis, with only about 15-20% of all pancreatic cancer patients having borderline resectable tumors. Because most patients are non-operable, the only remaining treatment options are generally conventional chemotherapy, radiation and targeted therapy separately or combined. Gemcitabine is the current standard chemotherapy regimen for advanced pancreatic cancer. The concept of intratumoral drug delivery has been known for several decades. Some successful examples have clearly shown the clinical feasibility of such treatment options, with significant reduction in both toxicity and tumor growth, but not in pancreatic cancer patients. Our data suggests that UMIPIC offers an ideal percutaneous intratumoral approach for chemical de-bulking of advanced pancreatic tumors.
Aim: In advanced pancreatic cancer patients we compared the clinical effectiveness of hapten-enhanced chemoimmunotherapy by ultra-minimum incision personalized intratumoral chemoimmunotherapy (UMIPIC) with intratumoral chemotherapy (ITCT). We then analyzed the role of hapten as an immune booster.
Method: Patients with advanced pancreatic cancer (92 cases) were randomly divided into two groups. UMIPIC is a proprietary therapeutic regimen which is composed of three intratumoral injections of a compound with an oxidant, a cytotoxic drug and hapten (hapten is utilized in modulation of chemoimmunotherapy). ITCT uses an oxidant and a cytotoxic drug without hapten. Both UMIPIC and ITCT use the same clinical therapeutic procedure.
Results: The UMIPIC-Therapy group (n=57) had 33 with response data and 25 with survival data. The ITCT group (n=26) had 14 with response data and 20 with survival data. In the UMIPIC and ITCT groups, clinical benefit rates were 82.88% and 92.86% respectively (no significant difference). The median survival times were 6.45 months vs 4.98 months ($P<0.05$) and the 6-month survival rate 64% vs 45% ($P>0.05$), respectively. While the six-month survival rate showed no statistical difference ($P>0.05$), the one-year survival rate of the UMIPIC-Therapy group

reached 28% compared with the ITCT group of only 5%, a statistically significant difference (P<0.05).
Conclusion: coagulation (like de-bulking) eliminates at least some (more than 90%) of the neoplastic cells in the tumor targeted with anti-neoplastic agents, which kill the left-over live neoplastic cells not initially killed by UMIPIC. The *in situ* "vaccination" further eliminates live neoplastic cells, which results in better therapeutic efficacy than other therapeutic options. Therefore, it is reasonable to conclude that UMIPIC is a relatively safer and more efficacious option, especially for patients with advanced stage disease. More effective controls of the disease is strongly needed. We hope to continue to investigate UMIPIC-Therapy with two cytotoxic drugs with different hapten under clinical study for better effectiveness. We believe it has shown to be safe, easy to operate and repeat, simply based on the patient tumor size, without side effects.

B94 Postoperative intravenous chemotherapy combined with intraperitoneal perfusion chemotherapy versus intravenous chemotherapy alone for gastric cancer: A systematic review and meta-analysis. Yang Sheng. Department of Medical Oncology, Fujian Medical University Union Hospital, Fujian, China.

Background: Gastric cancer is a common cancer with relatively poor survival rates. The present meta-analysis compared the effect and safety of postoperative intravenous (IV) plus intraperitoneal (IP) chemotherapy with IV chemotherapy alone for gastric cancer.
Methods: CINAHL, Medline, Embase, Web of Science, the Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov databases were searched for relevant RCTs. Two review authors independently screened studies, extracted data and assessed the risk of bias. GRADE system was used to assess the quality of evidence.
Results: A collection of 392 citations yielded eligible 5 studies involving 1072 participants. Combined IV and IP chemotherapy following gastric resection were found to have a statistical improvement for overall survival rate at 1 year (RR=1.10, 95% CI 1.04 to1.17, P=0.005); overall survival rate at 3 years, (RR=1.22 95% CI 1.11 to1.35, P=0.001); and for overall survival rate at 5 years, (RR=1.42, 95% CI 1.12 to 1.80, P=0.004). The evidence was judged to be moderate. Low evidence supported a lower rate of distant metastasis (RR=0.41, 95% CI 0.19 to 0.89, P=0.002) and moderate evidence supported a lower peritoneal metastasis rate in the treatment group (RR=0.41, 95%CI 0.26 to 0.62, P=0.001). For adverse effects, our meta-analysis showed a higher incidence of neutropenia (RR=1.32, 95%CI 1.18 to 1.48, P=0.001), peripheral edema (RR=3.63, 95% CI 2.28 to 5.77, P=0.001) and neuropathy (RR=3.52, 95%CI 2.66 to 4.65, P=0.001) in the treatment group.
Conclusion: Postoperative Combination of IP chemotherapy and IV chemotherapy was demonstrated as more effective in improving 1-5 year overall survival rates and in preventing the distant or peritoneal metastasis. However, it may elicit more adverse effects in neutropenia, peripheral edema and neuropathy. As the quality of included studies was judged to have some risk of bias, our confidence to these findings is limited. More data from rigorous studies is needed.

B96 Not all types of tumor-infiltrating lymphocytes amity with breast cancer: A systematic review and meta-analysis. Zhigang Zhang[1,3§]; Xiuyang Yu[1,2§]; Yang Liu[1]; Zhen Wang[1]; Jing Zhao[1,2]; Ping Wu[1]; Zhigang Chen[1,2]; Fuming Qiu[1,2]; Jian Huang[1,2]*. [1]Cancer Institute (Key Laboratory of Cancer Prevention & Intervention, National Ministry of Education, Provincial Key Laboratory of Molecular Biology in Medical Sciences), [2]Department of Oncology, Second Affiliated Hospital, [3]Department of Gynecology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. [§]These authors contributed equally to this work. *Corresponding Author: Professor Jian Huang, MD, PhD, Cancer Institute and Department of Oncology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China.

Background**:** Breast cancer is the most common invasive cancer to affect women in the world. Researches previously concern more about the biological features of breast cancer itself, but recently pay more attentions to tumor microenviroment, like tumor-infiltrating lymphocytes (TILs). Accumulating evidences have shown that TILs exhibit both inhibitory and stimulatory effects on breast cancer, but the conclusion still remains unclear. Here we searched the available studies and conducted a meta-analysis aimed to evaluate the value of total or a subtype of TILs as a prognostic marker for breast cancer and to determine their relationship with several clinicopathological variables.
Methods: A comprehensive search strategy was used to search relevant literatures in PubMed, MEDLINE and the Web of Science, by using the following tags: “tumor-infiltrating lymphocytes and breast cancer” or “tumor-associated lymphocytes and breast cancer”. The correlation between TILs and breast cancer clinicopathological features and prognosis was analyzed by using Rev Man 5.3.
Result: Eighteen eligible studies consisting of 12,805 participants were included. We found that high value of total TILs had no relationship with breast cancer grade, expression of hormone receptor and HER-*2, whereas some subtypes of* TILs, such as higher PD-1^{+}TILs or Foxp3^{+}TILs values were observed in patients with HR(-) (pooled RR =1.34, 95% CI: 1.18-1.52) and HER-2(+) cancer (pooled RR =0.65, 95% CI: 0.46-0.92) respectively. Interestingly, TILs were correlated with response to neoadjuvant chemotherapy (pooled RR =0.49, 95% CI: 0.39-0.61), except for HR(-) patients (pooled RR =0.79, 95% CI: 0.59-1.06). Moreover, over-expression of total TILs were associated with better prognosis, but without statistical significance (RR = 0.88, 95% CI = 0.67–1.16), whereas both PD^{+} TILs and Foxp3^{+}TILs subtypes predict a worse prognosis.
Conclusion: This meta-analysis confirms that high value of total TILs were not associated with breast cancer clinicopathological features, but can predict a favourable outcome for neoadjuvant chemotherapy except for HR(-) type. Higher total TILs may be a potential prognostic indicator, while some subtypes like PD-1^{+}TILs and Foxp3^{+}TILs show a poor prognosis.

B103 Rapamycin-Induced tumor response in a patient with metastatic epithelioid angiomyolipoma. Peng Li, Hua-jie Dong, Wei-wei Cheng, Tao Ye,Hong-jun Lu,Tong Zhou
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Purpose: Renal angiomyolipoma (AML) is a benign mesenchymal tumor of the kidney composed of blood vessels, smooth muscle cells, and adipose tissue. The epithelioid variant of renal angiomyolipoma is a rare disease and is characterized by a predominance of perivascular epithelial cells and HMB-45 expression. Unlike benign renal AML, E-AML behaves in an

aggressive manner. Mutations in TSC genes and the activity of mammalian target of rapamycin (mTOR) are associated with the pathogenesis of angiomyolipoma. Therefore, mTOR inhibitor therapy may be considered in patients with E-AML.
Methods: A 64-year-old woman with a history of epithelioid angiomyolipoma of the kidney was admitted to our hospital with newly found lung nodules. Four years previously, he had undergone right radical nephrectomy at our institution. Percutaneous needle biopsy of the left lower lober nodule, A positron emission tomography–CT scan showed no metastatic disease other than in the lung. The patient refused surgery, so the treatment with everolimus was initiated at 10 mg everyday.
Results**:** After one week of everolimus treatment a CT scan showed the left lower lung nodule had decreased in size of 50%, and after three weeks, a follow-up CT scan showed the left lower lung nodule had decreased in size of 70%.
Conclusion: Currently, there is no standard therapy for EAML, and cytotoxic chemotherapy agents showed minimal response or no response. As in AML, the mTOR pathway is upregulated in EAML. Based on this result, mTOR inhibitors may be an appropriate
option in patients with unresectable E-AML.