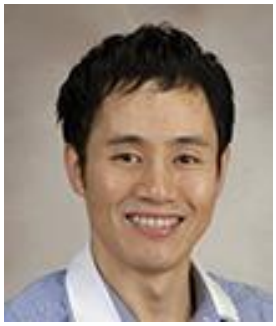
Profile of an Early-Career Researcher

Kyoji Tsuchikama, PhD



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Antibody-drug conjugates (ADCs) are an emerging class of chemotherapeutic agents for cancer therapy. Promising clinical outcomes, with 4 FDA-approved ADCs and more than 70 ADCs in clinical trials, have attracted a great deal of attention from many researchers and clinicians. Dr. Kyoji Tsuchikama is a principal investigator leading a medicinal chemistry lab at the University of Texas Health Sciences Center at Houston (UTHealth). One of his research interests is directed towards establishing novel chemical platforms for effective targeted therapeutics, including ADCs. The engine of his laboratory, driving their effort in complex ADC research, is the diverse expertise and background of his lab members, which include organic chemistry, peptide chemistry, medicinal chemistry, chemical biology, pharmacology, radiochemistry, and skills for performing animal studies.

Dr. Tsuchikama studied organic chemistry, with a focus on transition metal-catalyzed reactions, during his undergraduate and graduate work with Prof. Takanori Shibata at Waseda University in Japan. After earning a Ph.D. in organic chemistry in March 2010, he received fellowship support from the Japan Society for the Promotion of Science (JSPS) to begin postdoctoral work on bacterial quorum sensing, under supervision of Prof. Kim D. Janda at The Scripps Research Institute. With experience at the interface of chemistry and biology in hand, Dr. Tsuchikama started his independent career at UTHealth in July 2014. He has received support for his research programs from the UT System (Reagents' Health Research Scholars Award) and the Department of Defense (Breast Cancer Research Program Breakthrough Award).

His laboratory has been committed to developing novel linker technologies for constructing efficacious ADCs. One of their recent achievements is a method for enzymatic conjugation using branched linkers that can accommodate two payload units (*Organic & Biomolecular Chemistry* **2017**, 15, 5635-5642). Despite extensive efforts to advance ADC linker and conjugation chemistries, most of the commonly used ADC linkers possess linear structures and can accommodate only single payloads. The clinical potential of branched ADC linkers that can accommodate multiple payloads has not been fully explored. In particular, although apparently easy to achieve, it has been technically challenging to construct homogeneous ADCs using branched linkers, due largely to the lack of efficient conjugation methods.

Dr. Tsuchikama and his lab members have developed an efficient method for conjugating branched linkers using microbial transglutaminase (MTGase). MTGase-mediated linker conjugation has been previously used to attach simple linear linkers to the side chain of glutamine 295 within the antibody heavy chain. Tsuchikama and his lab have now optimized conjugation conditions and linker design to achieve quantitative installation of relatively bulky branched linkers containing two azide

groups, using the same enzyme. Subsequent coupling of payload modules by click reactions provides homogeneous ADCs with higher drug-to-antibody ratios (DARs) than conventional linear linkers, thus enhancing ADC cytotoxic potency with a minimal modification to the antibody structure. The lab is currently developing second-generation branched linkers that enable modular assembly of various homogeneous ADCs equipped with two different payloads.

Another notable achievement by his group is the discovery of a glutamic acid-valine-citrulline tripeptide linker (*Nature Communications* **2018**, 9:2512). A dipeptide comprised of valine-citrulline (VCit) is used as an enzymatically-cleavable linker in approximately 50% of ADCs that have been clinically approved or tested. This linker releases conjugated payloads in a traceless manner upon cathepsin-mediated cleavage in the target cancer cell. While stable in human plasma, VCit linkers are unstable in mouse plasma, due to susceptibility to the carboxylesterase Ces1c. Considering that most preclinical studies use mouse models, this instability creates an obstacle for the preclinical evaluation of the therapeutic potential and safety profiles of VCit-based ADCs. Dr. Tsuchikama and co-workers discovered that a glutamic acid-valine-citrulline (EVCit) linker can significantly improve ADC stability and efficacy in mouse models. A model anti-HER2 ADC constructed using this linker exhibited much greater *in vivo* stability than the corresponding VCit-based ADC, which rapidly lost payload molecules due to premature linker cleavage. Further, the EVCit ADC showed significantly improved therapeutic efficacy in xenograft mouse models of human breast cancer compared with the VCit variant. The use of the EVCit linker could serve as a simple, but powerful, solution to salvage many types of ADCs that were previously abandoned at the preclinical evaluation stage due to linker instability in mice. These findings also highlight the potential of this novel ADC linker to minimize failure rates in future preclinical studies.

In collaboration with many cancer biologists and immunologists, ongoing work in the Tsuchikama group is further refining these novel linker technologies to generate next-generation ADCs for difficult-to-treat cancers and other diseases.