Social Cooperation Research Program

Project Division of Cancer Biomolecular Therapy
がん生体分子治療社会連携研究部門

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Our division has been conducting basic research projects for development of innovative cancer therapy using immunologic and gene therapy approaches. The reagents, modalities, and concepts developed in this division have been clinically applied as translational research projects. We believe that bidirectional information exchange between the bench and the bedside would be one of the most important requirements for the successful development of novel and effective therapies.

I. Development of cancer immunotherapy using the blockade of MFG-E8

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The secreted protein, milk fat globule-EGF factor 8 (MFG-E8), stimulates disease progression through coordinated αvβ3 integrin signaling in tumor and host cells. MFG-E8 enhances tumor cell survival, invasion, and angiogenesis, and contributes to local immune suppression.

We have shown that systemic MFG-E8 blockade cooperates with cytotoxic chemotherapy, molecularly targeted therapy, and radiation therapy to induce destruction of various types of established mouse tumors. The combination treatments evoke extensive tumor cell apoptosis that is coupled to efficient dendritic cell cross-presentation of dying tumor cells. Our previous findings suggest that systemic MFG-E8 blockade might intensify the antitumor activities of existing therapeutic regimens through coordinated cell-autonomous. Our subsequent studies on human samples suggest that MFG-E8 derived from the tumor cells might affect the outcome of the chemotherapy for cancer patients. Based on these findings, our recent projects include the investigation on the significance of tumor-derived and non-tumor derived MFG-E8 using MFG-E8 gene knock-out mice. Furthermore, we are now seeking the mechanisms for the upregulation of immune-regulatory molecules including PD-L1 and MFG-E8 using human cancer cell lines. We believe that these efforts will results in the development of new class of anti-tumor therapies.

II. Development of fully retargeted herpes simplex virus (HSV) vectors for oncolytic virotherapy

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Herpes simplex virus (HSV) vectors are promising agents for oncolytic virotherapy. Uchida established a fully retargeted HSV platform that mediates virus entry exclusively via tumor-associated antigens in the lab of Prof. Joseph Glorioso at the University of Pittsburgh. Entry of HSV is initiated by the binding of glycoprotein D (gD) to one of its receptors, herpesvirus entry mediator (HVEM) or nectin-1. This interaction results in a conformational change in gD, triggering

sequential activation of gH and gB to execute fusion between the viral envelope and cell membranes. We inserted single-chain antibodies (scFv) against a number of different cell surface molecules such as epidermal growth factor receptor (EGFR), carcinoembryonic antigen (CEA), and epithelial cell adhesion molecule (EpCAM), into the retargeted HSV platform that encodes a gD ablated for binding to natural receptors and a gB containing entry-enhancing mutations we previously identified. As a result, we observed specific virus entry into cells expressing the cognate target antigen for each of the retargeted constructs. Our results indicate the adaptability of our system to different targeting ligands, leading to a new generation of broadly applicable and effective oncolytic HSV vectors (receptor-retargeted oncolytic HSVs; RR-oHSVs). Furthermore, we introduced syncytial mutations into the gB and/or gK genes of RR-oHSVs and found that gD retargeting does not abolish the hyperfusogenic activity of syncytial mutations and that these mutations do not eliminate the dependence of HSV entry and spread on a specific gD-receptor interaction. We investigated the *in vivo* anti-tumor effects of the RR-oHSVs that harbor the syncytial mutations (RRsyn-oHSVs) using human cancer xenografts in immunodeficient mice. With only a single intra-tumoral injection of RRsyn-oHSV at very low doses, all treated tumors regressed completely. Furthermore, intra-venous administration of RRsyn-oHSV resulted in robust anti-tumor effects even against large tumors. We found that these potent anti-tumor effects of RRsyn-oHSV may be associated with the formation of long-lasting tumor cell syncytia not containing non-cancerous cells that appear to trigger death of the syncytia. These results strongly suggest that cancer patients with distant metastases could be effectively treated with our RRsyn-oHSV. Additionally, we are developing novel oncolytic vectors that are retargeted to tumor-associated antigens that have been shown to be expressed specifically on cancer cells.

III. Establishment of highly functional monoclonal antibodies through novel screening methods for targeted cancer therapy

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Monoclonal antibodies (mAbs) have become an established therapeutic modality in clinical oncology. In order to identify cell-surface molecules that may be useful for targeting various types of cancers, our group established a unique screening approach that employs an adenoviral vector harboring fiber proteins engineered to bind antibodies, Adv-FZ33. This approach led to the successful identification of an array of potential target molecules for cancer treatment. Immunotoxins (antibody-drug conjugates; ADC) are a promising class of cancer therapeutics composed of a cytotoxic agent linked covalently to a cancer-targeted antibody. To systematically hunt for cell-surface molecules that may be efficiently targeted by immunotoxins, our group created another method for screening highly functional cancer-targeted mAbs and cognate antigens. The receptor-binding domain of the Diphtheria toxin (DT) was replaced with the antibody-binding domain (3C) derived from the Streptococcal protein G. The resultant mutated toxin protein (DT-3C) was used for selection of mAbs for specific cell killing activity as components of immunotoxins. Our novel screening system is advantageous in that the selected antibodies bind to intact cancer cells and get internalized efficiently, which has been critically required for therapeutic applications but elusive thus far. Furthermore, we have successfully taken advantage of some of these in-house monoclonal antibodies for development of novel fully retargeted HSV vectors. Additionally, we have created an HSV-based probe for screening of Abs that could mediate HSV entry by recognition of unknown receptors. We have found that one of the Abs selected by this screening method is capable of mediating HSV entry when incorporated into gD as an scFv. Interestingly, the antigen recognized by the Ab has been found to be epiregulin, a molecule that is known as a growth factor expressed and shed from cancer cells. This was unexpected because this molecule has been commonly investigated as a soluble "ligand" that acts as a growth factor, and thus not as a membrane-bound "receptor". Thus, we expect that this novel Ab-screening system may lead to a new generation of RR-oHSV vectors.

Publications

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