IDENTIFICATION OF ONCOGENE-INDUCED SURFACE PROTEIN IL1RAP AS AN IMMUNOTHERAPY TARGET IN MULTIPLE CANCERS

Hai-Feng Zhang*, Wei Li, Christopher Hughes, Rajesh Kumar, Alberto Delaidelli, Yue Zhou Huang, Michael Lizardo, Rimas Orentas, Dimiter Dimitrov, Poul Sorensen.

1University of British Columbia, Vancouver, Canada; 2University of Pittsburgh, Pittsburgh, PA, USA; 3BC Cancer Research Centre, Vancouver, Canada; 4Seattle Children’s Research Institute, Seattle, WA, USA

Background Adoptive and antibody-based immunotherapies are highly successful in the clinic for the treatment of certain malignancies. However, effective immunotherapy targets remain elusive for various cancers, in particular metastatic diseases. Certain existing targets often encounter antigen loss, leading to tumor relapse. Thus, it is crucial to discover novel cell surface targets that are essential for tumor sustenance and metastasis, and thus less prone to immunotherapeutic escape.

Methods To uncover crucial cell surface proteins that drive anoikis-resistance and metastasis, we performed an integrated analysis of the global proteome and acute translatome in distinct oncogene-transformed and non-transformed cells in 3D cultures. Anoikis-resistance in vitro and metastasis in vivo were measured by 3D spheroid cultures and a murine renal subcapsular implantation metastasis model, respectively. Candidate surface targets from the screens were evaluated by immunohistochemistry using tissue microarrays (TMAs) of multiple human cancers, and TMAs consisting various adult and pediatric normal tissues. Phage-display biopanning was performed to identify single-domain VH binders, based on which CAR-T cells and a panel of humanized IgG1 antibody-drug conjugates (ADCs) were engineered. The CAR-T cells and ADCs were tested both in vitro and in vivo using murine tumor xenograft models.

Results The screens identified that distinct oncoproteins such as mutant KRasG12V, KRasG13D, ETV6-NTRK3, and EWS-FLI1 each upregulate IL1RAP (IL-1 receptor accessory protein) to suppress tumor cell anoikis. IL1RAP is highly expressed on cell surface in multiple malignancies, including Ewing sarcoma (EwS), melanoma, pancreatic ductal adenocarcinoma, high-grade astrocytoma, glioblastoma, and ALK+ anaplastic large cell lymphoma. Genetic IL1RAP inactivation in EwS triggers anoikis and ferroptosis in vitro and impedes metastasis in vivo. Importantly, IL1RAP is minimally expressed in pediatric and adult normal tissues and blood cells, nominating IL1RAP as a promising surface target for immunotherapy. We identified a panel of novel and highly specific IL1RAP binders via phage-display biopanning, and engineered IL1RAP CAR-T cells and anti-IL1RAP ADCs conjugated to cytoxic payloads such as pyrrolobenzodiazepine (PBD) dimer and Duocarmycin SA. IL1RAP CAR-T cells and anti-IL1RAP ADCs induced massive cell killing in a panel of EwS cells in vitro. Although the efficacy of IL1RAP CAR-T cells in murine EwS xenografts was limited by an immune suppressive tumor microenvironment, the anti-IL1RAP ADCs effectively limited EwS tumor progression at low doses such as 0.1mg/kg.

Conclusions Therefore, we have defined surface IL1RAP as an immunotherapy target in EwS and potentially other human malignancies, and our pre-clinical studies suggest anti-IL1RAP ADCs as a highly promising immunotherapy strategy.

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