Scientific Abstract

SU2C Natural Killer Cells Convergence Research Team:

"Integrating Experimental and Computational Pipelines to Develop Biomarkers of Tumor Cell Resistance to NK Cells"

[This abstract was provided by the scientists when their application was accepted.]

Natural killer (NK) cells have potent anti-tumor cytotoxic properties, operate across HLA barriers, and their in vivo activity against allogeneic tumor cells is not confounded by GVHD, but it is typically less potent than their activity in vitro, even after potent ex vivo activation. The precise molecular mechanisms/biomarkers determining whether tumor cells (in vivo or in vitro) will be sensitive vs. resistant to NK cells have been examined in the past but remain incompletely understood. Despite the perception or, perhaps, hope that NK cells can kill tumor cells in a manner agnostic to their underlying genetics, data from various groups, including our own papers and preliminary data suggest that the underlying genetic features of tumor cells can significantly impact the response of tumor cells to NK cells alone; as well as influence their response to compounds with immune-modulatory properties.

Identification of candidate biomarkers of NK cell resistance through CRISPR/Cas9-based screens and studies in large panels of molecularly annotated cell lines: To systematically identify and validate candidate mechanisms regulating tumor cell response to NK cells, we performed (unpublished observations) orthogonal screens in 2 systems: (i) genome-wide CRISPR gene editing screen (GECKO library) in a highly NK cell-sensitive tumor cell line and identified candidate genes for which their knockout confers NK cell resistance; and (ii) quantified the cytotoxic effect of primary NK cells or an NK cell line (at different time points and effector to tumor, ratios) against a pool of 568 solid tumor lines, each with a distinct "DNA barcode" (PRISM system), quantified NK cell cytotoxicity based on the relative abundance of these barcodes in treated vs. control cells; and correlated the response of each cell line with transcriptional, mutational and other molecular features of the cell lines covering a broad range of different types of solid tumors. It was reassuring that the candidate "hit" genes from each screen included some easily recognizable regulators of pathways that NK cells use to kill tumor cells; as well as biologically plausible, but previously under-appreciated, candidates, which we plan to further evaluate.

In vivo model of bone marrow (BM)-like scaffolds with "humanized" stroma to support human patient-derived xenografts (PDX): Several investigators (Labs of Drs Mitsiades and Groen) have extensive experience with this preclinical in vivo model, which has become a major model system to enable in vivo expansion of patient-derived tumor cells from blood cancers and bone- metastazing solid tumors, including tumor cells (e.g. standard risk myeloma or leukemia samples) which do not typically survive and expand in vitro or in vivo. In this model, we pre-populate biphasic calcium phosphate

scaffolds in vitro with primary human BMSCs from donors or patients, under conditions of osteogenic differentiation, and implant the scaffolds s.c. in NSG mice: this creates an in vivo niche which recapitulates the human BM. In this model, patient-derived cells from various blood cancers (e.g. multiple myeloma [MM], leukemias) and bone-metastasizing solid tumors survive, expand, and maintain the clonal architecture of the initial clinical sample; while their response or resistance to treatments is concordant to the clinical outcomes of the same treatments in the respective patient(s). This model addresses limitations of conventional PDX models, e.g. the limited, if any, cross-reaction of several BMSC-derived mouse cytokines (e.g. IL-6), with their receptors on human tumor cells; and it will be one of the main in vivo systems for the testing and validation of new markers identified from our proposed studies.