

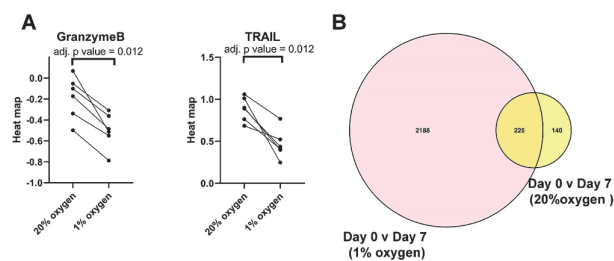
Immune Cell Biology

638 HYPOXIA REPROGRAMS NATURAL KILLER CELLS AND IMPAIRS THEIR THERAPEUTIC POTENTIAL

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Background Natural killer (NK) cell-based immunotherapies, from biologics to cell products, are being studied in the clinic across many cancer settings. These treatments have had therapeutic success for hematological malignancies but their impact on solid tumors remains limited. To succeed in the solid tumor setting, NK cells must enter the tumor microenvironment, with its low oxygen concentration (hypoxia), and retain functionality. Hypoxia is known to impair NK cell function, but a greater understanding of the mechanisms driving this impairment could lead to improvements in NK cell immunotherapy for solid tumors.

Methods We used an advanced incubator system: AVATARTM (Xcell biosciences), to finely tune the oxygen conditions in vitro to mimic the physiologic (5–12% oxygen) and hypoxic (1% oxygen) conditions found in vivo. Human NK cells were isolated from healthy donor blood and cultured with a low dose of interleukin 15 for up to 7 days, at 20% oxygen (standard incubator) or at 12%, 5% or 1% oxygen, to replicate the physiological conditions found in blood, bone marrow or hypoxic tumor, respectively. Phenotypes were analyzed by mass cytometry. Confocal and live cell imaging examined the cytotoxic process. Metabolic processes were assessed by flow cytometry and Seahorse assay. RNAseq and ATACseq were performed.



Abstract 638 Figure 1 NK cells are altered by exposure to hypoxia. Human NK cells from healthy donor blood were cultured in standard incubators (20% oxygen) or hypoxia (1% oxygen) for 7 days. (A) The relative abundance of granzyme B and TRAIL were compared on these NK cells at day 7 by time of flight mass cytometry. Analyzed by differential expression analysis through Astrolabe Diagnostics. Each line represents a donor. (B) Venn diagram of overlap between ATACseq differential expression analysis peaks. 2,413 regions were open at day 7 compared to day 0, when cultured under hypoxia (red circle); 365 regions were open at day 7 compared to day 0, when cultured in standard incubators (yellow circle).

Results NK cells were capable of natural cytotoxicity and antibody-dependent cellular cytotoxicity at physiological oxygen concentrations (5–20% oxygen), but killing was impaired under hypoxia (1% oxygen). Examination of the cytotoxic process revealed conjugate formation, polarization of granules to the synapse and granule release were not impaired by hypoxia. However, granzyme B (a component of cytotoxic

granules) and the death receptor TRAIL were decreased in NK cells exposed to hypoxia (figure 1A). RNAseq revealed upregulation of histone demethylases under hypoxia, with a shift in metabolism and decrease in the cell cycle. Glycolysis was upregulated under hypoxia and there was a concomitant increase in reactive oxygen species. ATACseq revealed profound epigenetic regulation of NK cells exposed to hypoxia, with limited changes occurring in NK cells cultured in 20% oxygen (figure 1B). Activation, adhesion, killing, proliferation and cytokine secretion were all pathways differentially regulated under hypoxia compared to 20% oxygen.

Conclusions NK cells exposed to hypoxia fail to kill tumor cells. Mechanistically, a lack of granzyme B and death receptors contribute to this deficit. ATACseq reveals epigenetic signatures associated with NK cell function that may allow interventions crucial to overcome barriers to solid tumor immunotherapy.

Acknowledgements We would like to acknowledge the services of the Minnesota Supercomputing Institute, the University Imaging Centers and the University Flow Cytometry Resource, all University of Minnesota.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.638>