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ACTIVATION OF IL-22 SIGNALING CORRELATES WITH HIGHER CD155 EXPRESSION AND STRATIFIES POOR OUTCOMES OF EARLY-STAGE LUNG AND BREAST CANCER PATIENTS

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Background CD155 (poliovirus receptor, PVR) is an immunosuppressive molecule overexpressed in lung adenocarcinoma (LUAD) and breast cancers (BRCA). However, no mutation has been identified that could be linked to such overexpression, and therefore it is likely regulated on the transcriptional level. Previously we identified interleukin-22 (IL-22) signaling as one of the pathways that upregulate CD155 expression in mouse models of lung and breast cancer. However, it is difficult to assess the activity of the IL-22 axis in the publicly available datasets since IL-22 signaling involves several components that must be considered: IL22, IL22RA1 and IL10RB, which encode heterodimeric IL-22 receptors found on tumor cells, and IL22RA2, which encodes soluble IL-22 binding protein (IL-22BP), an antagonist of IL-22 secreted by myeloid cells. The expression of IL22 itself is often missing in the available data due to the insufficient depth of sequencing, which prompts scientists to utilize one of the available components of the axis as a surrogate.

Materials and Methods Here we used agglomerative clustering, a bottom-up method of hierarchical clustering, to stratify the dataset by gene expression patterns in an unsupervised way. For this, we used LUAD and BRCA sequencing datasets from The Cancer Genome Atlas (TCGA). In the current analysis, we focused on HER2+ samples of the BRCA dataset. For clustering, we utilized PVR, IL22RA1, IL22RA2, and IL10RB gene expression. In identified clusters, we compared overall (OS) and restricted mean survival time (RMST) for the first five-year follow-up.

Results In both cohorts, we identified three clusters that are characterized by the following patterns of gene expression: cluster 0 (IL22RA1high, IL22RA2low, IL10RBmedium, PVRlow), cluster 1 (IL22RA1low, IL22RA2high, IL10RBhigh, PVRhigh), and cluster 2 (IL22RA1low, IL22RA2low, IL10RBlow, PVRmedium). Here, cluster 0, identified by a high expression of IL-22 receptor and CD155, and low expression of IL-22BP, was characterized by the poor OS in both cohorts. Moreover, the average difference in RMST between clusters 1 and 0 constituted 361 days in lung and 93 days in HER2+ breast cancer. This difference could be explained by the prevalence of advanced-stage patients in the lung cancer but not in the breast cancer cohort. Moreover, we identified that this difference in survival between clusters stems from differences in early (I and II), but not late-stage (III and IV) patient entries.

Conclusions Here we identified that early-stage lung and HER2+ breast cancer patients could be stratified according to their IL22RA1, IL22RA2, IL10RB, and PVR expression with cluster 0 predicting lower OS and shorter RMST. Mechanistically, the activity of such a pathway defines the immunosuppressive axis we identified previously.

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CATHEPSIN V INHIBITION PREVENTS THE ACTIVATION OF CYSTATIN F AND INCREASES IMMUNE CELL CYTOTOXICITY

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Background Cysteine cathepsins C, H, and L are important mediators of granule-dependent cytotoxicity of natural killer cells and cytotoxic T lymphocytes as they enable activation of granzymes and perforin, which execute cytotoxic effects on cancer cells. Cystatin F plays a central role in the regulation of cathepsins in cytotoxic immune cells. This type II family cystatin can be translocated to endo/lysosomes or secreted and further internalized by bystander cells due to the glycosylation. In the lysosomes it is activated from inactive dimeric form to active monomer by cathepsin V, which cleaves 15 N-terminal amino acids from cystatin F. Cystatin F is normally expressed by immune cells, however, in tumor microenvironment it was found to be increased also in non-immune cells. The increased levels of cystatin F may contribute to the immunosuppressive status of the tumor microenvironment. We evaluated the effects of cathepsin V inhibition on the cytotoxicity of immune effector cells NK-92 and TALL-104.

Materials and Methods To discover cathepsin V inhibitor, molecular docking was used to evaluate interactions of small molecular compounds from commercial libraries with cathepsin V. A set of selected compounds was evaluated by enzyme kinetics for the inhibition of recombinant cathepsin V, selectivity and reversibility of binding to the target. The most potent, selective, and reversible acting compound was tested in functional assays. The effect of cathepsin V inhibition on cystatin F activation was tested with western blot. Calcein-AM release assay was used to evaluate the effect on immune cell cytotoxicity.

Results After molecular docking and biochemical evaluation, we selected the most potent, selective, and reversible ureido methylpiperdine carboxylate derivative as inhibitor of cathepsin V. Next, we tested the effect of the selected compound on cystatin F activation in cytotoxic immune cells. The cystatin F dimer-to-monomer ratio was increased after treatment with both broad-spectrum peptidase inhibitor E-64d and after treatment with the selective reversible cathepsin V inhibitor. As expected, treatment of immune effector cells with E-64d decreased cytotoxic function, as this inhibitor impairs the activities of all cathepsins including cathepsins C, H, and L. However, treatment of cytotoxic cells with cathepsin V inhibitor increased their cytotoxicity.

Conclusions Selective inhibition of cathepsin V prevents the monomerization and activation of cystatin F. By targeting the activating protease of cystatin F, we can reduce the detrimental effects of cystatin F on cytotoxic cells in the tumor microenvironment.


P09.05 EXPLORING THE THERAPEUTIC VULNERABILITIES OF CATHEPSIN S HYPERACTIVE TUMORS

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Background We recently discovered recurrent cathepsin S (CTSS) alterations, i.e., either somatic gain-of-function mutations (at the hotspot Y132) or amplification/overexpression of CTSS that result in aberrant hyperactivity of this lysosomal cysteine protease and promote tumor growth in follicular lymphoma (FL). In addition, CTSS is frequently overexpressed in other lymphomas and other cancers (e.g., Gloma, triple-negative breast cancer, renal cancer). Intriguingly, when released from the lysosome, CTSS is tightly linked with the regulation of cell death pathways.

Materials and Methods BH3 profiling allows determining, to what extent a cell is primed towards cell death. It measures the extent of mitochondrial membrane permeabilization (MOMP) in response to BH3 peptides. Thus, BH3 profiling can record the net response of the pro- and anti-apoptotic interactions of the >15 apoptotic BCL2 family members in high-throughput (384-well plate flow-cytometry). Using BH3 profiling, we aimed to exploit the therapeutic vulnerabilities of CTSS hyperactive tumors, with a focus on compounds that induce lysosomal membrane permeabilization (LMP).

Results Our preliminary data suggest that LMP is a promising novel therapeutic strategy in tumors with CTSS hyperactivity. Specifically, dose-response curves for cell viability indicated a higher sensitivity to LLOMe treatment in DG75 CTSS knock-out (CTSSKO) lymphoma cells with reexpression of CTSS WT (KO+CTSSWT), and highest sensitivity in DG75 with overexpression of CTSS Y132D (KO+CTSSY132D). Similar results were obtained in single-cell derived clones from Karpas422 lymphoma cells (CTSS KO vs WT vs Y132D). Release of cathepsins into the cytosol can lead to MOMP and apoptosis through the cleavage of BCL2 family members, including proteolytic inactivation of anti-apoptotic MCL1 and cleavage of BID which produces proapoptotic truncated BID (tBID). Note, CTSS is the only cathepsin that remains enzymatically active at neutral pH for hours. Western blot confirmed increased cleavage of BID, MCL-1 and PARP-1 in LLOMe treated CTSSWT and CTSSY132D cells, indicating that CTSS release by LMP can more efficiently induce apoptosis in CTSS hyperactive lymphomas. BH3 profiling confirmed that CTSS hyperactivity is associated with a dependency on MCL-1 and BCL-2 following LMP. In addition, CTSS hyperactive glioblastoma cell lines U-87 and U-251 also showed MCL-1 dependency following LMP, indicating that cytosolic CTSS hyperactivity primes for MCL-1 dependency across many cancer types.

Conclusions Releasing CTSS from the lysosome is novel, attractive concept that can be combined with conventional, apoptosis-inducing therapies.