ACTIVATION OF IL-22 SIGNALING CORRELATES WITH
CATHEPSIN V INHIBITION PREVENTS THE ACTIVATION
OF IL-22 RECEPTOR (IL22RA1, IL22RA2, IL10RB, AND PVR) WITH
Cytokine Activity

Materials and Methods

which prompts scientists to utilize one of the available components that must be considered: IL22, IL22RA1 and IL10RB.

cluster 0, identified by a high expression of IL-22 receptor

CD155 (poliovirus receptor, PVR) is an immunosuppressive molecule overexpressed in lung adenocarcinoma (LUAD) and breast cancers (BRCA).

Background

Conclusion

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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OUTCOMES OF EARLY-STAGE LUNG AND BREAST CANCER PATIENTS

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 (IL22RA1<sub>high</sub>, IL22RA2<sub>low</sub>, IL10RB<sub>medium</sub>, PVR<sub>high</sub>), cluster 1 (IL22RA1<sub>low</sub>, IL22RA2<sub>high</sub>, IL10RB<sub>high</sub>, PVR<sub>medium</sub>), and cluster 2 (IL22RA1<sub>low</sub>, IL22RA2<sub>low</sub>, IL10RB<sub>medium</sub>, PVR<sub>medium</sub>). Here,
Exploring the Therapeutic Vulnerabilities of Cathepsin S Hyperactive Tumors

**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 granymes 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 into 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 methyliiperidine 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.


**Initial**

**Exploring the Therapeutic Vulnerabilities of Cathepsin S Hyperactive Tumors**

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