ACTIVATION OF IL-22 SIGNALING CORRELATES WITH CATHEPSIN V INHIBITION PREVENTS THE ACTIVATION OF HER2+ BREAST CANCER PATIENTS

D Briukhovetska*, J Jobst, S Endres, S Kobold. LMU, Munich, Germany

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: IL-22, 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, IL10RBmed, PVRhigh), cluster 1 (IL22RA1low, IL22RA2high, IL10RBhigh, PVRLow), and cluster 2 (IL22RA1low, IL22RA2low, IL10RBlow, PVRmedium). Here, cluster 0 is characterized by the expression of IL-22 receptor and CD155, and low expression of IL-22BP, which is 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: IL-22, 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.
EXPLORING THE THERAPEUTIC VULNERABILITIES OF CATHEPSIN S HYPERACTIVE TUMORS

1O Weigert, 1TALL-104.
2Dana-Farber-Cancer-Institute, Boston, MA, USA
3TUM, München, Germany
4Universität Göttingen, Göttingen, Germany

PO0905

None.

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 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 proform to active monomer by cathepsin V, which has a pH optimum of 4.5. In the cytosol it is activated from dimeric form to active monomer by 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 1-[2-(methylamino)ethyl]-2-cyano-3-formyl-1H-pyrrole-4-carboxylate 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 EC50 concentration of 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 propeptide of cystatin F, we can reduce the detrimental effects of cystatin F on cytotoxic cells in the tumor microenvironment.