

Increasing natural killer cell cytotoxicity by targeting cystatin F

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Natural killer (NK) cells show strong cytolytic function against tumor cells and virus-infected cells. Due to their effective lysis of tumor cells and tumor stem cells NK cell-based immunotherapies were tested in several clinical trials delivering promising results. Increasing evidence suggests that tumor microenvironment regulate the phenotype and inactivates the cytolytic function of NK cells. Understanding the mechanisms of NK cell inactivation is crucial for development of more effective antitumor therapy. Cystatin F is endogenous inhibitor of cysteine peptidases cathepsins. It is produced and secreted as a disulphide-linked dimer inactive as an inhibitor of C1 cathepsins. Due to this N-linked glycosylation, it is targeted to endo/lysosomes where it is proteolytically cleaved by cysteine peptidase cathepsin V and activated to become a strong inhibitor of major granzyme convertases, cathepsins C and H. The secreted dimeric protein can be taken up from the microenvironment via mannose-6-phosphate receptors and directed to the endo/lysosomes of recipient cell. Using recombinant cystatin F proteins, we showed that dimeric and activated N-terminally truncated cystatin F were taken up by both NK-92 cells and primary human NK cells and translocated to endo/lysosomes. There they inhibited the activity of cathepsins C and H, and decreased the activity of effector granzymes A and B leading to a decrease in cytotoxic efficiency of NK-92 cells and primary human NK cells. Therefore, altered extra- and intra-cellular availability of active cystatin F could significantly affect NK cells' cytolytic function. We confirmed that N-glycosylation pattern affects the secretion, uptake and subcellular sorting of cystatin F in different cell lines. By targeting its expression and activation through inhibition of cystatin F activating peptidase or by modulating its glycosylation, we will be able to enhance/maintain the cytotoxicity of NK cells and increase their antitumor activity.