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ORAL ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Targeting SLC3A2 Sensitizes AML Cells Towards NK Cell-Mediated Killing

Kishan Bellamkonda, PhD[1], *Ramprasad Ramakrishnan, PhD*[1], *Somadri Ghosh*[2], *Katrin Reinbach*[1], *Marcus Järås, PhD*[1]

[1] Division of Clinical Genetics, Lund University, Lund, Sweden
[2] Division of Clinical Genetics, Lund University, Lund, Sweden

Acute myeloid leukemia (AML) is an aggressive hematological malignancy with poor prognosis; hence, new therapeutic strategies are urgently needed. Natural Killer (NK) cells play a key role in tumor immune surveillance, but their anti-tumor activity in AML is often attenuated due to immunosuppressive effects of the malignant cells. Thus, strategies to restore NK cell function has therapeutic potential by boosting endogenous NK cells. In particular, the identification of cell surface proteins on AML cells that inhibit NK cell-mediated killing may reveal new targets for antibody-based therapies. To identify such targets, we performed a pooled CRISPR screen directed to 1389 cell surface genes in Mono-mac-6 (MM6), a human AML cell line. The MM6 cells were co-cultured with primary human NK cells for four days in media supplemented with the cytokines IL-2 and IL-15. Among the top hits were several genes coding for MHC class I molecules, which are known negative regulators of NK cells, demonstrating that the screen was robust. Notably, the screen also identified that *SLC3A2* disruption sensitized the MM6 cells towards NK cell-mediated killing. *SLC3A2* encodes the heavy chain of the transmembrane protein CD98 (CD98hc), which plays a key role in integrin signaling, regulation of intracellular calcium and the transport of L-type amino acids. CRISPR-mediated deletion of *SLC3A2* in MM6 cells resulted in a two-fold downregulation of the transcripts of several inhibitory ligands of NK cells, including HLA-A, HLA-B, HLA-C and HLA-E. Upon co-culture, loss of *SLC3A2* expression in MM6 cells induced an upregulation of the degranulation marker CD107a on NK cells ($p<0.01$), resulting in increased killing of the AML cells.
CD98hc consists of two functional domains - one responsible for integrin signaling and the other responsible for amino acid transport. To identify which of these processes affect the sensitivity of AML cells towards NK cell-mediated killing, we performed rescue experiments by overexpression of mutated *SLC3A2* cDNAs following *SLC3A2* knockdown in MM6 cells. Overexpression of *SLC3A2* wild-type or an integrin signaling deficient *SLC3A2* cDNA rescued the inhibitory effect on NK cells. In contrast, the *SLC3A2* variant that lacked the amino acid transportation domain failed to inhibit NK cells. These findings suggest that it is the amino acid transportation function of *SLC3A2* that regulates the sensitivity of AML cells to NK cells.
To validate these findings, we cultured MM6 cells in media deprived of three key amino acids (leucine, isoleucine and phenylalanine) transported across the plasma membrane by CD98. Consistent with our previous findings, culturing the MM6 cells in the amino acid deprived media resulted in a two-fold downregulation of HLA class I molecules ($p<0.001$) accompanied by an increased killing by NK cells.
To assess the clinical relevance of these findings, we measured CD98hc expression on AML patient samples and corresponding normal bone marrow cells. CD98 levels were about 1.8-fold higher ($p<0,001$) in the AML samples ($n = 30$) compared to normal bone marrow cells ($n = 5$). Treating the AML patient cells with a monoclonal antibody targeting CD98 resulted in a 1.63-fold increase ($p<0.0001$) in NK cell-mediated killing. Corresponding treatments using normal bone marrow cells resulted in a 1.36-fold ($p<0.01$) increase in NK cell-mediated killing. Intriguingly, the CD98 antibody also negatively affected the viability (1.41-fold, $p<0.0001$) of AML patient cells in the absence of NK cells, this effect was not observed on normal bone marrow cells.
Taken together, we here performed CRISPR screening on AML cells co-cultured with NK cells and identified *SLC3A2* as a novel regulator of NK cells. Mechanistically, it is the amino acid transport function of *SLC3A2* that regulate the sensitization of AML cells towards NK cell killing. Targeting of CD98 using a monoclonal antibody selectively increased NK cell-mediated killing of AML patient cells compared to normal bone marrow cells. These findings highlight CD98 as a new promising target on AML that boost NK cell-mediated tumor immune surveillance.

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