SPPL3 and its substrate glycosyltransferases regulate the N-glycosylation of SLC3A2 and alter the affinities of anti-SLC3A2 immunotherapies in AML [Abstract]

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SPPL3 und seine Substrat-Glykosyltransferasen regulieren die N-Glykosylierung von SLC3A2 und verändern die Affinitäten von anti-SLC3A2 Immuntherapien in der AML

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Introduction: SLC3A2 is a cell surface transmembrane glycoprotein, which is frequently overexpressed in acute myeloid leukemia (AML) and negatively impacts patient survival. Next to mediating uptake of essential amino acids in complex with SLC7A5, SLC3A2 is crucial for integrin signaling, thus promoting interaction of leukemic cells with their microenvironment. Accordingly, anti-SLC3A2 immunotherapies have proven to inhibit growth and propagation of leukemic cells in preclinical models. Despite its importance as therapeutic target, no systematic screens on regulators of SLC3A2 have yet been reported.
Methods: Leukemia cell lines were infected with a genome-wide CRISPR/Cas9 knockout library, stained with anti-SLC3A2 antibody, and subjected to FACS to isolate the populations with high or low SLC3A2 signal. sgRNA distribution was analyzed bioinformatically. For main hits, sgRNA constructs were cloned in lentiviral vectors, AML lines were infected and the effects on SLC3A2 abundance, glycosylation and subcellular localization were assessed by western blot, flow cytometry, lectin blotting and immunofluorescence. Binding of anti-SLC3A2 antibodies and anticalins was assessed by flow cytometry and functional assays.
Results: The genome-wide genetic screens identified the Golgi-resident intra-membrane protease SPPL3 and its substrate glycosyltransferases MGAT1, 2 and 5, as opposite modifiers of SLC3A2. Whereas SPPL3 knockout decreased SLC3A2 detection, knockout of respective glycosyltransferases increased SLC3A2 detection by anti-SLC3A2 antibodies. Mechanistically, we found loss of MGAT1, 2, or 5 to significantly reduce N-glycosylation levels of SLC3A2, whereas knockout of SPPL3 increased SLC3A2 glycosylation. However, neither SPPL3 nor the glycosyltransferases directly affected SLC3A2 abundance or subcellular localization. The altered signal in flow cytometry hence resulted from changes in glycosylation only, which has been described for few other surface proteins. Target detection by an anti-SLC3A2 directed Anticalin likewise improved upon knockout of MGAT1,2 or 5, thus strengthening our findings.
Conclusion: Inhibition of the Golgi-resident glycosyl transferases MGAT1, 2, and 5 reduces SLC3A2 N-glycosylation and increases affinities of anti-SLC3A2 directed immunotherapies. Inhibiting glycosylation, which in itself has anti-cancer activities, could therefore synergize with anti-SLC3A2 directed therapies in AML.

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