Immunotherapy against a novel neuroligin-4X /β-neurexin checkpoint reverses natural killer cell exhaustion and halts liver fibrogenesis

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Backgrounds and Aims: The molecular mechanisms underlying impaired natural killer (NK) cell cytotoxicity in advanced liver fibrosis remain unclear. This study aimed to identify immunological checkpoint affecting NK cell dysfunction, particularly in the context of their interaction with hepatic stellate cells (HSCs). **Methods:** MASLD patients recruited with histologically confirmed Metavir fibrosis scores across a range of severities. Peripheral NK cells were screened via DNA microarray, revealing elevated expression of *Neuroligin-4X (NLGN4X)*. These cells were further characterized for exhaustion and cytotoxic markers before and after treatment with NLGN4X siRNA or a neutralizing antibody. Liver tissue-resident NK (trNK) cells from early and advanced fibrosis stages were sorted based on NLGN4X expression and assessed for proliferation and activation markers. Primary HSCs derived from MASLD liver biopsies were evaluated for expression of β-neurexin (β-Nrxn), the known ligand for NLGN4X. *In vivo* experiments included wild type (WT) and NLGN4X knockout ($NLGN4X^{-/-}$) mice treated with carbon tetrachloride (CCl_4) to induce fibrosis, administration of anti-NLGN4X antibody to WT fibrotic models, and adoptive transfer of $NLGN4X^{-}$ or $NLGN4X^{+}$ trNK cells into immunodeficient fibrotic recipient mice. Additionally, dual-targeting Luji peptides were tested for anti-fibrotic efficacy. **Results:** NLGN4X was overexpressed on peripheral and trNK cells in advanced fibrosis and correlated with impaired cytotoxicity. Activated HSCs showed elevated β-Nrxn expression. Treatment with anti-NLGN4X antibody upregulated phosphorylation of AKT, ERK, and P70S6K, thereby restoring NK activation and ameliorating fibrosis *in vitro* and *in vivo*. Silencing of NLGN4X also restored NK cell cytotoxicity against HSCs *in vitro*. $NLGN4X^{-/-}$ mice exhibited enhanced trNK activity and resistance to CCl_4-induced fibrosis. Furthermore, adoptive transfer of sorted $trNK^{NLGN4X-}$ cells into immunodeficient recipient mice delayed fibrosis progression compared to $trNK^{NLGN4X+}$ cells. Our designed Luji peptides blocking both NLGN4X and β-Nrxn significantly attenuated fibrotic changes *in vitro* and *in vivo*. **Conclusion:** The NLGN4X/β-Nrxn axis represents a novel immune checkpoint that modulates NK cell function and liver fibrosis progression. Targeting this pathway offers a promising therapeutic strategy in advanced liver fibrosis.