Luji-1 reduces fibrosis by blocking Neuroligin-4 and β-neurexin and overexpressing thyroid hormone receptor-β, suggesting Rezdiffra sensitization and synergism in combination

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Background and Aims: The anti-fibrotic effect of Natural Killer (NK) cells against active hepatic stellate cells (HSCs) is impaired in advanced fibrosis. Following uncover of a novel immune inhibitory checkpoint, Luji-1 designed as dual antagonist peptide to HSCs ligand β-neurexin (βNRXN) and NK Neuroligin-4 receptor (NLGN4). Rezdiffra, a thyroid hormone receptor-β (THR-β) agonist, added 11% fibrosis improve in metabolic dysfunction-associated steatohepatitis (MASH). Here-in, THR-β modulation by Luji-1 assessed on addition to its anti-fibrotic effects.

Method: Luji-1, scrambled-peptide (both 8 µg/ml), L-Thyroxin (T4, 10 nM) and combinations were in vitro incubated 6 hours with LX2 [HSCs cell line] monocultures in 1% FCS [quiescent] and 10% [activated]. Similar incubations assessed in peripheral-blood NK cells from F1/F2 and F3/F4 MASH donors monocultured in 10% FCS. Luji-1 vs scrambled-peptide treated NK cells were then cocultured 6 hours with LX2. For the in vivo study, biweekly i.p CCl₄ (0.5 µl /g body weight) fibrosis model induced in C57/Bl mice for 6 weeks; and biweekly Luji-1 (8 µg/mice) treatment provided along last 2 weeks.

Results: Using flow cytometry, active LX2 monocultured cells significantly overexpressed αSmooth Muscle Actin (αSMA) and βNRXN (suggesting pro-fibrotic features), but reduced THR-β expressions by 8.3-folds compared to quiescent state. Treated LX2 cells with Luji-1, T4 and combination significantly reduced αSMA expressions (61%, 31% and 85%, respectively). As Luji-1 induced 3.2-folds THR-β overexpression, the achieved synergism by combination was significant. Monocultured F3/F4 NK cells with scrambled-peptide were impaired (low CD107a); associated with 3.2-folds NLGN4 overexpress and 2.4-folds THR-β down express as compared to active F1/2 counterparts and quiescent healthy donors (P<0.05). Luji-1 or T4 promoted F3/F4 NK cytotoxicity against activate HSCs and enhanced THR-β expressions. Both Luji-1 and T4 achieved a significant synergistic increase of NK cytotoxic effect. In the in vivo CCl₄ model, Luji-1 significantly activated NK cells (increased CD107a and F-actin expressions) and improved all markers of liver inflammation and fibrosis (serum liver enzymes, histology H&E and serious red stains, liver extracts for αSMA and Collagen). Luji-1 downregulated NLGN4 and βNRXN expressions while increased THR-β expressions in liver extracts.
Conclusion: Luji-1 improved liver injury via: (1) direct βNRXN inhibition in myofibroblasts and decreased their activation, (2) NK cells stimulation against myofibroblasts directly via NLGN4 inhibition in NK cells and indirectly by blocking the myofibroblast βNRXN that serves as NK inhibitor and (3) THR-β over expressions that sensitizes Rezdiffra in combination.