

16 HEPARAN SULFATE SIDE CHAINS ARE ESSENTIAL FOR THE MODULATION OF IL-1 SIGNALS BY SYNDECAN-4 IN RA SYNOVIAL FIBROBLASTS

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Background and objectives Activated synovial fibroblasts (SFs) are key players during RA disease progression. They exhibit a tumour-like invasive behavior, which is most likely induced by a continuous stimulation with ECM molecules, growth-factors and inflammatory cytokines, like IL-1. Syndecan-4 (sdc4), a transmembrane heparan sulfate proteoglycan, has been implicated recently in the modulation of cytokine signaling and has been found to be upregulated in RA-SF. Specifically, sdc4 is shown to be involved in Erk signalling upon IL-1 stimulation in chondrocytes. However, the role of sdc4 side chains and the underlying mechanisms of sdc4 signaling are largely unknown.

Materials and methods In order to investigate the influence of heparan sulfate side chains on sdc4 signaling, different sdc4 side chain mutants were designed using overlap PCR. The authors mutated the serine residues, which constitute the heparan sulphate attachment sites to alanines, thereby preventing side chain assembly. Laser scanning fluorescence microscopy of transiently transfected Cos-7 cells was performed to analyse the membrane localisation of the generated mutants. The multimerisation pattern of the different sdc4 mutants was analysed using crosslinking upon IL-1 stimulation and subsequent Western blot analysis. Furthermore, fibroblasts lacking sdc4, the IL-1 receptor (IL1-RI) or both were stimulated with IL-1 to unravel the interaction of the IL-1RI and sdc4 in IL-1 signaling. Erk1/2 was chosen as readout.

Results The authors showed that all sdc4 side chain lacking mutants exhibited normal intracellular trafficking into the cell membrane. While wild type sdc4 showed normal multimerisation, the side chain mutants exhibited an impaired sdc4 complex formation, particularly with respect to tetramers. Furthermore, the authors found by our cross-linking experiments that sdc4 undergoes complex formation upon IL-1 stimulation. Analysing downstream signaling, sdc4^{-/-} cells showed less IL-1 induced Erk1/2 phosphorylation, while IL-1 induced Erk1/2 phosphorylation was virtually abolished in cells without IL-1RI. The loss of both proteins completely prevented IL-1 dependent Erk1/2 phosphorylation. When comparing the effects of IL-1 α and IL-1 β on Erk1/2 phosphorylation, the authors found that IL-1 β led to faster and more pronounced increase in phosphorylation levels compared to IL-1 α .

Conclusion In conclusion, heparan sulfate side chains are essential for multimerisation of sdc4 and IL-1 stimulation promotes sdc4 complex formation. Additionally, sdc4 seems to be involved in IL-1 signaling and has an additive or even regulatory effect on IL-1 signaling. Therefore, sdc4 complex formation in RA-SF might be an important step in signal transduction during RA disease progression.