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BONE FRACTURE REPAIR, BUT NOT FETAL SKELETAL DEVELOPMENT IS SUPPORTED BY SYNDECAN-4

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Objectives The heparan sulphate proteoglycan syndecan-4 (Sdc4) has been strongly associated with osteoarthritis, a disease that mimics key aspects of early cartilage remodelling during endochondral ossification. The role of Sdc4 in embryonic and adult bone formation, however, remains unclear. Therefore, the authors used *Sdc4*^{-/-} mice to analyse the distribution and functional role of Sdc4 in endochondral ossification of mouse embryos and in adult fracture repair, which recapitulates endochondral ossification, but like osteoarthritis involves an inflammatory component.

Methods In *Sdc4*^{-/-}/*LacZ* knock-in animals, *Sdc4* promoter activity was analysed using β -galactosidase stainings. E16.5

embryos were used for histological (alcian blue/alizarin red) and immunohistological (PCNA, Col10a1, ADAMTS-4, BC-3, Sdc2) stainings. Calcified bone area was quantified using whole mount stainings. Histological (Masson-Goldner, alcian blue) and immunohistological (Col10a1, Sdc2, PCNA) stainings at day 7, 14 and 28 fracture calli were performed. Callus size and cartilage area were quantified using image J. Chondrocytes were isolated from neonatal knee joints and embryonal cartilage. Proliferation was investigated using MTT assay. Gene expression analysis for Sdc-2, Sdc-4 with and without stimulation using tumour necrosis factor α and WNT3a was performed using quantitative RT-PCR.

Results Sdc4 promoter activity was detectable in all stages of chondrocyte differentiation, and Sdc4 deficiency inhibited chondrocyte proliferation both in vivo and in vitro. Moreover, aggrecan turn over in the epiphysial cartilage was decreased transiently in vivo, but this did not lead to a growth phenotype at birth. In contrast, fracture healing in adult mice was markedly delayed in Sdc4^{-/-} animals and accompanied by increased callus formation. Analysing the discrepancy between the mild embryonic and the severe adult phenotype, the authors found a compensatory upregulation of Sdc2 in the developing cartilage of Sdc4^{-/-} mice that was absent in adult tissue. Stimulation of chondrocytes with Wnt3a in vitro, led to an increased expression of Sdc2, while stimulation with TNF α resulted in an upregulation of Sdc4 but a decreased expression of Sdc2.

Conclusion The authors conclude that Sdc4 is functionally involved in endochondral ossification and that the loss of Sdc4 impairs adult fracture healing due to the inhibition of compensatory mechanisms under inflammatory conditions.