Syndecan-1 deficiency impairs neoangiogenesis at the chondro-osseous interface in bone and fracture healing

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INTRODUCTION: Neoangiogenesis drives the replacement of mineralized cartilage by trabecular bone during bone growth regulated by molecules like e.g. VEGF, OPG and RANKL. The Heparan sulfate proteoglycan Syndecan-1 (Sdc1) while interacting with VEGF and OPG, plays a role in the communication of osteoclasts and osteoblasts and in the development of blood vessels. To understand the function of Sdc1 in endochondral ossification we analysed bone structure and vessel development in bone growth and fracture healing in mice deficient in Sdc1.

METHODS: Femora of C57BL/6 WT (n=11) and Sdc1-/- (n=13) mice (male/female) were used for native bone analysis at 4 month age. Female mice (WT n=6-14, Sdc1-/- n=6-8, per time point) underwent midshaft femur fracture stabilized using an intramedullary nail and healed for up to 28 days. Bone structure was analyzed using microCT scans with a resolution of 9µm. Width of the growth plate was determined from Alcian blue stainings in sagittal slices of femurs. Fracture callus composition was quantified after Alcian Blue staining as callus area, fibrous tissue, cartilage and trabecular bone in 5µm thick slices. Vascularization was visualized using an anti-Endomucin antibody in 80µm thick cryosections and vessel buds were counted at the chondro-osseous border (growth plate, fracture callus). Animal experiments were conducted as approved by the Landesamt für Naturschutz, Umweltschutz und Verbraucherschutz, North Rhine-Westphalia, Germany (Ref.No: 81-02.05.50.20.002/ 81-02.04.2019.A164). Bone marrow isolates (WT/Sdc1-/-) were used to generate endothelial progenitor cells by sequential cultivation on fibronectin *in vitro*. Microvessel development was analysed 4h after plating on matrigel and amount of vessel, sprouts and microvessel loops were counted. Statistical analysis was performed with GraphPadPrism software using 2way-ANOVA, Significance: p=0.05 *).

RESULTS SECTION: Bone structure in 4-month-old male Sdc1 deficient mice was significantly reduced compared to age matched male WT, whereas female mice of both genotypes did not differ. The width of the growth plate was not affected significantly by genotype or sex. Sdc1 deficient mice showed a significantly decreased number of vessel buds at the chondro-osseous border at the growth plate at the age of 4 month compared to WT mice in male and female mice. During fracture healing, callus development was delayed with regard to cartilage area at day 7 and trabecular bone area at day 14. A decreased number of vessel buds invading at the borderline of cartilage to bone in the callus were counted in Sdc1 deficient callus tissue. Quantification of microvessel outgrowth of endothelial cells from bone marrow in matrigel revealed a decreased amount of sprouting, but increased length of microvessels of Sdc1-/- cells compared to WT.

DISCUSSION: Syndecan-1 has a striking impact on neoangiogenesis at the chondro-osseous border of the native bone as well as during bone healing in the callus area. Syndecan-1 plays a role in blood vessel outgrowth and sprouting *in vitro* suggesting an important function of Syndecan-1 in blood vessel invasion into the cartilage area during bone growth and fracture callus remodeling. This emphasizes the importance to further characterize the mechanism, how Syndecan-1 regulates the process of endothelial invasion during enchondral ossification.

SIGNIFICANCE/CLINICAL RELEVANCE: Although the high significance of neoangiogenesis during endochondral ossification is beyond controversy, many details of the regulatory mechanisms and the complex interaction of different cell types at the chondro-osseous interface remain unclear. Elucidating the role of Syndecan-1 acting as a co-receptor of signaling molecules and adhesion molecule on the cell surface has great potency to target angiogenesis during bone and cartilage remodeling.

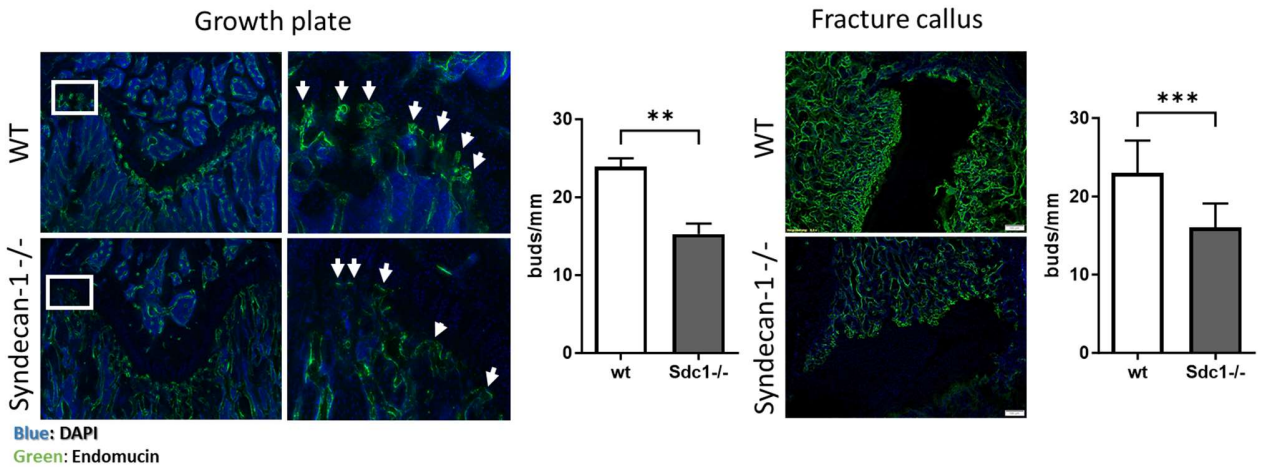

Figure: Neoangiogenesis at the chondro-osseous border of the femur growth plate or in the fracture callus of femur fractures. Syndecan-1 deficient and WT mice were sacrificed at the age of 14 weeks, after 14days of fracture healing (femur midshaft fracture stabilized with an intramedullary nail). Vessel buds were stained using antiEndomucin-antibody in green (DAPI as nucleus staining) and quantified as buds/mm of the chondro-osseous borderline. Syndecan-1 deficient mice showed decreased number of vessel buds pointing towards an impaired neoangiogenesis