ABSTRACT

The IGF1R pathway plays an essential anabolic role in skeletal growth. The deletion of both the receptor as well as its ligands IGF1 or IGF2 in mice leads to dwarfism.

The aim of this study was to analyze how particularly auto- and paracrine IGF1R signaling affects cartilage development with a special focus on the cartilage extracellular matrix structure.

Chondrocyte-specific deletion of Igf1r in mice led to perinatal lethality and proportional dwarfism with shorter and narrower long bones. This phenotype appeared already early in development of the Col2Cre;Igf1r$^{fl/fl}$ metatarsals, suggesting that mesenchymal condensation and/or chondrogenesis are affected. Albeit the cartilaginous epiphysis of the Col2Cre;Igf1r$^{fl/fl}$ tibia was normally organized and no drastic changes in the proliferative or hypertrophic zone could be observed, mineralization and bone formation was reduced. This implies that either the terminal differentiation of the chondrocytes or the osteoblast function is impaired. This result is reflected by the reduced mineralization found in metatarsals cultured in the presence of the IGF1R inhibitor picropodophyllin. Here, reduced expression of Osterix was observed which provides a potential mechanism for a delay in terminal differentiation and reduced mineralization. Compared to the deletion of Igf1r *in vivo*, the inhibition of receptor activity *ex vivo* led to additional effects on hypertrophy, cellular organization and apoptosis. This suggests a potential role of the IGF1R signaling in perichondrial cells as these are affected by the inhibition *ex vivo* but not by the deletion *in vivo*. Apart from the impact on the expression of several extracellular matrix proteins, both the deletion and the inhibition of the IGF1R strongly decreased the extractability of collagen II. This could be rescued *ex vivo* by incubation with β-aminopropionitrile, an inhibitor of the enzymatic collagen crosslinking, indicating that crosslinking might be increased upon defective IGF1R signaling.

Analysis of the embryonic trachea revealed a drastic decrease in tracheal lumen diameter of Col2Cre;Igf1r$^{fl/fl}$ mice due to a smaller size of the cartilaginous rings. Additionally, altered expression of extracellular matrix proteins could be found in the trachea potentially leading to tracheal instability. A resulting tracheomalacia could therefore further restrict lumen size. It is likely that the narrowing of the tracheal lumen causes increased airway resistance which might result in perinatal death of the Igf1r-deficient animals due to respiratory failure.

Overall, these results show that IGF1R signaling is important for cartilage development in both long bones and in the trachea. Lack of IGF1R signaling affects not only early developmental steps, but also terminal hypertrophic differentiation and mineralization, potentially via regulation of Osterix. In addition, this study demonstrates the importance of IGF1R signaling for cartilage matrix structure, strongly suggesting a role of the IGF1R in the regulation of collagen crosslinking.