Summary

Decline of mitochondrial respiratory chain (mtRC) capacity is a hallmark of aging and disease. Previously we showed that the loss of mtRC function causes a premature aging associated with cartilage degeneration and short stature, but how this dysfunction leads to skeletal abnormalities remained poorly understood.

Here, a transgenic mouse model with cartilage-specific mitochondrial DNA alterations was used to impair respiratory chain activity in chondrocytes only. To unravel the consequences of mtRC dysfunction for chondrocyte metabolism and downstream signaling pathway activation a combined mass spectrometry approach was applied to femur head cartilage. Metabolite analysis revealed an anaplerotic replenishment of TCA cycle metabolites upon mtRC dysfunction, associated with an accumulation of TCA cycle metabolites and amino acids. The latter were sensed by mTORC1-mediated signaling network and translated into enhanced SREBP-mediated lipogenesis, as demonstrated by phosphoproteome, immunoblot and lipidomic analysis. Immunofluorescence experiments confirmed the chronic activation of mTORC1 signaling and accumulation of $SQSTM1^{+}$ autophagic vesicles in the enlarged resting zone of the femur head cartilage. Here, expanded ER cisternae and a disturbed Golgi apparatus were detected by electron microscopy. The results indicate that mTORC1-mediated inhibition of the autophagic flux may affect the secretory capacity of chondrocytes with mtRC dysfunction. High-resolution single cell RNA sequencing (scRNA-seq) analysis indeed revealed a unique extracellular matrix (ECM)-related transcriptional response with increased expression of matrilin-1 *(Matn1)* and thrombospondin-1 *(Thbs1)* in chondrocytes. Downstream validation experiments demonstrated that the inactivation of the mtRC dysfunction results in an expanded resting zone characterized by extracellular accumulation of MATN1 and THBS1 proteins, increased collagen crosslinking, matrix stiffness and aberrant ossification. Hence, chronic mtRC dysfunction redirects the metabolism and persistently activates mTORC1 signaling to inhibit autophagy and disturb ER/Golgi-mediated ECM secretion in cartilage. These processes were also studied during cartilage-mediated fracture healing and here first experiments indicate that ECM-homeostasis is disturbed in the fracture callus of the transgenic mouse model with cartilage-specific mitochondrial DNA alterations.

Overall, this study provides a comprehensive understanding of how mtRC dysfunction in cartilage contributes to skeletal growth defects and impaired fracture healing processes.