Abstract

Mitochondria play a major role in energy production in almost every cell type. Due to the low oxygen concentration in the avascular cartilage tissue ATP production in chondrocytes mainly relies on glycolysis whereas oxidative phosphorylation in the mitochondria seems to be less important. However, over the last years increasing *in vitro* studies indicated a connection between mitochondrial defects, altered oxidative phosphorylation and pathological cartilage degeneration in humans. Thus mitochondria may play an essential role in cartilage homeostasis. In this study a cartilage-specific mtDNA deletor mouse expressing the dominant-negative Twinkle^{K320E} helicase in chondrocytes was generated. These mice should progressively accumulate mtDNA deletions in chondrocytes and therefore allow for the first time the *in vivo* characterization of mitochondrial function in cartilage development and homeostasis.

Expression of the Twinkle^{K320E} helicase induced a strong reduction in mtDNA copy number and respiratory chain complexes already in newborn cartilage. While the skeleton of newborns showed no obvious changes, older mice developed a progressive growth retardation and a waddling gait. A detailed histomorphological analysis of endochondral bone development demonstrated that the maturation of the growth plate was disturbed. The growth plate was enlarged in juvenile mice and fused prematurally in four months old mutants. Expansion of the growth plate was accompanied by an increase in apoptosis, a decrease in collagen X expression and a reduction of alkaline phosphatase activity in the hypertrophic zone. Mitochondrial mass, membrane potential, NADH content, extracellular pH and ROS production were already altered in chondrocytes isolated from newborn mice. However, differences in mitochondrial morphology, ATP content, proliferation, apoptosis and autophagy were only apparent in chondrocytes isolated from one month old mutants. Changes in calcium homeostasis could not be detected but the amount of the vitamin D receptor was strongly reduced. In addition, an increased expression of PTHrP could be detected in the proliferative zone of the growth plate of juvenile animals. The changes in vitamin D receptor and PTHrP expression may promote the fusion of the growth plate in CreTW^{K320E} mice.

In summary, an essential function of mitochondria in postnatal cartilage development and homeostasis could be demonstrated *in vivo* and underlying mechanisms could be determined. Moreover, mitochondrial dysfunction was identified as a novel molecular determinant for premature growth plate closure.