Abstract

Cartilaginous tissue originates from the condensation of mesenchymal cells, which differ in chondrocytes of the temporary cartilage of the growth plate or the permanent articular cartilage of the joint surfaces and the trachea. MicroRNAs (miRNAs) are posttranscriptional regulators of gene expression and may play an important role in the regulation of cartilage differentiation. However, so far no miRNAs have been identified that influence important pathways of cartilage development. Previously, we identified miR-322 to be differentially expressed within the growth plate. In this work the role of miR-322 for skeletal development should be determined. First, the increase in miR-322 expression during chondrogenic differentiation was confirmed by qPCR analysis in ATDC5 micromass cultures. Transfection studies with miR-322 *mimic* oligonucleotides showed that the increase in miR-322 concentrations in primary epiphyseal chondrocytes (PEC) correlates with a decrease in proliferation. Bioinformatic analysis identified the insulin signaling pathway as a potential regulated signaling pathway, mediating the antiproliferative effect of the miR-322 in chondrocytes. Biochemical characterization of the miR-322 specific target gene levels showed that the miR-322 increases the protein levels of the central kinase MEK1 to inhibit the phosphorylation of the downstream kinase ERK1/2 in PECs and proliferation. Actinomycin D mediated mRNA degradation studies showed that *Mek1* mRNA levels increased due to a slow degradation of mRNA mediated by miR-322 binding to the 3'UTR of *Mek1* mRNA. The direct interaction of the miR-322 with the target sequence in the 3'UTR of the Mek1 mRNA was confirmed by CRISPR/Cas9-based deletion studies. LacZ reporter gene expression analysis in a miR-322 specific mouse model showed that the miR-322 is expressed in the tracheal and growth plate cartilage. Within the growth plate miR-322 was mainly expressed in prehypertrophic/hypertrophic chondroctyes. Cartilage-specific inactivation of the miR-322 in mice caused respiratory insufficiency due to a narrowing of the tracheal cartilage rings and lumen causing perinatal death. In addition the length of the long bones was slightly increased, accompanied by a reduction of the hypertrophic zone. At the molecular level, the loss of miR-322 *in vivo* was linked to a decrease in MEK1 protein levels and increased ERK1/2 phosphorylation levels. These results demonstrate that the miR-322 can bind to the 3'UTR of the *Mek1* mRNA to increase the stability of the mRNA and subsequently the protein amount of MEK1. This increase suppresses the activation of the ERK1/2 signaling pathway and inhibits the proliferation of the chondrocytes in the growth plate and tracheal cartilage.