Characterization of the multi-zinc finger transcription factor Trps1 during chondrocyte differentiation

Trps1 is a multi-zinc finger transcription factor, regulating chondrocyte proliferation and differentiation. Mutations in the *Trps1* gene sequence results in defects of bone formation, like malformations of the facial bones, dwarfism and a premature closure of the growth plate. But how Trps1 is regulating the endochondral ossification is not exactly known so far. To elucidate the details of Trps1 function, Co-Immunoprecipitation (Co-IP) interaction studies were performed. In this study could be shown, that endogenous Trps1 interacts on protein level with endogenous Hdac4, Hdac6 and Hsp90. Gelfiltration experiments with protein complexes of cell lysates show that Trps1 elutes not as a monomer but in higher order complexes between 307 and 687 kDa. Hdac1, Hdac4 and Hdac6 elute partly in the same fractions, so that it can be assumed that these proteins could act together in the same transcription complexes.

The analysis of H3K9 and H3K18 acetylation and Hdac activity in cell cultures and mouse limb extracts points up an activating Trps1 action on Hdac activity. Trps1 deficiency results in hyperacetylated chromatin. This could lead to defects in mitosis, which are common in *Trps1*$^{-/-}$ mice.

In a mass spectrometric analysis of Trps1 Co-IPs new potential Trps1 interacting proteins could be identified. These are the transcription factor SUPT6H, the Nuclear Body Protein SP110, the RNA Helicase P110, as well as proteins of the heat shock group like Hsp70 and DnaJ.

Hdac inhibition, due to TSA and NaB treatment, during differentiation of chondrogenic ATDC5 cells results in a stronger and faster differentiation. But the analysis of the H3K9 acetylation during the differentiation process showed, despite Hdac inhibition, the acetylation is enhanced in the first week, only. From the second week on the acetylation is steadily reduced. Either the Hdac inhibition pulse is only needed in the first week for an accelerated differentiation, or the inhibitors act by deacetylating other proteins like Runx2, which can be deacetylated by Hdacs. So the enhanced acetylation in the first week could be a secondary effect due to an enhanced differentiation, which resulted from deacetylation of other proteins.