

Abstract

“Tricho-rhino-phalangeal syndrome” (TRPS) is an autosomal-dominant inherited human disorder caused by mutations in the *TRPS1* gene and is characterized by craniofacial and skeletal abnormalities. In mice, inactivation of the homologous gene reproduces the human phenotype, revealing a role for Trps1 in regulating chondrogenesis. The Trps1 protein is a transcription factor with nine predicted zinc finger (zf) domains. Previous *in vitro* studies have shown that Trps1 interacts with Gli3, Hdac4 and Runx2, main transcriptional regulators of chondrocyte proliferation and differentiation. Based on these results, we hypothesize that first the Trps1 zf domains mediate binding with further, yet unidentified, interacting partners and second Trps1 functions as an adaptor in multiprotein complexes.

To specify the molecular mechanism of Trps1 function, coimmunoprecipitations (Co-IPs) were performed. While the chondrogenic regulators Mef2c, Sin3a and Sox9 did not bind to Trps1, the heat shock protein Hsp90 β and the histone deacetylase Hdac1 were found as new Trps1-interacting partners. In contrast, no interaction was observed with Hdac2, the closest homolog of Hdac1, although both Hdacs are expressed in all chondrocytes and are known to be part of the same complexes. For Trps1 and Hdac1, the interacting domains were mapped to a region encompassing the GATA- and IKAROS-zfs of Trps1 and the deacetylase domain of Hdac1. Although this domain is present in all Hdac proteins, only Trps1-interacting Hdacs 1, 4 and 6 have sumoylation sites. The function of this sumoylation in Trps1-Hdac1 interaction has to be analysed in further experiments. As Trps1 GATA- and IKAROS-zfs mediate the interaction with Hdac1 as well as Runx2, a multiprotein complex formation appears possible. This multiprotein complex formation is supported by FRET experiments showing no direct interaction between Trps1 and Hdac1, indicating that they are connected via other proteins.

To analyze complex formation around Trps1, gelfiltrations were performed on lysates of ATDC-5 chondrogenic cells. Trps1 and its interacting partners were detected in protein complexes > 150 kDa. After isolating Trps1 complexes by Co-IPs, Hdac4, Hdac1 and Hsp90 β were present in complexes > 500 kDa. Beyond that, changes in Trps1 multiprotein complex composition during chondrogenesis were analysed in differentiated ATDC-5 cells. Trps1 protein amount declines during this process leading to a reduction of Trps1 complexes. Hdac1 and Hsp90 β still interact with

Trps1 in complexes > 500 kDa, while the Hdac4 protein amount was insufficient to precipitate with Trps1. The aforementioned complex sizes strongly suggest the existence of further, yet unidentified, interacting partners. The identification of these proteins will contribute to a more detailed understanding of the mechanism regulating endochondral bone formation.