**VIPERIN regulates chondrogenic differentiation via CXCL10 protein secretion**

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**Purpose**

Viperin participates in the cell’s innate immune response against viruses. Viperin mRNA was previously identified as a substrate for RNase MRP. Mutations in the RMRP snoRNA subunit of RNase MRP are the cause of cartilage-hair hypoplasia (CHH), a rare human dwarfism condition. It is not understood how CHH-pathogenic mutations in RMRP snoRNA lead to impaired skeletal development and it is thought that aberrant processing of RNase MRP substrate RNAs is involved. We therefore hypothesized that viperin plays a role in chondrogenic differentiation.

**Methods**

Viperin was immunohistochemically detected in E15.5 murine growth plates. ATDC5 cells were differentiated in the chondrogenic lineage. Viperin expression was reduced by transfecting a siRNA duplex, and increased by transfecting a FLAG-viperin plasmid. Primary healthy and CHH human dermal fibroblasts were obtained with informed consent, expanded and trans-differentiated towards chondrocytic cells in chondrogenic medium by seeding on aggrecan coating. Chondrogenic differentiation of ATDC5 and fibroblasts was determined by gene and protein expression analyses using RT-qPCR, immunoblotting, and GAG assays. Protein secretion was determined using a secretable luciferase and TGF-β bioactivity using a CAGA12-luciferase plasmid. Secretome proteomes were determined by LC-MS/MS and label-free quantitation of proteomics data. CXCL10 was measured using an ELISA.

**Results**

Viperin was expressed in the developing growth plate with dispersed viperin positivity in the upper zone. During the first 3 days of ATDC5 chondrogenic differentiation *viperin* expression was hardly detectable. From day 4 through day 14, *viperin* expression was more than a 100-fold increased, with a prominent peak expression at day 5 and 6 in differentiation. Total protein secretion capacity was reduced after *viperin* knockdown and increased following *viperin* overexpression. ATDC5 differentiation in conditioned medium from viperin knock down donor cultures led to an increased chondrogenic capacity. The opposite was found for conditioned medium from viperin overexpression cultures. Mass-spectrometry secretomics analysis revealed six proteins that were differentially expressed in the secretome of differentiating ATDC5 cells with reduced viperin expression. In the secretome of differentiating ATDC5 cells in which viperin was overexpressed there were eight differentially expressed proteins. CXCL10 was the only protein that was detected in both differential secretome proteomes. It was decreased in viperin knock down- and increased in viperin over expression-conditioned medium. CXCL10 levels during ATDC5 differentiation mirrored viperin expression dynamics. ATDC5 chondrogenic differentiation was attenuated by exogenously added CXCL10. Conditioned medium obtained from differentiating ATDC5 cultures in which viperin levels were reduced displayed a TGF-β/SMAD3-activity promoting action, while we found the opposite for *viperin* over-expressed conditions and CXCL10 dose-dependently reduced TGF-β/SMAD3 activity. In concert with a CHH-associated pathological defective RNase MRP activity, viperin expression was increased in chondrogenic trans-differentiated CHH fibroblasts, accompanied with an increase of secreted CXCL10 and decreased expression of TGF-β target genes PAI1 and SMAD7.

**Conclusions**

We discovered that viperin is expressed in differentiating chondrocytic cells, regulates their protein secretion and the outcome of the chondrogenic differentiation program through influencing TGF-β/SMAD3 activity via CXCL10. Disturbances in this viperin-CXCL10-TGF-β/SMAD3 axis were also found in chondrocytic cells of CHH patients. Our data for the first time demonstrates that the anti-viral protein viperin controls chondrogenic differentiation by influencing the secretion of soluble proteins and clarifies its involvement in impaired chondrogenic differentiation in CHH patient cells.