

CCN2 regulates chondrocyte differentiation by binding to GDF5 and its receptor.

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AUTHOR DISCLOSURES

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ABSTRACT INTRODUCTION: CCN2 (Cellular Communication Network factor 2) is a secretory protein binding with various factors. We previously reported that GDF5, a member of the BMP family, binds to CCN2 by surface plasmon assay and the binding may enhance the activity of GDF5 in chondrocytic cell line. Here, we further investigated whether CCN2 binds to the GDF5 receptor and how the presence of CCN2 affects the binding of GDF5 to its receptor.

METHODS: To clarify the effect of CCN2 on the bioactivity of GDF5 in chondrocytes, we cultured ATDC5 cells, a mouse prochondrocyte cell line, and examined gene expression using RT-qPCR. We also examined the ability of ATDC5 cells to produce proteoglycans by Alcian blue staining. Furthermore, we analyzed downstream signaling of GDF5 using Western blotting assay. In addition, in order to investigate the mechanism by which CCN2 affects the physiological activity of GDF5, solid-phase binding assays were performed and examined the binding of CCN2 to GDF5 and its receptors, as well as the effect of CCN2 on receptor dimerization. We also investigated the binding affinity between CCN2 and BMPRII using the surface plasmon resonance assay.

RESULTS: CCN2 inhibits GDF5-induced promotion of cartilage differentiation marker gene expression in chondrocytes. CCN2 suppresses GDF5-induced phosphorylation of Smad1/5/8 in ATDC5 cells. CCN2 binds not only GDF5 but also its receptor BMPRs. CCN2 inhibits GDF5-induced promotion of BMPRII-BMPRIa heterodimerization.

DISCUSSION: The results of this study suggest that CCN2 may suppress the physiological activity of GDF5 in chondrocytes through binding to GDF5 and BMPRs or by inhibiting the dimerization of BMPRIa and BMPRII.

SIGNIFICANCE/CLINICAL RELEVANCE: The results of this study reveal the role of CCN2 on the physiological activity of GDF5 and may be useful in developing treatments for cartilage damage.

