FIBROBLAST GROWTH FACTOR 9 (FGF9) REGULATES PROLIFERATION AND PHENOTYPE EXPRESSION OF CHONDROCYTES

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Introduction: Growth factors have been shown to modulate the development, growth, and maintenance of skeletal tissues. However, the effect of FGF9, a recently identified member of the FGF family, on human chondrocytes is not well known. Recently, elevated levels of FGF9 have been detected in the cartilaginous formation and synovial fluid of patients affected with primary synovial chondromatosis. Synoviocytes from these patients have been discovered to produce FGF9 in culture (1). Elevated levels of FGF9 have also been detected in the osteochondromas of patients affected with hereditary multiple exostosis (2). The role of FGF9 on chondrocyte proliferation, their ability to synthesize ECM and the extraneous cartilage formation is unclear. The present study was undertaken to test the hypothesis that FGF9 enhances the proliferation and phenotype expression of human chondrocytes.

Materials and Methods: Human nasal osteoblasts, synovial fibroblasts, nasal and articular chondrocytes (1x10^5/ml) were incubated with heparin (2µg/ml) and 100 ng/ml FGF9, or heparin and control media alone, in 24 well plates for 0.5 to 9 days. Heparin was added to promote FGF9 high-affinity receptor binding (3). Proliferative capacity was evaluated by ¹H-thymidine uptake and the expression of cyclin D1 and CDK4 at the message and protein levels. Viable cells were enumerated using trypan blue uptake. Levels of cartilage-specific ECM components (collagen-type-II, aggrecan), collagen-type-I, and the FGF9 receptor (FGFR3), were determined by the reverse-transcription polymerase chain-reaction (RT-PCR), electrophoretic mobility-shift assay (EMSA), and immunostaining. Statistical analysis was performed using the Student’s paired t test with Intercooled Stata 6.0 software.

Results: FGF9 significantly increased DNA synthesis in chondrocytes obtained from human articular cartilage (200% increase, p<0.01) and nasal septum (100% increase, p<0.01) as indicated by higher ¹H-thymidine uptake. In contrast, FGF9 did not significantly increase ¹H-thymidine uptake in human osteoblasts and synoviocytes. Since FGF9 specifically increased chondrocyte DNA synthesis, we investigated the effect of FGF9 on articular chondrocyte number. In the FGF9 treatment cultures, there was a 50% increase (p<0.01) at day 6 and a 130% increase (p<0.001) at day 9 in the number of articular chondrocytes (Figure 1). All cells remained more than 90% viable through day 9. The mitogenic effect correlated with increased mRNA and protein levels (Figure 3) of cyclin-D1 and CDK4. Compared to control medium alone, FGF9 enhanced the expression of collagen-type-II, aggrecan, and FGFR3 at the message and protein levels by 24 hours. In contrast, incubation with FGF9 inhibited collagen-type-I expression through day 9 at the message and protein levels (Figures 2 and 3).

Discussion and Conclusions: The present study demonstrated for the first time that FGF9 stimulates well-differentiated, normal, human chondrocyte proliferation and hyaline cartilage extracellular matrix (ECM) synthesis in an in-vitro monolayer culture system. Our results also showed that FGF9 is not mitogenic to osteoblasts or synovial fibroblasts. Normally, dedifferentiation occurs when chondrocytes are isolated and transferred to a culture dish. As chondrocytes expand and form a monolayer of fibroblastic cells, collagen type II and aggrecan synthesis diminish, and collagen-type-I synthesis increases (4). In our study, treatment of articular chondrocytes in a monolayer culture system with FGF9 increased collagen-type-II and aggrecan expression with the upregulation of FGFR3. FGF9 also induced chondrocyte proliferation and downregulated levels of collagen-type-I. The stimulation of chondrocyte proliferation and ECM synthesis by FGF9 and its downregulation of collagen-type-I synthesis imply that FGF9 could play a role in cartilage repair as well as in in-vitro expansion of chondrocytes for re-implantation.

References:


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