INTRODUCTION:
Growth Differentiation Factor-5 (GDF-5), also known as Bone Morphogenetic Protein-14 (BMP-14), belongs to a subgroup of the BMP family. Although BMP was originally discovered as a protein capable of inducing new bone formation when implanted subcutaneously in vivo, it is well known that BMPs are essential for the differentiation of mesenchymal cells into various types of connective tissues. Several studies have indicated the role of GDF-5 in tendon and ligament formation. Studies looking at the effect of GDF-5 implantation demonstrated induction of tendon/ligament-like tissues in ectopic sites.[1] Moreover, studies using mice which carry mutation on GDF-5 gene lead to a delay in tendon healing.[2,3] And it is also shown that administration of exogenous GDF-5 enhanced tendon healing in several animal models.[4,5] However, understanding of the mechanism for the effect of GDF-5 is still limited at the molecular level. Recently it was reported that GDF-5 modulated the expression of Vascular Endothelial Growth Factors (VEGF) and their receptors in vitro, using bone marrow stromal cells.[6] Here, we test the hypothesis that GDF-5 may modulate VEGF related molecules in tendon cells. Accordingly, the aim of this study was to determine the effects of GDF-5 on expression profiles of VEGF ligand and receptor genes in vitro using rat tendon derived cells.

MATERIALS & METHODS:
Cell Culture: Patella tendon cells were obtained from adult male Sprague-Dawley rats by modifying published procedures.[7] Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml Penicillin/Streptomycin, and -D showed a peak at 3 hr or 6hr after the GDF-5 application.

Effect of GDF-5 on VEGFs gene expression:
Several studies have indicated the role of GDF-5 in tendon and ligament formation. Studies looking at the effect of GDF-5 implantation demonstrated induction of tendon/ligament-like tissues in ectopic sites.[1] Moreover, studies using mice which carry mutation on GDF-5 gene lead to a delay in tendon healing.[2,3] And it is also shown that administration of exogenous GDF-5 enhanced tendon healing in several animal models.[4,5] However, understanding of the mechanism for the effect of GDF-5 is still limited at the molecular level. Recently it was reported that GDF-5 modulated the expression of Vascular Endothelial Growth Factors (VEGF) and their receptors in vitro, using bone marrow stromal cells.[6] Here, we test the hypothesis that GDF-5 may modulate VEGF related molecules in tendon cells. Accordingly, the aim of this study was to determine the effects of GDF-5 on expression profiles of VEGF ligand and receptor genes in vitro using rat tendon derived cells.

RESULTS:
Effect of GDF-5 on VEGF-A mRNA expression:
GDF-5 significantly increased the VEGF-A mRNA expression at 3 hr, followed by gradual decline to baseline level by 24 hr (Fig. 1). For splice variants of VEGF-A, predominant isoforms detected by electrophoresis were VEGF-A 164 and 120. The expression level of VEGF-B, -C, and -D was not altered significantly by GDF-5 treatment. However, VEGF-C and -D showed a peak at 3 hr or 6hr after the GDF-5 application. The expression of VEGF has been detected during proliferation and angiogenesis in vitro.[9] The expression of VEGF has been detected during proliferation and remodeling phases of tendon healing.[8] Also, exogenous application of VEGF-A to tenocytes, has been shown to increase expression of TGF-β, in vitro.[9] GDF-5 also has been shown to enhance tendon healing in several animal models. Previously, increased tensile strength after GDF-5 application in rat Achilles tendon defects was reported.[10] More recently, the effect of exogenous GDF-5 on MCL in rat knee joints was shown.[5] The present results suggest that these beneficial effects of GDF-5 may be modulated in part by the induction of VEGF related genes in tendons.

DISCUSSION:
These results demonstrate that GDF-5 modulates VEGF related genes in tendon cells. Among 9 genes studied, expression level for VEGF-A and VEGF3 showed a significant increase after 3 hr.

Several growth factors have been studied in relation to tendon healing after injury, such as IGF-1, TGF-β, PDGF, bFGF, and VEGF. The expression of VEGF has been detected during proliferation and remodeling phases of tendon healing.[8] Also, exogenous application of VEGF-A to tenocytes, has been shown to increase expression of TGF-β, in vitro.[9] GDF-5 also has been shown to enhance tendon healing in several animal models. Previously, increased tensile strength after GDF-5 application in rat Achilles tendon defects was reported.[10] More recently, the effect of exogenous GDF-5 on MCL in rat knee joints was shown.[5] The present results suggest that these beneficial effects of GDF-5 may be modulated in part by the induction of VEGF related genes in tendons.

Predominant expression of VEGF-A164 and 120 splice variants was used and the products analyzed on an agarose gel by electrophoresis.

Statistical analysis: Data are presented as mean ± S.D. Significance was defined as probability values less than 0.05 with Turkey-Kramer multiple comparisons test.

REFERENCES:

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