GDF-5 DEFICIENCY IN MICE RESULTS IN DELAYED ACHILLES TENDON HEALING

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INTRODUCTION: The Growth and Differentiation Factors (GDFs) represent a distinct subset of the TGF-β family best known for their role in joint formation and endochondral bone growth [1-4]. Several recent pieces of evidence suggest that the GDFs may also be involved in tendon and ligament formation and repair [4,5]. The goal of this study was to examine the effect of GDF-5 deficiency on tendon healing by characterizing the repair process in mice deficient in this particular growth factor. We hypothesized that GDF-5 deficient mice would exhibit an impaired healing response to Achilles tendon defects when compared to control mice.

METHODS: The animal model used for these studies was the GDF-5 (-/-) brachypodism (hp-J) mouse with an A/J background strain (obtained from Jackson Labs, Bar Harbor, ME). Two different control groups were used: phenotypically normal heterozygous (+/-) littermates served as controls for all quantitative biochemical analyses, while A/J mice with two functional copies of the GDF-5 gene served as controls for qualitative histologic analyses due to their greater availability. Prior to using the A/J’s as control animals, an additional study was performed which demonstrated that the time-course of healing was identical in the +/- and A/J control groups based on biochemical composition of the healing tissue over time. All animals were 8 weeks old at the start of the study, and a total of 32 animals per genotype group were examined. All experiments were approved by our animal care review board.

Midsubstance tenotomies were performed on the left Achilles tendon in all animals, and the tendon was then repaired using 6-0 suture. The right Achilles tendon served as an internal, sham operated control for each animal. Mice were sacrificed at 3, 5, 7, 9, 11, 14, 28, and 42 days after surgery. For assessment of biochemical composition of the repair tissue, left and right Achilles tendons were digested in papain overnight at 60°C. DNA content was determined using the Hoechst dye method, and GAG content was determined using a colorimetric DMMB assay. Hydroxyproline (OHP) was determined subsequent to acid hydrolysis using a DMBA color reaction. All samples and appropriate standards were processed in duplicate and average values were used for analysis. Each set of biochemical analyses was repeated at least twice on separate occasions to verify the results. Data were expressed as a ratio of repair side values normalized to sham side values at each time point, and analyzed statistically using a two-factor ANOVA with genotype and time-post-op as the two factors. A value of p<0.05 was chosen as the cutoff for statistical significance. Additional samples were processed at each time point for routine histology using H&E as well as Safranin-O, Hematoxylin, & Fast Green trichrome staining on serial 7µm paraffin sections. 3-5 mid-sagittal sections were then analyzed histomorphometrically to determine quantitative trends in the percentage of healing tissue occupied by vasculature and adipocytes using point counting methods on approximately 5 non-overlapping images per tissue section.

RESULTS: Based on all biochemical measures (normalized to internal control sham values), GDF-5 deficient mice displayed a delay of 5-9 days in attaining peak values of normalized DNA, GAG, and Hydroxyproline levels in healing Achilles tendon tissue when compared to control animals (Figure 1). Statistically, the time-dependent changes in all three biochemical measures were significantly affected by the GDF-5 mutation (p < 0.001). Histologically, GDF-5 deficient Achilles tendons also exhibited a delay in peak areal cell density and collagen re-organization (qualitatively assessed). Trends in the percentage of healing tissue occupied by blood vessels/arteries as well as fatty tissue indicated that GDF-5 deficient tendons were delayed by approximately one week in the initial phase of vascularization (Figure 2). GDF-5 deficient healing tissue also exhibited a higher percentage of fatty tissue compared to controls throughout the entire healing process.

DISCUSSION: These data demonstrate that GDF-5 deficiency results in impaired Achilles tendon healing in mice: tendons from mutant (-/-) mice exhibit a delay of approximately one week in attaining peak values of DNA, GAG, and OHP levels during repair. Mutant Achilles also exhibited a delay in remodeling of the repair tissue, with increased presence of adipocytes and less organized collagen organization several weeks after tenotomy. The observed abnormalities may be due to a delay in cell recruitment and differentiation in the early stages of tendon repair in the absence of GDF-5. These results support the hypothesis that GDF-5/BMP-14 plays a role in Achilles tendon repair. In addition, the observed trend of delayed vascularization in the mutant tissue is consistent with the reported angiogenic capability of GDF-5 [6]. Our findings suggest that GDF-5/BMP-14 may be a viable candidate for eventual therapeutic use to augment tendon and ligament repair in humans.


ACKNOWLEDGMENTS: Funded in part by a grant from the NIH (AR45828) to BM.