INTRODUCTION: CD44 is a polymorphic type I transmembrane glycoprotein that is expressed on most cell types including fibroblasts and leukocytes. Its main role is in mediating the uptake and clearance of hyaluronic acid (HA), but it is also associated with early inflammatory events and matrix remodeling. Interestingly, CD44 is highly expressed in wounded adult tendons but is almost absent in wounded fetal tendons that heal without scarring [1]. Using CD44 knockout mice, previous studies have demonstrated decreased macrophage recruitment and decreased expression of proinflammatory cytokines at the site of wound injury in CD44 knockout wounds [2]. In addition, we previously demonstrated improved mechanical properties at 3 and 6 weeks post injury in the patellar tendon of a CD44 knockout mouse [3]. Collectively, these studies demonstrate that CD44 plays a crucial role in inflammation and wound healing. Therefore, the purpose of the current study was to investigate the effect of CD44 on growth factors and matrix components early in the healing of the patellar tendon. We hypothesized that proinflammatory cytokines would be down regulated and extracellular matrix proteins would be up regulated in the CD44 knockout mice [4].

METHODS: C57BL/6 wild type (WT, n=16) and CD44 knockout (KO, n=16) mice (C57BL/6 background) at twelve weeks of age were used (IACUC approved). Four mice from each genotype were sacrificed without injury to serve as uninjured controls (day 0). Patellar tendons from the remaining twenty-four animals were injured bilaterally [5]. Briefly, incisions were made adjacent to the tendon. A rubber-coated backing was placed underneath the tendon to provide support against a 0.75 mm diameter biopsy punch used to create a full thickness, partial width (~60%) transection. Skin incisions were sutured and mice were allowed normal cage activity until sacrifice at 1, 3 and 7 days post-operatively. Injured and uninjured tendons were subjected to quantitative real-time polymerase chain reaction (QPCR) (ΔΔCT method relative first to GAPDH within each specimen and then to the uninjured tendons within each genotype).

RESULTS: The relative quantity of TGFβ3, bFGF and DCN was increased at least two fold at each time point post injury in the CD44 KO compared to when WT (Figure 1). Similarly, the relative expression of Col1a3, Has2 and PDGFB was increased at least two fold at 1 and 3 days post injury. Relative expression was also increased at least two fold for BGN at 1 day post injury and for TGFβ1 at 3 days post injury in the CD44 KO. There were no appreciable differences in Col1a2 and IL1β (data not shown).

DISCUSSION: In support of our hypothesis, we demonstrated that the extracellular matrix components DCN, Col3a1 and Has2 had increased expression. Contrary to our hypothesis, we did not show decreased expression in proinflammatory cytokines IL-1β and TGFβ1 but we did demonstrate increased expression of TGFβ3, bFGF and PDGFB. The effects of cytokines and matrix components on cells are interdependent. For example, administration of bFGF to injured tendon increased expression of type III collagen [6]. Similarly, application of TGFβ3 to skin wounds reduced scar formation [7]. Conversely, DCN is closely related to both collagen metabolism and TGF-β activity [8]. As noted above, we have previously demonstrated that CD44 KO mice with the same patellar tendon injury had improved healing at 3 and 6 weeks post injury [3]. The possible synergistic effects in the current study may have created an improved tendon healing environment, leading to enhanced mechanical properties in the CD44 KO mouse.

An absence of CD44 during injury may cause a prolonged presence of HA [9] which is a prominent feature in scarless fetal healing [10]. Similarly, HA-treated cells stimulated type III collagen and TGFβ3 expression [11], both of which are also indicative of scarless fetal healing. TGFβ1, which is up regulated in fibrotic scar, was also increased by HA; however, this effect was no longer present when cells were pretreated with CD44 silencing RNA. Therefore, an absence of CD44 may promote an environment that favors scarless wound healing.

In summary, we demonstrated early changes in gene expression during tendon healing of a CD44 KO mouse. This study, coupled with our previous study [3], demonstrates the importance of understanding the role of CD44 in wound healing. The absence of CD44 may create a permissive environment for scarless healing which leads to improved mechanics. Future studies will examine compositional and structural changes post injury to further elucidate the role of CD44. Understanding how the absence of CD44 affects wound healing provides insight into the process of improved tendon healing and may serve as a target for development of novel treatment modalities.

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