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TGF- β 2 enhances expression of equine bone marrow-derived mesenchymal stem cell paracrine factors with known associations to tendon healing

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Abstract

Background: Mesenchymal stem cells (MSCs) secrete paracrine factors and extracellular matrix proteins that contribute to their ability to support tissue healing and regeneration. Both the transcriptome and the secretome of MSCs can be altered by treating the cells with cytokines, but neither have been thoroughly investigated following treatment with the specific cytokine transforming growth factor (TGF)- β 2.

Methods: RNA-sequencing and western blotting were used to compare gene and protein expression between untreated and TGF- β 2-treated equine bone marrow-derived MSCs (BM-MSCs). A co-culture system was utilized to compare equine tenocyte migration during co-culture with untreated and TGF- β 2-treated BM-MSCs.

Results: TGF- β 2 treatment significantly upregulated gene expression of collagens, extracellular matrix molecules, and growth factors. Protein expression of collagen type I and tenascin-C was also confirmed to be upregulated in TGF- β 2-treated BM-MSCs compared to untreated BM-MSCs. Both untreated and TGF- β 2-treated BM-MSCs increased tenocyte migration in vitro.

Conclusions: Treating equine BM-MSCs with TGF- β 2 significantly increases production of paracrine factors and extracellular matrix molecules important for tendon healing and promotes the migration of tenocytes in vitro.

Keywords: Mesenchymal stem cell, Tenocyte, TGF- β , Equine, Tendon, RNA-sequencing, Paracrine factors, Extracellular matrix

Background

Mesenchymal stem cells (MSCs) are fibroblast-like cells that can be isolated from various tissues including bone marrow, adipose, peripheral blood, and cord blood. Although MSCs can be directed to differentiate into cartilage, fat, and bone in vitro, there is little evidence to support that MSCs engraft and differentiate

into new tissue once injected in vivo [1]. Instead, MSCs secrete numerous paracrine factors that recruit and support the differentiation of endogenous progenitor cells, promote angiogenesis, inhibit apoptosis, and modulate local immune responses [2]. Extracellular matrix (ECM) proteins secreted by MSCs may also directly contribute to tissue healing or be used to generate acellular scaffolds for tissue engineering [3–5]. MSCs are therefore of interest as a regenerative therapy to enhance the endogenous healing of injured tissues.

Due to the lack of inherent healing capacity within the tendon, improved therapies for tendon regeneration are

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needed to promote return to functionality after injury. Bone marrow-derived MSCs are currently used to treat tendon injuries in horses and are actively being investigated in preclinical research and clinical trials for treating human tendon injuries as well. Horses with superficial digital flexor tendon (SDFT) injuries treated with autologous MSCs had reduced reinjury rates, improved fiber alignment, and less inflammatory cell infiltrate compared to horses that did not receive MSCs [6–8]. In a rat Achilles tendon repair model, tendons treated with BM-MSCs had higher maximum load to failure and stiffness compared to control tendons as well as increased expression of pro-tendon healing factors, scleraxis and tenomodulin [9]. However, results from human clinical trials using MSCs to treat musculoskeletal injuries have failed to meet the expectations set by preclinical animal models. A number of factors have likely contributed to this including immunocompatibility, cell quality and dose, and inconsistent culture protocols [10–12]. Current trends in MSC research are therefore focused on improving the consistency and quality of MSCs to increase their therapeutic efficacy.

There is considerable interest in *in vitro* priming or “licensing” of MSCs to enhance their secretome and increase the expression of paracrine factors specific for the type of injury or disease being treated. Previous research has focused primarily on priming MSCs with inflammatory cytokines like IFN- γ , TNF- α , or IL-1 β to increase expression of growth factors and anti-inflammatory cytokines from MSCs [13]. We previously published that allogeneic equine BM-MSCs cultured with TGF- β 2 have reduced MHC I surface expression and cell-mediated cytotoxicity *in vitro* while retaining their abilities to suppress T cell proliferation and secrete PGE2 and TGF- β 1 [14]. However, TGF- β is known to affect the expression of other cytokines, growth factors, and extracellular matrix molecules that may affect the MSC functions and the efficacy of MSC therapy [15, 16].

The purpose of this study was to determine how TGF- β 2 changes the transcriptome of MSCs with an emphasis on secreted factors relevant to tendon healing in equine BM-MSCs. Through use of RNA-sequencing, western blotting, and a tenocyte migration assay, we discovered that TGF- β 2-treated BM-MSCs have enhanced expression of extracellular matrix molecules including collagen and tenascin-C and, similar to untreated MSCs, increase tenocyte migration, all of which may improve their therapeutic efficacy for *in vivo* tendon healing.

Methods

Animal use and welfare

A total of 13 systemically healthy horses were utilized in this study for sternal bone marrow harvest as approved

under protocol #19–628 for sedated bone marrow collection or #20–454 for postmortem collection by the Institutional Animal Care and Use Committee of North Carolina State University. All horses were between the ages of 6–16 years of age, were Thoroughbred or Thoroughbred crosses, and an equal number of mares and geldings were used in each experiment. The SDFT obtained for tenocyte isolation was collected from a 16 year old Thoroughbred horse euthanized for reasons unrelated to this study as approved under protocol #20–454.

MSC isolation and culture

Bone marrow aspirates were collected aseptically from the sternum of horses using 11-gauge Jamshidi bone marrow aspirate biopsy needles under standing sedation with local lidocaine anesthesia or immediately post-euthanasia. Bone marrow aspirates were purified via Ficoll-Paque Plus (GE Healthcare) gradient centrifugation. Isolated cells were cultured in standard media containing 1 g/dl glucose DMEM (Corning), 10% fetal bovine serum (Atlanta Biologicals), 2 mM L-glutamine, 100 U/ml penicillin and streptomycin, and 1 ng/ml recombinant human basic fibroblast growth factor (bFGF)(Corning). When cultures reached 80% confluency, MSCs were passaged with Accutase cell-dissociation solution (Innovative Cell Technologies) and plated at a density of 6,250 cells per cm² on 100 mm tissue culture plates. BM-MSCs were passaged for 2 to 4 passages prior to use. Cell count and viability at each passage were determined using a Cellometer[®] Auto 2000 and ViaStain[™] AOPI Staining Solution (Nexcelom Bioscience LLC). BM-MSCs were treated by adding 1 ng/ml recombinant human TGF- β 2 (BioLegend) to the media as previously described for 3 passages prior to RNA-sequencing or for 72 h for all other analyses [17]. We have previously published that MSCs isolated and cultured using this protocol are positive for CD29, CD44, and CD90 and negative for LFA-1 and CD45 [17].

RNA-sequencing

Total RNA was extracted from paired P3 or P4 untreated and TGF- β 2-treated BM-MSCs from four horses using the RNeasy Mini kit (Qiagen). Libraries were generated and poly(A) enriched using 1 μ g of RNA as input. Indexed samples were sequenced using a 150 base pair paired-end protocol on a HiSeq 2500 (Illumina) according to the manufacturer’s protocol. Sequence reads were trimmed to remove possible adaptor sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were mapped to the EquCab 3.0 using the STAR aligner v.2.5.2b. Unique gene hit counts were calculated using featureCounts from the Subread package

v.1.5.2. Using DESeq2, a comparison of gene expression between the untreated and TGF- β 2-treated BM-MSCs was performed. The Wald test was used to generate p values and log₂ fold changes. Genes with an adjusted p value of <0.05 and log₂ fold change >1 were determined to be differentially expressed for each comparison. The quantification and poly(A) selection of mRNA, library preparation, sequencing, and bioinformatics were outsourced to GENEWIZ, Inc.

Protein lysate collection and western blotting

BM-MSCs from four additional horses not used for RNA-sequencing were cultured on 100 mm tissue culture plates in control media or media containing 1 ng/ml TGF- β 2 for 72 h. The plates were washed with ice cold phosphate buffered saline (PBS) before adding 350 μ l of cold RIPA Lysis and Extraction Buffer (Thermo Scientific) with 1X Halt Protease Inhibitor Cocktail (Thermo Scientific). Plates were scraped on ice and the contents transferred to a microcentrifuge tube and incubated on ice for 15 min. Microcentrifuge tubes were then centrifuged at 14,000 g for 10 min. The protein lysate supernatant was collected and frozen at -80 °C until used. For western blotting, 20 μ g of protein lysate was boiled at 100 °C for 5 min with 1X Laemmli buffer and 5% 2-mercaptoethanol and resolved using an Any kD Criterion TGX gel (Bio-Rad). Protein was transferred to a PVDF membrane using a Trans-Blot Turbo Blotting System (Bio-Rad). The membrane was blocked with 5% non-fat dry milk before blotting the membrane with rabbit anti-human type I collagen antibody (Novus Biologicals, NBP168942) or rabbit anti-human tenascin-C antibody (Millipore Sigma, AB19011) [18] both at 1:500 in tris-buffered saline with 0.1% Tween-20 detergent. A mouse anti-human beta-actin antibody was used as a loading control at 1:10,000 (Invitrogen, clone 15G5A11/E2). The membrane was probed overnight at 4 °C before adding mouse anti-rabbit IgG-HRP secondary antibody (Cell Signaling Technology) at 1:2000 or goat anti-mouse IgG-HRP secondary antibody (Invitrogen) at 1:2000 for 1 h at room temperature. The membrane was incubated with Clarity Western ECL Substrate (Bio-Rad) for 5 min before imaging. Images were captured by a luminescent image analyzer with a CCD camera (Bio-Rad). Quantification of expression levels was calculated using ImageJ software (NIH), and values were expressed in arbitrary units relative to beta-actin (ACTB).

MSC and tenocyte co-culture

To determine the effects of paracrine factors from TGF- β 2-treated BM-MSCs on tendon, tenocytes were isolated from a forelimb equine SDFT similar to previously published methods [19]. The SDFT palmar to

the third metacarpal bone was aseptically dissected and the tendon was transported in sterile PBS with 100 U/ml penicillin and streptomycin and transferred to a tissue culture hood. Under aseptic conditions, 5 mm \times 5 mm \times 5 mm tendon explants were created ensuring the center of the SDFT was isolated and paratenon excluded. Explants were digested for 12–18 h in media containing 1 g/dl glucose DMEM (Corning) supplemented with 10% fetal bovine serum (Atlanta Biologicals), 100 U/ml penicillin and streptomycin, 2 mM L-glutamine, 20 μ g/ml α -ketoglutaric acid, and 50 μ g/ml ascorbic acid and 0.3% collagenase type I (Gibco). Following digestion, media was filtered through a 100 μ m Nylon cell strainer before centrifuging the cell solution at 800 g. Viability and cell count were obtained, and cells were cultured on 100 mm tissue culture plates at 8800 cells per cm² in tenocyte media (as previously mentioned, without collagenase). Tenocytes were passaged with trypsin-EDTA (0.25%) when plates became confluent and subcultured to P2 prior to co-culture.

Untreated or TGF- β 2-treated BM-MSCs from five additional horses and on horse also used for western blotting were seeded onto Transwell[®] polycarbonate membrane inserts (MilliporeSigma) in tenocyte media at a density of 20,000 cells per cm². Tenocytes were similarly plated in Transwell inserts at 20,000 cells per cm² as a control and to standardize nutrient deprivation between treatment groups. Cells were allowed 24 h to attach on inserts before adding to scratch wound wells for co-culture. Tenocytes were plated at a density of 6,250 cells per cm² in the wells of a 12-well tissue culture plate and allowed to grow to confluency. Twenty-four hours prior to tenocyte wounding, tenocytes were transitioned to serum-free tenocyte media. A single vertical scratch wound was created in each well using a 200 μ l pipette tip. The media was then aspirated, wells were washed twice with PBS, and serum-free tenocyte media was replaced. Images were taken at baseline and then every 12 h for 48 h at three separate locations along each scratch wound using an IX83 inverted microscope and cellSens[™] software (Olympus). All images were obtained with the Transwell inserts removed, and to ensure the same location was imaged at each time point, a plate map was created and saved within cellSens[™] software. Using Adobe Photoshop, the pixel area of the scratch wound at each location for all treatment groups was measured at baseline (time 0) and then each subsequent time point. The percent closure was calculated by averaging the three locations for each treatment group at each time point using the equation ((Average Area Time 0 - Average Area Time x)/Average Area Time 0) \times 100 where "x" represents time.

Statistical analysis

The functional enrichment analysis of RNA-sequencing data was performed using g:Profiler (version e104_eg51_p15_3922dba) with g:SCS multiple testing correction method applying significance threshold of 0.05 [20]. Western blot data were normalized by log transformation and analyzed using paired one-tailed t tests with a null hypothesis of no difference using JMP Pro 15 (SAS Institute Inc.). The percent wound healing data for the tenocyte scratch assays were analyzed using a one-way repeated measures ANOVA followed by a Tukey's test for multiple comparisons using Prism 9.0 (GraphPad).

Results

Differentially expressed genes in TGF- β 2-treated BM-MSCs

To identify global changes in gene expression after TGF- β 2 treatment, RNA-sequencing was performed on matched untreated and TGF- β 2-treated BM-MSCs from four donor horses. We observed 2467 significant differentially expressed genes (DEGs) in total with 1148 genes upregulated and 1319 genes downregulated (Fig. 1a). The genes with the largest increase in expression were INHBE, a member of the TGF- β superfamily, IFGBP5, an insulin-like growth factor binding protein, and RELN, a glycoprotein involved in neuronal migration. The genes with the largest decrease in expression were SCARA5, a ferritin receptor, PDE1A, a phosphodiesterase involved in cAMP signaling, and C30H1orf115, an uncharacterized homolog of the human gene C1orf115. Gene ontology (GO) enrichment analysis was performed to identify the top overrepresented terms in the entire DEG set. The top ten molecular functions (MF), biological processes (BP), and cellular components (CC) for this data are listed in Fig. 1b. Among the overrepresented GO terms relevant to tendon healing were extracellular matrix, collagen-containing extracellular matrix, tissue development, cell adhesion molecule binding, and growth factor activity. For the top 200 upregulated genes, all significant GO terms for MF, BP, CC, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and human phenotype ontology (HP) are shown in Additional file 1: Fig. S1.

Collagen expression is upregulated in TGF- β 2-treated BM-MSCs

Twenty-eight different types of collagens have been described, although types I, II, III, IV, and V are the most common [21, 22]. Normal, healthy tendon is composed

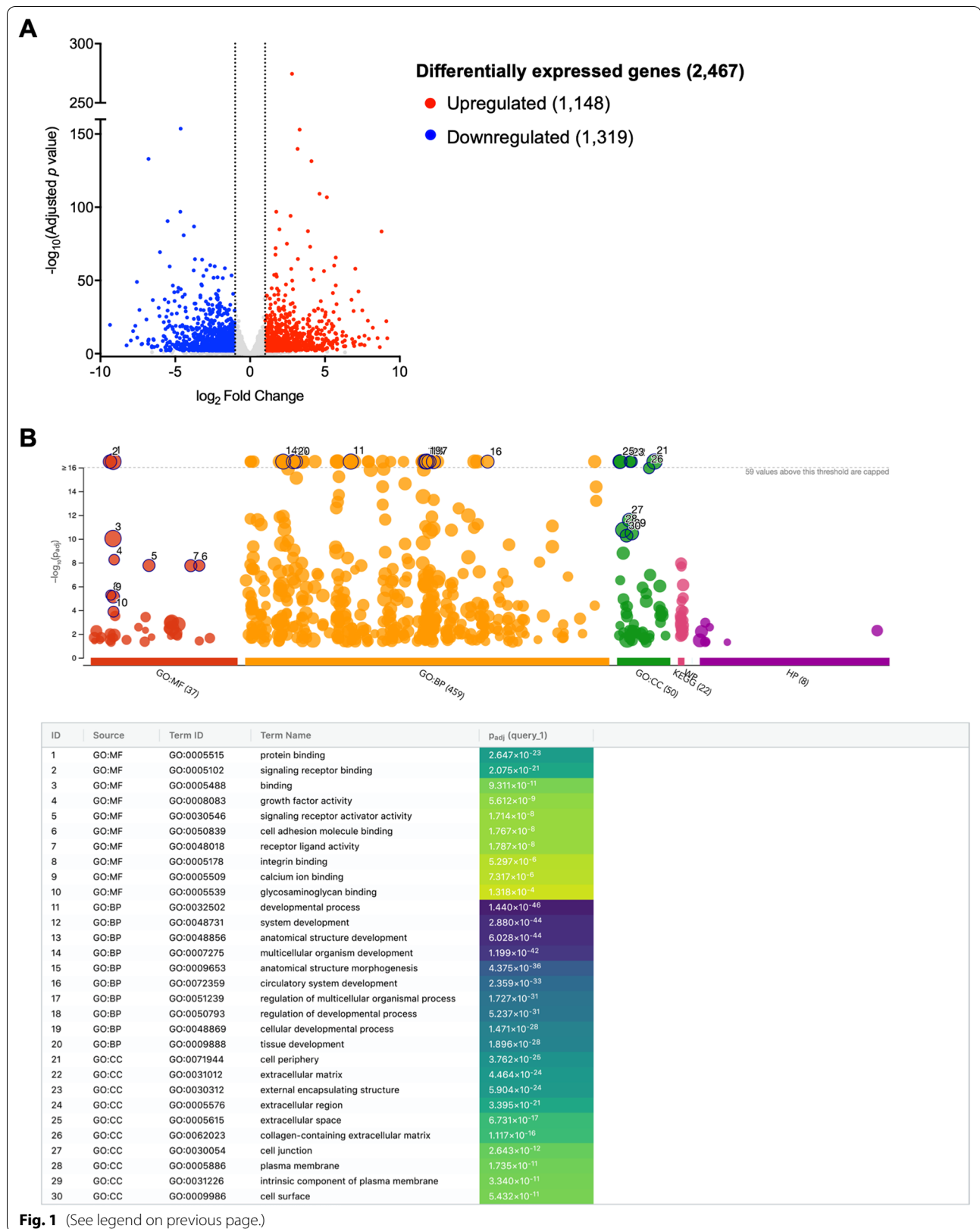
primarily of type I collagen. Regenerative therapies for tendons aim to increase type I collagen and limit persistence of type III collagen that is secreted during the proliferative phase of tendon healing, but is structurally weaker. In BM-MSCs treated with TGF- β 2, collagen types I, IV, V, VIII, XI, XII, XV, and XVIII were significantly upregulated compared to untreated BM-MSCs (Fig. 2a). COL1A1 and COL1A2 were by far the most abundant collagen transcripts detected in both untreated and TGF- β 2-treated equine BM-MSCs followed by COL5A1, COL5A2, and COL12A1. COL11A1, which has been shown to regulate type I collagen fibril assembly [23, 24], had the greatest Log₂ fold change in TGF- β 2-treated BM-MSCs compared to untreated. Both subunits associated with type V collagen were upregulated, which may support collagen fibrillogenesis [25]. Gene transcripts of type II and type III collagens were not detected by RNA-sequencing. Protein expression of alpha-1 type I collagen from untreated and TGF- β 2-treated BM-MSCs from 4 different donor horses was measured by western blot (Fig. 2b). Prominent bands could be detected around both 180 kDa and 35 kDa, which likely correspond to the procollagen alpha-1 chain and procollagen type I carboxy-terminal propeptide, respectively [26, 27]. All horses expressed significantly more total alpha-1 type I collagen from TGF- β 2-treated BM-MSCs than untreated ($p = 0.0001$) (Fig. 2b). This significant increase in collagen production by TGF- β 2-treated BM-MSCs may be beneficial in improving healing in tissues like tendons where collagen production is critical to the structure, integrity, and function of the tissue.

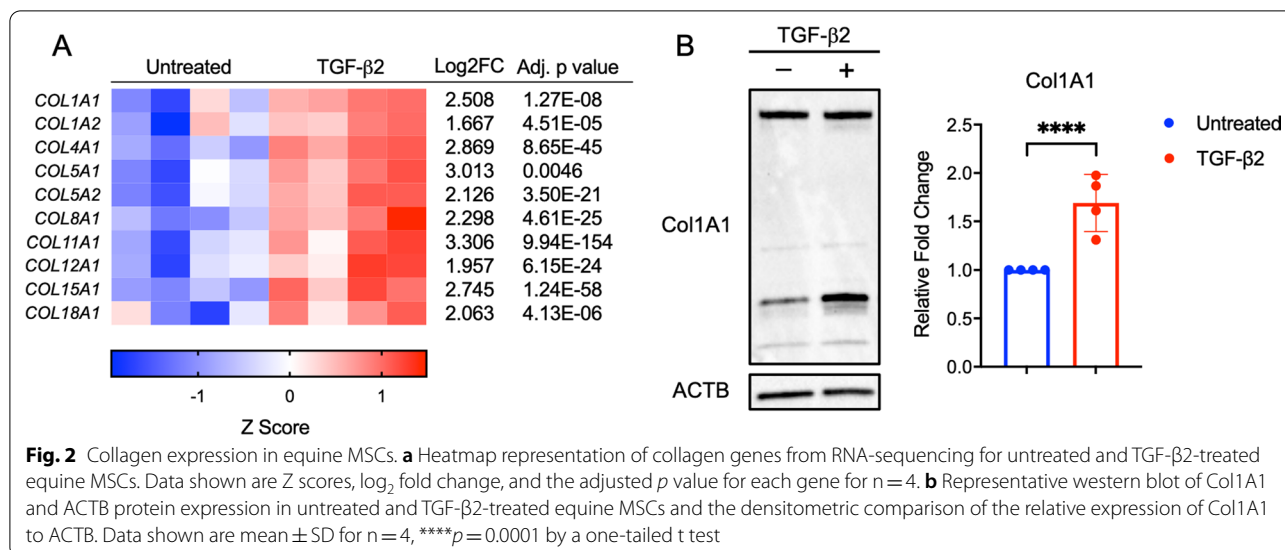
Extracellular matrix genes and enzymes are differentially expressed in TGF- β 2-treated MSCs

Numerous other non-collagenous ECM genes linked to healthy tendon or tendon healing were upregulated in TGF- β 2-treated BM-MSCs including tenascin-C, biglycan, elastin, and fibronectin (Fig. 3a). Tenascin-C is a known marker of tenogenic differentiation and tendon ECM; it plays an important role in cell migration during wound healing, collagen fibril organization, and creates a de-adhesive ECM environment during injury repair [28, 29]. Protein expression of tenascin-C was confirmed to be significantly upregulated in TGF- β 2-treated BM-MSCs compared to untreated BM-MSCs ($p = 0.0005$) (Fig. 3b). Of the tendon relevant ECM genes, only tenascin-X (TNXB) was downregulated in TGF- β 2-treated

(See figure on next page.)

Fig. 1 RNA-sequencing and functional enrichment analysis of differentially expressed genes (DEGs). **a** Volcano plot depicting the log₂ fold change and $-\log_{10}$ (adjusted p value) for gene transcripts in TGF- β 2-treated equine bone marrow-derived MSCs detected by RNA-sequencing. Significantly upregulated DEGs are shown in red and significantly downregulated DEGs in blue. **b** Manhattan plots from g:Profiler illustrating GO term enrichment analysis of DEGs in TGF- β 2-treated equine MSCs. The top 10 enriched pathways for each GO term (MF: molecular function; BP: biological process; CC: cellular component) are listed below





BM-MSCs. Along with increases in collagen expression, increases in ECM protein expression from TGF- β 2-treated BM-MSCs may assist and enhance tendon repair and healing.

Also important to the ECM in tendon healing are the enzymes that assist with remodeling, collagen cross-linking, and regulation of growth factors. Matrix metalloproteinase-9 (MMP9) is suggested to play a role in early tendon remodeling [30] and was downregulated in TGF- β 2-treated BM-MSCs (Fig. 3c). Tissue inhibitors of metalloproteinase, TIMP3 and TIMP4, were also downregulated in TGF- β 2-treated BM-MSCs, although expression of TIMP1 and TIMP2 was unaffected by TGF- β 2 treatment. LOX and LOXL2, which encode lysyl oxidase family members and are involved in cross-linking of collagen and elastin, had high levels of transcript detected in BM-MSCs and were significantly upregulated in TGF- β 2-treated BM-MSCs. The timing of expression and coordination between secreted ECM subunits and enzymes that contribute to tendon ECM remodeling, maintenance, and healing are not well understood, so it is still unclear how significantly the changes in these secreted factors might affect the healing tendon.

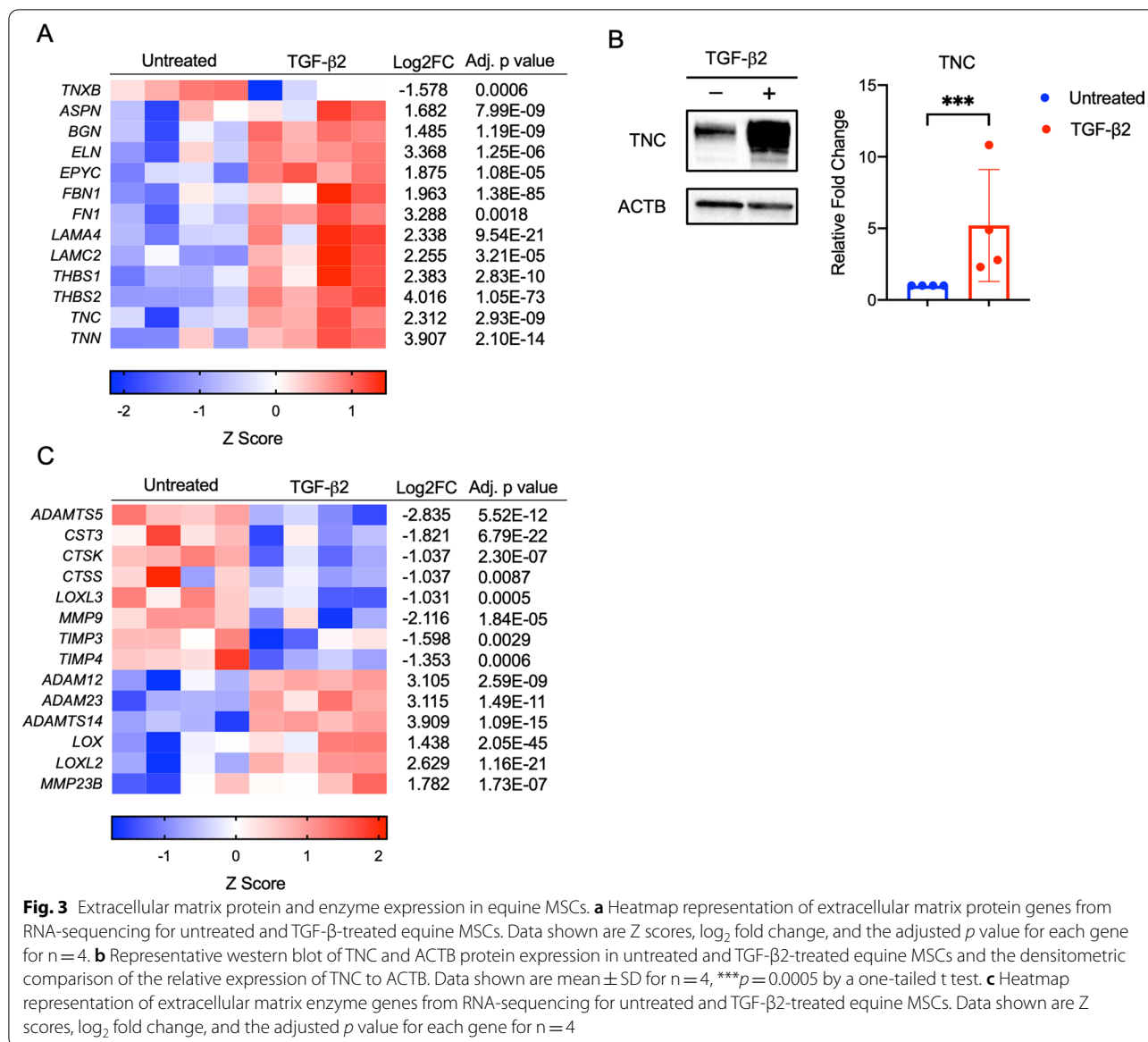
Cytokines and growth factors are differentially expressed in TGF- β 2-treated BM-MSCs

Cytokines and growth factors regulate tendon healing by stimulating angiogenesis, extracellular matrix production, tenocyte proliferation, and modulating immune responses. Gene expression of several important cytokines and growth factors relevant to tendon healing was upregulated in TGF- β 2-treated BM-MSCs compared to untreated controls (Fig. 4). Cytokines with the greatest

upregulation in TGF- β 2-treated BM-MSCs included bone-derived neurotrophic factor (BDNF), interleukin-11 (IL-11), insulin-like growth factor-1 (IGF-1), and acidic fibroblast growth factor (FGF1). We attempted to measure IGF-1 protein expression from BM-MSCs using a commercial anti-human IGF-1 ELISA kit and an anti-human IGF-1 antibody in a western blot, but no protein could be detected (data not shown). Human IGF-1 and equine IGF-1 have 98% homology, but high levels of transcripts of five IGF binding proteins, IGFBP2, IGFBP4, IGFBP5, IGFBP6, and IGFBP7, were also detected in untreated and TGF- β 2-treated BM-MSCs. IGFBPs can block antibody binding to IGF-1, which may have affected our ability to measure protein expression in cell culture supernatant and cell lysate [31, 32]. ANGPT1 and ANGPTL4, which may promote angiogenesis and tenocyte migration and cell cycle progression in injured tendons [33, 34], were downregulated in TGF- β 2-treated BM-MSCs, but VEGFA, VEGFB, and VEGFC isoforms were not differentially expressed. PGE2 and TGF- β 1 have been identified in the horse as the primary cytokines responsible for immunomodulation [35], and these were not differentially expressed in TGF- β 2-treated BM-MSCs. This is supported by our previous work that did not find any significant difference in the concentrations of PGE2 and TGF- β 1 in modified one-way mixed leukocyte reactions with untreated and TGF- β 2-treated BM-MSCs [14].

BM-MSCs increase tenocyte migration

Migration of tenocytes to the site of tendon damage following injury is an important initial step in the repair process [36]. To determine if the changes in the

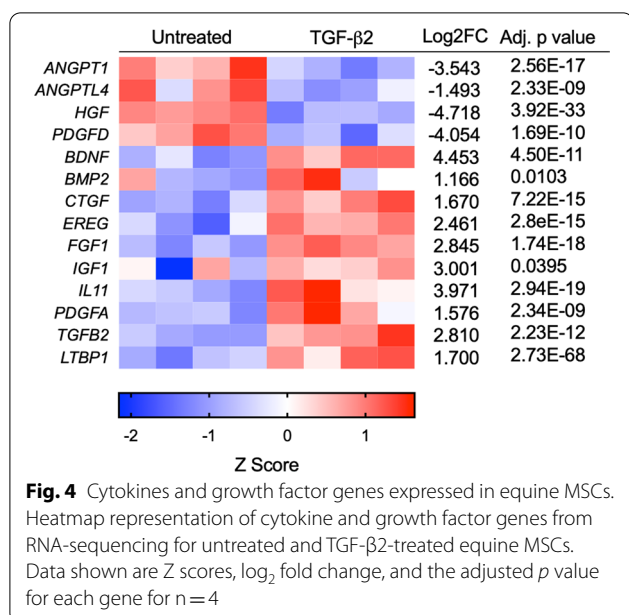


secretome of TGF-β2-treated BM-MSCs affect tenocyte migration, untreated and TGF-β2-treated BM-MSCs were indirectly co-cultured with tenocytes in a standard scratch assay. Tenocyte scratch wound closure was significantly improved with untreated BM-MSCs at 24 and 48 h and with TGF-β2-treated BM-MSCs at 24, 36, and 48 h (Fig. 5a). A significant difference between untreated and TGF-β2-treated BM-MSC groups was measured only at 36 h (Fig. 5b). An effect on tenocyte migration was noted between BM-MSCs from different horses (*p* < 0.0001), which is consistent with the known variation in the quantity of paracrine factors secreted from outbred species like humans and horses [37, 38]. Based on these results, the secretome of TGF-β2-treated BM-MSCs does

not appear to be detrimental to tendon migration following wounding in an in vitro scratch wound assay.

Discussion

Treatment of tendon injuries is a major challenge in human and veterinary orthopedic medicine, and the goals of regenerative therapies are to improve the tendon biochemical composition and biomechanical strength while reducing clinical reinjury rates. Advances in the understanding of MSC biology have led us to explore modulating cell function in an effort to improve MSC therapy for tendon injuries. In this study, we investigated changes in gene and protein expression of paracrine factors and ECM components related to tendon healing in equine



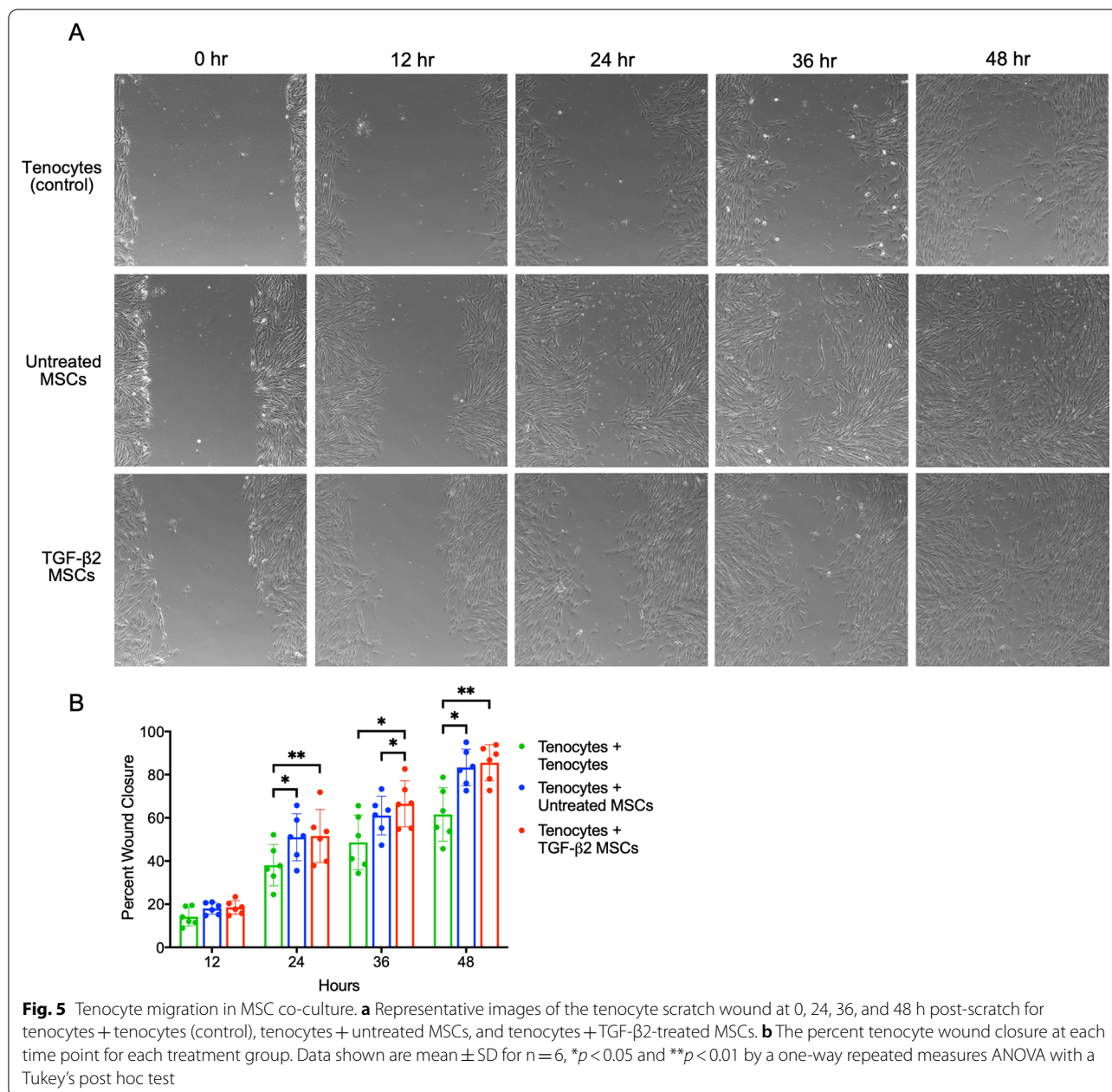
BM-MSCs treated with TGF-β2. Using RNA-sequencing, we identified gene expression of numerous secreted factors important for tendon healing including COL1A1 and COL1A2, TNC, LOX, IGF-1, and FGF1 which were upregulated in TGF-β2-treated BM-MSCs compared to untreated MSCs. Further, when grown together in co-culture, both untreated and TGF-β2-treated BM-MSCs enhanced migration of equine tenocytes.

The clinical outcome of MSC therapy is dependent on both the quality and viability of the cells [39–42]. As seen in both the RNA-seq results and western blots, there were significant differences in the production of paracrine factors and ECM components between individual horses. This is consistent with reports that the quality of autologous MSCs can be affected by individual genetic backgrounds, health status, and age [43–45]. As shown in this study, the quantity of paracrine factors important for tendon healing can be increased by treating BM-MSCs with TGF-β2. Additionally, BM-MSCs from older horses may grow more slowly, but treating BM-MSCs with TGF-β2, provided bFGF is also included in the culture media, increases the yield of cells even from older horses [17]. Treating BM-MSCs with TGF-β2 could therefore potentially improve the efficacy of autologous therapy from patients who might otherwise be less than ideal candidates for MSC therapy including older patients. TGF-β2 also reduces MHC class I expression on MSCs, which reduces the cell-mediated immunogenicity of allogeneic BM-MSCs [14, 17]. In this study, we used different horses across experiments and still saw similar increases in both gene and protein expression of type I collagen and tenascin-c in the TGF-β2-treated MSCs.

Even though BM-MSCs were treated with TGF-β2 for a much shorter period of time than the BM-MSCs used for RNA-seq, both type I collagen and tenascin-c protein expression was significantly upregulated. This is consistent with previously published in vitro and in vivo studies that have shown significant improvements in wound healing or clinical signs following treatment with MSCs licensed with cytokines for 24 or 48 h [40, 46–48]. Therefore, a short duration treatment of TGF-β2 is likely sufficient to significantly enhance the secretome of BM-MSCs and improve the efficacy of both autologous and allogeneic BM-MSC therapy, although more studies are needed to confirm this.

Type I collagen is the primary collagen of healthy tendon ECM. With aging and injury, the ECM contains higher levels of type III collagen along with more disorganized fibers [49]. Therefore, a strategy to improve tendon healing would include a shift to decrease deposition of type III collagen and concurrent remodeling into type I collagen. In western blots, TGF-β2-treated BM-MSCs had increased expression of the pro-alpha-1 chain and procollagen type I carboxy-terminal propeptide (PICP), which can only originate from synthesized type I collagen and is used as a marker of type I collagen production [50]. Increased PICP in the BM-MSC cultures may be due to both upregulation of type I procollagen and BMP1, which cleaves the carboxy terminus of type I procollagen [51]. BMP1 was upregulated in TGF-β2-treated BM-MSCs, although the log₂ fold change was slightly less than 1. TGF-β2 treatment also enhanced BM-MSC gene expression of two of the three type V collagen alpha chains, which are incorporated into the type I collagen triple helix during collagen maturation in tendon tissue [22]. Tendon ECM development may be further supported through BM-MSC secretion of tenascin-C, which has been shown to enhance tendon stem cell migration and remodeling and accelerate restoration of tissue matrix after exogenous administration [52]. Therefore, TGF-β2-treated BM-MSCs may improve the composition of the tendon ECM during healing through direct secretion of both the collagenous and non-collagenous tendon ECM.

While many of the secreted factors upregulated following treatment could impart benefit to a healing tendon, others may be associated with fibrosis under certain conditions. IL-11 is a poorly understood pleiotropic cytokine belonging to the IL-6 family which plays a role in stromal cell proliferation, tissue healing and homeostasis, as well as fibrosis, but its role in tendon injury and repair is not well characterized [53–55]. Like TGF-β, BMP-2 drives extracellular matrix expression and can promote fibrosis in chronic tendon inflammation, although it also promotes tenocyte migration [56, 57]. HGF, an inhibitor of myofibroblast differentiation and TGF-β-induced fibrosis



[58], is downregulated in TGF- β 2-treated BM-MSCs. Due to the inherent complexity of biologic systems, the timing and cell specificity of cytokine and cytokine receptors involved in fibrosis, and our minimal understanding of what is required for a tendon to remodel to its native composition and strength [59], there are still many unknowns about the full effects of TGF- β 2-treated BM-MSCs on tendon healing. Further research into how TGF- β 2-treated BM-MSCs affect tenocyte gene expression and function is necessary to better predict any potential adverse effects. Additionally, while we do not

see any overt morphologic changes in the BM-MSCs after adding TGF- β 2 [17], in vivo testing in preclinical animal models is needed to confirm that TGF- β 2-treated BM-MSCs do not promote ectopic bone or cartilage formation as some of the upregulated secreted factors are also involved in osteo-chondrogenic development.

Co-culture assays with BM-MSCs and tenocytes demonstrated that BM-MSC paracrine factors enhanced tenocyte migration. Other studies have established the role of migration in improved tendon healing [36, 57] and that MSC paracrine factors enhance cellular migration

and proliferation of tenocytes as well as corneal epithelial cells and dermal fibroblasts [60–62]. Chen et al. determined that this tenocyte-specific response in improved cell migration was due to activation of the ERK1/2 pathway by MSC paracrine factors, although the exact factors responsible were not elucidated [60]. FGF1, TGF- β 2, and IL-11, all which were upregulated in TGF- β 2-treated BM-MSCs, can activate MAP kinase signaling as can angiotensin, prostaglandins, and platelet derived growth factors [63–66]. In this study, both untreated and TGF- β 2-treated BM-MSCs significantly improved migration of tenocytes. While TGF- β 2-treated BM-MSCs only showed an enhanced effect on migration over untreated BM-MSCs at a single time point, 36 h, it is not clear from this in vitro study if this difference would significantly enhance healing in vivo. However, migration is a single outcome measure and future studies investigating if TGF- β 2-treated BM-MSCs improve tendon healing will focus more in depth on modulation of tenocyte function including ECM production and secretion of paracrine factors.

Genome-wide transcriptional analysis of equine BM-MSCs has not been extensively investigated despite the horse being an important large animal translational model for testing MSC regenerative therapies in musculoskeletal injuries [67, 68]. Harman et al. used single-cell RNA-seq to compare equine MSCs between tissue sources, but the focus of the reported data was on differences in phenotypic markers and expression of C-X-C motif chemokine ligand 6 (CXCL6) [69]. Although some cell functions can be inferred across species, differences in MSC phenotype and function have been previously identified in the horse including differences in important immunomodulatory factors [38, 70, 71]. Through RNA-sequencing, we revealed which secreted factor genes relevant to tendon healing are and are not expressed in equine BM-MSCs allowing for future comparisons between other species including humans and mice. We have also shown that TGF- β 2 enhances gene expression of collagens, ECM matrix proteins, matrix remodeling enzymes, and growth factors in BM-MSCs. MSCs licensed with inflammatory cytokines typically have significant increases in immunomodulatory factors rather than collagen and other ECM-related proteins. As immunomodulatory factors play a critical role in tissue healing, treating BM-MSCs with both TGF- β 2 and an inflammatory cytokine prior to treatment may enhance the paracrine function of MSCs in additional ways that are relevant to treatment of tendon injuries and should be further investigated. One limitation of this study is that it focuses primarily on the changes in gene expression in BM-MSCs following TGF- β 2 treatment. However, the results from the RNA-seq do give us a better understanding of what

proteins equine BM-MSCs potentially secrete, which allows us to better plan future experiments to characterize the entire secretome. Additional research into the changes in protein expression of BM-MSC secreted factors is also necessary to understand mechanisms of BM-MSC-mediated tissue repair and the differences in the BM-MSC secretome between the horse and the human for translational relevance.

Conclusion

In this study, we demonstrated that treating equine BM-MSCs with TGF- β 2 significantly increased production of secreted factors associated with tendon healing and maintained the ability of BM-MSCs to promote tenocyte migration. Increasing the expression of growth factors, collagens, and other extracellular matrix molecules from BM-MSCs by treating the cells with TGF- β 2 prior to in vivo use may improve the consistency of secreted factor production and cell quality. More in vitro research into which BM-MSC secreted factors are most important for improving tendon healing is still needed as are preclinical animal models to investigate if manipulation of the secretome of BM-MSCs using TGF- β 2 results in enhanced therapeutic efficacy for tendon injury.

Abbreviations

BP: Biological processes; CC: Cellular components; ECM: Extracellular matrix; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; MF: Molecular function; MHC: Major histocompatibility complex; MSC: Mesenchymal stem cell; PICP: Procollagen type I carboxy-terminal propeptide; SDFT: Superficial digital flexor tendon; TGF- β : Transforming growth factor- β .

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13287-022-03172-9>.

Additional file 1. Figure S1. GO terms, adjusted p value, and $-\log_{10}(\text{adjusted p value})$ for the top 200 upregulated genes in TGF- β 2-treated BM-MSCs.

Acknowledgements

The authors would like to thank the NCSU Laboratory Animal Resources staff for their help with animal care and handling and James Robertson for help with statistical analysis.

Author contributions

AB, LS, and DK contributed to study conception and design. AB and DK collected the data. DK, LS, IE, RB, and AB analyzed and interpreted the data. AB and DK performed statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by National Institutes of Health Grants K01OD027037 (AB), Morris Animal Foundation Grant D18EQ-055 (LS, AB), T32OD011130 (DK) and T35OD11070 (IE).

Availability of data and materials

The raw sequence data and normalized cells counts reported in this paper have been deposited in the Gene Expression Omnibus (GSE207394). All other data are available upon reasonable request.

Declarations**Ethics approval and consent to participate**

The Institutional Animal Care and Use Committee of North Carolina State University approved the use of horses in these studies.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 3 July 2022 Accepted: 7 September 2022

Published online: 16 September 2022

References

- Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol*. 2014;15:1009–16. <https://doi.org/10.1038/ni.3002>.
- da Silva ML, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev*. 2009;20:419–27.
- Hingert D, Nawilajroen P, Aldridge J, Baranto A, Brisby H. Investigation of the effect of secreted factors from mesenchymal stem cells on disc cells from degenerated discs. *Cells Tissues Organs*. 2020;208:76–88.
- Yang Y, Lin H, Shen H, Wang B, Lei G, Tuan RS. Mesenchymal stem cell-derived extracellular matrix enhances chondrogenic phenotype of and cartilage formation by encapsulated chondrocytes in vitro and in vivo. *Acta Biomater*. 2018;69:71–82. <https://doi.org/10.1016/j.actbio.2017.12.043>.
- Dong R, Bai Y, Dai J, Deng M, Zhao C, Tian Z, et al. Engineered scaffolds based on mesenchymal stem cells/preosteoclasts extracellular matrix promote bone regeneration. *J Tissue Eng*. 2020;11:2041731420926918.
- Godwin EE, Young NJ, Dudhia J, Beamish IC, Smith RKW. Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. *Equine Vet J*. 2012;44:25–32.
- Schnabel LV, Lynch ME, Van Der Meulen MCH, Yeager AE, Kornatowski MA, Nixon AJ. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res*. 2009;27:1392–8.
- Carvalho AM, Yamada ALM, Golim MA, Álvarez LEC, Hussni CA, Alves ALG. Evaluation of mesenchymal stem cell migration after equine tendonitis therapy. *Equine Vet J*. 2014;46:635–8.
- Maruyama M, Wei L, Thio T, Storaci HW, Ueda Y, Yao J. The effect of mesenchymal stem cell sheets on early healing of the Achilles tendon in rats. *Tissue Eng Part A*. 2020;26:206–13.
- Berglund AK, Fortier LA, Antczak DF, Schnabel LV. Immunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. *Stem Cell Res Ther*. 2017;8:288.
- Galipeau J, Sensébé L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell Stem Cell*. 2018;22:824–33.
- Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. *J Hematol Oncol*. 2021;14:1–24. <https://doi.org/10.1186/s13045-021-01037-x>.
- Lee BC, Kang KS. Functional enhancement strategies for immunomodulation of mesenchymal stem cells and their therapeutic application. *Stem Cell Res Ther*. 2020;11:1–10.
- Berglund AK, Long JM, Robertson JB, Schnabel LV. TGF- β 2 reduces the cell-mediated immunogenicity of equine MHC-mismatched bone marrow-derived mesenchymal stem cells without altering immunomodulatory properties. *Front Cell Dev Biol*. 2021;9:1–10.
- Verrecchia F, Mauviel A. Transforming growth factor- β signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol*. 2002;118:211–5. <https://doi.org/10.1046/j.1523-1747.2002.01641.x>.
- Kubiczkova L, Sedlarikova L, Hajek R, Sevcikova S. TGF-beta: an excellent servant but a bad master. *J Transl Med*. 2012;10:183.
- Berglund AK, Fisher MB, Cameron KA, Poole EJ, Schnabel LV. Transforming growth factor- β 2 downregulates major histocompatibility complex (MHC) I and MHC II surface expression on equine bone marrow-derived mesenchymal stem cells without altering other phenotypic cell surface markers. *Front Vet Sci*. 2017;4:84. <https://doi.org/10.3389/fvets.2017.00084/full>.
- Harman RM, He MK, Zhang S, van de Walle GR. Plasminogen activator inhibitor-1 and tenascin-C secreted by equine mesenchymal stromal cells stimulate dermal fibroblast migration in vitro and contribute to wound healing in vivo. *Cytotherapy*. 2018;20:1061–76. <https://doi.org/10.1016/j.jcyt.2018.06.005>.
- Nichols AEC, Settlage RE, Werre SR, Dahlgren LA. Novel roles for scleraxis in regulating adult tenocyte function. *BMC Cell Biol*. 2018;19:1–15.
- Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res*. 2019;47:W191–8.
- Ricard-Blum S. The collagen family. *Cold Spring Harb Perspect Biol*. 2011;3:1–19.
- Gelse K, Pöschl E, Aigner T. Collagens: structure, function, and biosynthesis. *Adv Drug Deliv Rev*. 2003;55:1531–46.
- Sun M, Luo EY, Adams SM, Adams T, Ye Y, Shetye SS, et al. Collagen XI regulates the acquisition of collagen fibril structure, organization and functional properties in tendon. *Matrix Biol*. 2020;94:77–94.
- Hansen U, Bruckner P. Macromolecular specificity of collagen fibrillogenesis: fibrils of collagens I and XI contain a heterotypic alloyed core and a collagen I sheath. *J Biol Chem*. 2003;278:37352–9. <https://doi.org/10.1074/jbc.M304325200>.
- Smith SM, Zhang G, Birk DE. Collagen V localizes to pericellular sites during tendon collagen fibrillogenesis. *Matrix Biol*. 2014;33:47–53. <https://doi.org/10.1016/j.matbio.2013.08.003>.
- Chung HJ, Steplewski A, Chung KY, Uitto J, Fertala A. Collagen fibril formation: a new target to limit fibrosis. *J Biol Chem*. 2008;283:25879–86. <https://doi.org/10.1074/jbc.M804272200>.
- Seo WY, Kim JH, Baek DS, Kim SJ, Kang S, Yang WS, et al. Production of recombinant human procollagen type I C-terminal propeptide and establishment of a sandwich ELISA for quantification. *Sci Rep*. 2017;7:1–13. <https://doi.org/10.1038/s41598-017-16290-9>.
- Riley GP, Harrall RL, Cawston TE, Hazleman BL, Mackie EJ. Tenascin-C and human tendon degeneration. *Am J Pathol*. 1996;149:933–43.
- Mackey AL, Brandstetter S, Schjerling P, Bojsen-Moller J, Qvortrup K, Pedersen MM, et al. Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. *FASEB J*. 2011;25:1943–59.
- Oshiro W, Lou J, Xing X, Tu Y, Manske PR. Flexor tendon healing in the rat: a histologic and gene expression study. *J Hand Surg Am*. 2003;28:814–23.
- Frystyk J, Freda P, Clemmons DR. The current status of IGF-I assays: a 2009 update. *Growth Horm IGF Res*. 2010;20:8–18.
- Baskerville CL, Bamford NJ, Harris PA, Bailey SR. Comparison and validation of ELISA assays for plasma insulin-like growth factor-1 in the horse. *Open Vet J*. 2017;7:75–80.
- Oike Y, Yasunaga K, Suda T. Angiopoietin-related/angiopoietin-like proteins regulate angiogenesis. *Int J Hematol*. 2004;80:21–8.
- Jamil S, Mousavizadeh R, Roshan-Moniri M, Tebbutt SJ, McCormack RG, Duronio V, et al. Angiopoietin-like 4 enhances the proliferation and migration of tendon fibroblasts. *Med Sci Sports Exerc*. 2017;49:1769–77.
- Carrade Holt DD, Wood JA, Granick JL, Walker NJ, Clark KC, Borjesson DL. Equine mesenchymal stem cells inhibit T cell proliferation through

- different mechanisms depending on tissue source. *Stem Cells Dev.* 2014;23:1258–65. <https://doi.org/10.1089/scd.2013.0537>.
36. Tsai WC, Hsu CC, Chou SW, Chung CY, Chen J, Pang JH. Effects of celecoxib on migration, proliferation and collagen expression of tendon cells. *Connect Tissue Res.* 2007;48:46–51.
 37. Hsiao STF, Asgari A, Lokmic Z, Sinclair R, Dusting GJ, Lim SY, et al. Comparative analysis of paracrine factor expression in human adult mesenchymal stem cells derived from bone marrow, adipose, and dermal tissue. *Stem Cells Dev.* 2012;21:2189–203.
 38. Carrade DD, Lame MW, Kent MS, Clark KC, Walker NJ, Borjesson DL. Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells. *Cell Med.* 2012;4:1–11.
 39. Krampera M. Mesenchymal stromal cell licensing: a multistep process. *Leukemia.* 2011;25:1408–14.
 40. Fan H, Zhao G, Liu L, Liu F, Gong W, Liu X, et al. Pre-treatment with IL-1 β enhances the efficacy of MSC transplantation in DSS-induced colitis. *Cell Mol Immunol.* 2012;9:473–81.
 41. Giri J, Galipeau J. Mesenchymal stromal cell therapeutic potency is dependent upon viability, route of delivery, and immune match. *Blood Adv.* 2020;4:1987–97.
 42. Rowland AL, Miller D, Berglund A, Schnabel LV, Levine GJ, Antczak DF, et al. Cross-matching of allogeneic mesenchymal stromal cells eliminates recipient immune targeting. *Stem Cells Transl Med.* 2021;10:694–710.
 43. Fafián-Labora J, Fernández-Pernas P, Fuentes I, De Toro J, Oreiro N, Sangiao-Alvarellos S, et al. Influence of age on rat bone-marrow mesenchymal stem cells potential. *Sci Rep.* 2015;5:16765.
 44. Nie Y, Lau C, Lie A, Chan G, Mok M. Defective phenotype of mesenchymal stem cells in patients with systemic lupus erythematosus. *Lupus.* 2010;19:850–9.
 45. Bagge J, MacLeod JN, Berg LC. Cellular proliferation of equine bone marrow- and adipose tissue-derived mesenchymal stem cells decline with increasing donor age. *Front Vet Sci.* 2020;7:1–14.
 46. Kim DS, Jang IK, Lee MW, Ko YJ, Lee DH, Lee JW, et al. Enhanced immunosuppressive properties of human mesenchymal stem cells primed by interferon- γ . *EBioMedicine.* 2018;28:261–73. <https://doi.org/10.1016/j.ebiom.2018.01.002>.
 47. Liu Q, Ji S, Xia T, Liu J, Liu Z, Chen X, et al. MCP-1 priming enhanced the therapeutic effects of human mesenchymal stromal cells on contact hypersensitivity mice by activating the COX2-PGE2/STAT3 pathway. *Stem Cells Dev.* 2020;29:1073–83.
 48. Broekman W, Amatngalim GD, de Mooij-Eijk Y, Oostendorp J, Roelofs H, Taube C, et al. TNF- α and IL-1 β -activated human mesenchymal stromal cells increase airway epithelial wound healing in vitro via activation of the epidermal growth factor receptor. *Respir Res.* 2016;17:1–12. <https://doi.org/10.1186/s12931-015-0316-1>.
 49. Lipman K, Wang C, Ting K, Soo C, Zheng Z. Tendinopathy: Injury, repair, and current exploration. *Drug Des Devel Ther.* 2018;12:591–603.
 50. Chalikias GK, Tziakas DN. Biomarkers of the extracellular matrix and of collagen fragments. *Clin Chim Acta.* 2015;443:39–47. <https://doi.org/10.1016/j.cca.2014.06.028>.
 51. Kessler E, Takahara K, Biniaminov L, Brusel M, Greenspan DS. Bone morphogenetic protein-1: the type I procollagen C-proteinase. *Science.* 1996;271:360–2.
 52. Xu K, Shao Y, Xia Y, Qian Y, Jiang N, Liu X, et al. Tenascin-C regulates migration of SOX10 tendon stem cells via integrin- α 9 for promoting patellar tendon remodeling. *BioFactors.* 2021;47:768–77.
 53. West NR. Coordination of immune-stroma crosstalk by IL-6 family cytokines. *Front Immunol.* 2019;10:1–16.
 54. Allanki S, Strlic B, Scheinberger L, Onderwater YL, Marks A, Günther S, et al. Interleukin-11 signaling promotes cellular reprogramming and limits fibrotic scarring during tissue regeneration. *Sci Adv.* 2021;7:eabg6497.
 55. Blache U, Wunderli SL, Hussien AA, Stauber T, Flückiger G, Bollhalder M, et al. Inhibition of ERK 1/2 kinases prevents tendon matrix breakdown. *Sci Rep.* 2021;11:1–9. <https://doi.org/10.1038/s41598-021-85331-1>.
 56. Morita W, Snelling SJB, Wheway K, Watkins B, Appleton L, Murphy RJ, et al. Comparison of cellular responses to TGF- β 1 and BMP-2 between healthy and torn tendons. *Am J Sports Med.* 2021;49:1892–903.
 57. Li Z, Shen XT, Cao L, Yuan ZQ, Chen SY, Zheng X, et al. Bone morphogenetic protein 2 improves patellar tendon healing by promoting migration and proliferation of tenocytes. *Chin Sci Bull.* 2011;56:1361–9.
 58. Cui Q, Wang Z, Jiang D, Qu L, Guo J, Li Z. HGF inhibits TGF- β 1-induced myofibroblast differentiation and ECM deposition via MMP-2 in Achilles tendon in rat. *Eur J Appl Physiol.* 2011;111:1457–63.
 59. Ellis JM, Schnabel LV, Berglund AK. Defining the profile: characterizing cytokines in tendon injury to improve clinical therapy. *J Immunol Regen Med.* 2022;16:100059. <https://doi.org/10.1016/j.regen.2022.100059>.
 60. Chen Q, Liang Q, Zhuang W, Zhou J, Zhang B, Xu P, et al. Tenocyte proliferation and migration promoted by rat bone marrow mesenchymal stem cell-derived conditioned medium. *Biotechnol Lett.* 2018;40:215–24.
 61. Sherman AB, Gilger BC, Berglund AK, Schnabel LV. Effect of bone marrow-derived mesenchymal stem cells and stem cell supernatant on equine corneal wound healing in vitro. *Stem Cell Res Ther.* 2017;8:120.
 62. Zhang W, Bai X, Zhao B, Li Y, Zhang Y, Li Z, et al. Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway. *Exp Cell Res.* 2018;370:333–42. <https://doi.org/10.1016/j.yexcr.2018.06.035>.
 63. Force T, Bonventre JV. Growth factors and mitogen-activated protein kinases. *Hypertension.* 1998;31:152–61.
 64. Chapnick DA, Warner L, Bernet J, Rao T, Liu X. Partners in crime: the TGF β and MAPK pathways in cancer progression. *Cell Biosci.* 2011;1:42.
 65. Lim WW, Corden B, Ng B, Vanezis K, D'Agostino G, Widjaja AA, et al. Interleukin-11 is important for vascular smooth muscle phenotypic switching and aortic inflammation, fibrosis and remodeling in mouse models. *Sci Rep.* 2020;10:1–18. <https://doi.org/10.1038/s41598-020-74944-7>.
 66. La Vallee TM, Prudovsky IA, McMahon GA, Hu X, Maciag T. Activation of the MAP kinase pathway by FGF-1 correlates with cell proliferation induction while activation of the Src pathway correlates with migration. *J Cell Biol.* 1998;141:1647–58.
 67. Oreff GL, Fenu M, Vogl C, Ribitsch I, Jenner F. Species variations in tenocytes' response to inflammation require careful selection of animal models for tendon research. *Sci Rep.* 2021;11:1–14. <https://doi.org/10.1038/s41598-021-91914-9>.
 68. Patterson-Kane JC, Becker DL, Rich T. The pathogenesis of tendon microdamage in athletes: the horse as a natural model for basic cellular research. *J Comp Pathol.* 2012;147:227–47. <https://doi.org/10.1016/j.jcpa.2012.05.010>.
 69. Harman RM, Patel RS, Fan JC, Park JE, Rosenberg BR, Van de Walle GR. Single-cell RNA sequencing of equine mesenchymal stromal cells from primary donor-matched tissue sources reveals functional heterogeneity in immune modulation and cell motility. *Stem Cell Res Ther.* 2020;11:1–15.
 70. Calloni R, Viegas GS, Türck P, Bonatto D, Pegas Henriques JA. Mesenchymal stromal cells from unconventional model organisms. *Cytotherapy.* 2014;16:3–16. <https://doi.org/10.1016/j.jcyt.2013.07.010>.
 71. Su J, Chen X, Huang Y, Li W, Li J, Cao K, et al. Phylogenetic distinction of iNOS and IDO function in mesenchymal stem cell-mediated immunosuppression in mammalian species. *Cell Death Differ.* 2014;21:388–96.

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