The combination of BMP12 and KY02111 enhances tendon differentiation in bone marrow-derived equine mesenchymal stromal cells (BM-eMSCs)

Aungkura SUPOKAWEJ1, Wasamon KORCHUNJIT1,4 and Tuempong WONGTAWAN2–4*

1Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand
2Akkhararatchakumari Veterinary College, Walailak University, Nakhon Si Thammarat 80160, Thailand
3Centre for One Health, Walailak University, Nakhon Si Thammarat 80160, Thailand
4Laboratory of Cellular Biomedicine, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand

The Wingless and Int-1 (WNT) and bone morphogenic protein/growth differentiation factor (BMP/GDF) signalling pathways contribute significantly to the development of the musculoskeletal system. The mechanism by which they contribute is as follows: BMP/GDF signalling usually promotes tendon differentiation, whereas WNT signalling inhibits it. We hypothesised that inhibiting WNT and subsequently stimulating BMP signalling may enhance the tenogenic differentiation of stem cells. The objective of this study was to determine whether a combination of WNT inhibitor (KY02111) and BMP12/GDF7 protein could enhance the differentiation of bone marrow-derived equine mesenchymal stromal cells (BM-eMSCs) into tenocytes. Cells were cultured in five treatments: control, BMP12, and three different combinations of BMP12 and KY02111. The results indicated that a 1-day treatment with KY02111 followed by a 13-day treatment with BMP12 resulted in the highest tenogenic differentiation score in this experiment. The effect of KY02111 is dependent on the incubation time, with 1 day being better than 3 or 5 days. This combination increased tenogenic gene marker expression, including SCX, TNMD, DCN, and TNC, as well as COL1 protein expression. In conclusion, we propose that a combination of BMP12 and KY02111 can enhance the in vitro tenogenic differentiation of BM-eMSCs more than BMP12 alone. The findings of this study might be useful for improving tendon differentiation protocols for stem cell transplantation and application to tendon regeneration.

Key words: BMP12, horse, KY02111, tendon

Introduction

Tendinitis as a result of an injury is one of the most common health issues in sport horses [39]. Even after conventional treatment (NSAIDs, physical therapy, and an exercise programme), tendon fibrosis persists, and the recovered tendon is weaker and more susceptible to re-injury due to increased numbers of abnormal collagen types and fibroblasts and a concurrent decrease in tenocyte number [2, 30].

Nowadays, equine mesenchymal stromal cells (eMSCs) are used to treat various equine diseases, including arthritis and tendinitis [12, 18, 28, 34]. The therapeutic effect of MSCs on the tendon is believed to be due to paracrine factors that modulate inflammation and promote cell differentiation, rather than to direct differentiation of MSCs [9, 13, 32]. Tendinitis treatment with eMSCs demonstrated a clinical outcome superior to conventional therapy in horses, as well as improved collagen organisation and decreased fibrosis [1, 12, 28].
While it is common to transplant undifferentiated MSCs, recent studies have demonstrated that targeted partial differentiation results in superior in vitro and clinical outcomes [6, 8, 29, 40]. Additionally, tenogenically induced MSCs have been shown to decrease the risk of ectopic bone formation following transplantation [19]. Tenogenic differentiation of equine mesenchymal stem cells in vitro is frequently accomplished by supplementing the culture medium with growth and differentiation factors such as bone morphogenetic protein/growth differentiation factor (BMP/GDF) and TGF family proteins [18, 32]. However, no standard protocol for tendon differentiation exists, and the tenocyte differentiation efficiency is still limited [22].

In vivo embryonic development has been used to gain a better understanding of mesoderm differentiation and to develop protocols for in vitro differentiation [36]. During embryonic development, osteochondroprogenitor cells are formed, and these cells can differentiate into osteogenic (bone) or tenochondrogenic progenitor cells. Tenochondrogenic progenitor cells can subsequently differentiate into progenitors of chondrocytes (cartilage) or tenocytes (tendon) [3, 7]. Notably, mesoderm differentiation is regulated by signalling pathways, including the Wingless and Int-1 (WNT)/β-catenin, Notch, and BMP/GDF signalling pathways [5, 36]. The WNT/β-catenin signalling pathway has been shown to inhibit tenogenesis [17] and chondrogenesis [14, 17] but to promote osteogenesis [16, 21]. Conversely, BMP/GDF signalling promotes tenogenesis [15, 27].

Our hypothesis was that increasing the tenochondrogenic progenitor and then stimulating the tenocyte lineage would enhance eMSC tenogenic differentiation. Inhibition of WNT signalling may be used to increase tenochondrogenic progenitors, whereas stimulation of the BMP/GDF signalling pathway may be used to promote tenogenic differentiation. The objective of this study was to determine whether the combination of a WNT inhibitor and BMP/GDF stimulator could enhance the differentiation of bone marrow-derived equine mesenchymal stromal cells (BM-eMSCs) into tendon. In this experiment, we used KY02111 as the WNT/β-catenin signalling pathway inhibitor [24] and BMP12 (also known as GDF7) as the BMP/GDF signalling pathway stimulator [32]. BMP12 has been shown in the majority of previous studies to promote tenogenic differentiation [15, 27].

**Materials and Methods**

This research project was approved by the Animal Care and Use Committee of the Faculty of Mahidol University. eMSC culture

BM-eMSCs in this experiment were obtained from a stem cell bank at Mahidol University. These cells have already been characterised and transplanted by our research team [18, 42]. Flow cytometry showed that they expressed for CD29 (90%), CD90 (90%), CD14 (14%), CD34 (2%), CD44 (4%), CD45 (0.5%), CD73 (11%), and CD105 (3%). PCR revealed that they are positive for CD90, POU5F1, CD14, and CD44 but negative for CD105, NANOG, SOX2, CD79a, and DRB. They were capable of differentiating into osteocytes, chondrocytes, adipocytes, and tenocytes [18]. This study was done in a period similar to the above two previous reports [18, 42].

Frozen eMSCs from passage 3 were used. Cell culture was performed in accordance with a previously published protocol [18, 42]. Briefly, cells were thawed at 37°C and then washed in a 15 ml tube with 5 ml culture medium (Minimum Essential Medium plus 10% foetal bovine serum; FBS). They were subsequently centrifuged at 1,500 × g for 5 min, and the supernatant was then removed. The pellet was resuspended in 5 ml of culture medium and then transferred to a T25 flask and cultured in a humidified incubator with 5% CO₂ at 37°C. The three MSCs lines used in this study were isolated from three thoroughbred horses. Each cell line was tested in triplicate.

**Strategy for tenogenic differentiation**

A summary of the strategy is shown in Fig. 1. First, dexamethasone, ascorbic acid, and glucose were added to the differentiation medium to promote osteochondrogenic differentiation [4, 10, 35, 45], followed by the addition of KY02111, the WNT inhibitor, to inhibit osteogenic differentiation but induce tenochondrogenic differentiation. Finally, BMP12 was introduced to promote tenogenic differentiation [15, 27].

The differentiation medium comprised Dulbecco’s Modified Eagle Medium with high glucose (Thermo Fisher Scientific, Waltham, MA, U.S.A.), 10% FBS (Sigma-Aldrich, St. Louis, MO, U.S.A.), 0.1 μM dexamethasone (Sigma-Aldrich), 50 μg/ml ascorbic acid (Sigma-Aldrich), and 1% antibiotic-antimycotic solution (Thermo Fisher Scientific). Additionally, 10 μM KY02111 (Selleckchem, Houston, TX, U.S.A.) and 200 ng/ml BMP12 (PeproTech, Cranbury, NJ, U.S.A.) were added at various time points during the experiment. eMSCs were seeded in 35 mm culture dishes at a density of 3 × 10⁴ of cells and incubated in a humidified atmosphere containing 5% CO₂ at 37°C. The medium was replaced with fresh medium every 3 days.

**Experimental design**

The eMSCs were divided into the following five groups (Fig. 2): 1) control group, in which cells were cultured in
differentiation medium without BMP12 and KY02111; 2) BMP group, in which cells were cultured in differentiation medium containing BMP12; 3) BMPKY1 group, in which cells were cultured in differentiation medium containing KY02111 for 3 days and BMP12 for 12 days; and 5) BMPKY5 group, in which cells were cultured in differentiation medium containing KY02111 for 5 days and BMP12 for 10 days. On day 15 (the end of cell culture), cells were harvested for gene and protein expression analysis.

Analysis of tenogenic markers
On days 14 to 15, BM-eMSCs were collected for the purpose of determining particular tenogenic markers. Gene expression of Tenascin-C (TNC), Decorin (DCN), Scleraxis (SCX), and Tenomodulin (TNMD) was detected and quantified using quantitative polymerase chain reaction (qPCR), while type I collagen (COL1) protein expression was observed and quantified using immunofluorescence microscopy.

RNA extraction and cDNA synthesis
To study gene expression, total RNA was extracted from eMSCs using a column-based nucleic acid extraction kit (Quick-RNA™, Zymo Research, Irvine, CA, U.S.A.). After extraction, the quantity and quality of RNA were determined with a Nanodrop spectrophotometer (Thermo Fisher Scientific). RNA was converted to cDNA using a SuperScript® VILO™ cDNA Synthesis Kit, according to the manufacturer’s instructions (Thermo Fisher Scientific).

qPCR
The qPCR was performed according to our previous studies [38, 42]. Briefly, qPCR was carried out on a Rotor-Gene Q instrument (Qiagen, Hilden, Germany). The PCR buffer was KAPA SYBR® Fast qPCR Master Mix (Kapa Biosystems, Wilmington, MA, U.S.A.). The following qPCR reaction protocol was used: Step 1 was pre-denaturation at 95°C for 1 min. Step 2 was cycling at 95°C for 5 sec (denaturation) and 60°C for 20 sec (annealing); this step was performed for 35 cycles. Step 3 was a final extension at 72°C for 3 min. Step 4 was post-PCR melting, in which the temperature was increased from 72°C to 99°C in 1-degree increments with a hold for 15 sec on the first step and for 4 sec on each subsequent step. The Rotor-Gene Q software was used to analyse the melting curve and measure comparative gene expression. Comparative gene expression was normalised with a reference gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and set relative
to the control group. The PCR reaction was performed in triplicate. Primer sequences are shown in Table 1.

**Immunofluorescence assays**

After 14 days of differentiation (Fig. 2), cells in each group were sub-cultured at an 80% confluence density in a chamber slide (Nalgene, Rochester, NY, U.S.A.), and culture was continued for 24 hr. Cells were fixed in 4% paraformaldehyde in phosphate buffer saline (PBS) for 10 min at room temperature and then washed twice with PBS for 5 min. Next, they were permeabilised with 0.3% Triton X-100 (Sigma-Aldrich) in PBS for 5 min and washed with PBS for 5 min. To block non-specific binding, cells were incubated with 3% bovine serum albumin (BSA; Sigma-Aldrich) in PBS at room temperature for an hour. After that, they were incubated overnight at room temperature with a primary antibody, mouse anti-collagen type I (1:500) antibodies (Millipore, CA, U.S.A.), in 1.5% BSA and then washed twice with PBS for 5 min. The cells were then incubated with a secondary antibody conjugated with Alexa Fluor 488 (goat anti-mouse; Abcam, Cambridge, U.K.) for 1 hr at 4°C in the dark. Following that, the unbound antibodies were removed by washing twice with 0.03% Triton X-100 in PBS at room temperature for 5 min. A coverslip was mounted with ProLong Gold Antifade Mountant with DAPI medium (Thermo Fisher Scientific). The stained cells were imaged and counted using a model BX51 fluorescence microscope (Olympus, Tokyo, Japan). Cells in three visual fields were counted and calculated as the percentage of positive (collagen type I) cells.

**Differentiation score**

In order to compare the treatments, we developed the following differentiation scoring system: the highest expression received a score of 3 (statistically different from expression scored as 2), medium expression received a score of 2 (statistically different from expression scored as 1), lowest expression received a score of 1, and no expression received a score of 0.

**Statistical analysis**

Mean ± standard error (SE) values were calculated for gene expression (TNC, DCN, SCX, and TNMD). Statistically significant differences in gene expression among the experimental groups were determined using repeated measures ANOVA followed by multiple comparisons using the Tukey post hoc test. Protein expression (COL1) is expressed as the percentage of positive cells, and the statistical significance of differences was calculated using the chi-square test. Differences with a $P$-value of $<0.05$ were considered statistically significant. The statistical analysis was performed using jamovi version 1.8.1, which is a publicly downloadable tool (https://www.jamovi.org).

**Results**

Gene and protein expression levels of tenogenic markers are shown in Table 2. Differentiation of eMSCs with the BMPKY5 group (incubated with KY02111 for 5 days) resulted in a significant increase ($P<0.05$) in TNC and DCN gene expression compared with the other groups. SCX and TNMD gene expression was significantly higher in the BMPKY1 group (incubated with KY02111 for 1 day) than in the other groups ($P<0.05$).

For COL1 staining (Fig. 3), the number of positive cells was significantly higher ($P<0.05$) in the treatments that contained KY02111 and BMP12 (BMPKY1, BMPKY3, and BMPKY5) than in the BMP and control groups.

The findings indicate that BMP12 or a combination of BMP12 and KY02111 promotes tendon differentiation more effectively than the control culture. The score for tendon differentiation was shown in Table 3, and the best score was obtained when the BMPKY1 treatment was used (KY02111 treatment for 1 day followed by 14 days of BMP12 treatment).

**Discussion**

We demonstrated in this study that a combination of KY02111 and BMP12 could enhance the tenogenic differentiation of eMSCs more than BMP12 alone. Supplementation with the WNT inhibitor, KY02111, for 1 day and then BMP12 for 14 days resulted in the highest tenogenic differentiation score in this experiment.

Other WNT inhibitors, such as SM04755 and IWR1, have been shown to induce tenogenic differentiation in a
SMALL MOLECULES INDUCE BM-EMSC DIFFERENTIATION

SM04755 has been shown to increase the expression of tenogenic markers, such as SCX, TNMD, TNC, Mohawk (MKX), thrombospondin 4 (THBS4), and type I and type III collagens [11], while IWR1 has been shown to increase the expression MKX, TNMD, and SCX [17]. Conversely, WNT stimulators such as BIO have been reported to reduce the expression of SCX, MKX, and TNMD in monolayer cultures of rat tendon cells, eMSCs, and human MSCs [17, 25]. The current study also demonstrated that the incubation time for the WNT inhibitor is critical because 1 day of incubation seemed superior to 3 or 5 days. This result could be explained by a previous study that reported that inhibiting the WNT signalling pathway for an extended period results in the apoptosis of rat tendon stem cells [41].

Apart from WNT pathway molecules, the roles of other small molecules in tendon differentiation have been investigated. For example, a combination of Oxo-M and 4-PPBP (FAK and ERK1/2 signalling pathway) synergistically increases the expressions of tenogenic markers (Col-I, Col III, Tn-C, Vim, Tnm, Scx) in tendon stem/progenitor cells (TSCs) [37]. Thus, not only is it essential to use small molecules to improve tendon differentiation for stem cell transplantation, but it also might be possible to use certain molecules as drugs to promote tendon regeneration following injury [11].

The present study and other previous studies found that BMPs play a crucial role in tendon development and regeneration. For instance, our study and others have demonstrated that BMP12 can promote tenogenic differentiation [15, 27] by stimulating SCX expression [20] via the Smad1/5/8 pathway [31]. Overexpression of BMP12 can promote tendon regeneration and formation [23]. Other BMP members, such as BMP-2 and BMP-7, also play a critical role in tendon healing via Smad pathways [44]. Among tenogenic markers, a transcription factor SCX is considered an early marker for tendon differentiation. SCX can directly induce the expression of other makers, such as TNMD, COL1, and DCN [33, 43], as well as indirectly stimulate TNC expression [26].

In conclusion, we propose that the use of a combination of WNT inhibitor (KY02111) and BMP stimulator (BMP12) along with the optimal incubation time can enhance tenogenic differentiation more than BMP12 alone. Further study is required to improve the differentiation protocol, including

Table 2. Comparison of gene expression and percentages of positive cells for tenogenic markers among treatments

| Group    | Comparative gene expression (Mean ± SE) | Percentage of positive cells |
|----------|----------------------------------------|-----------------------------|
|          | TNMD        | SCX          | DCN          | TNC          | COL1          |                        |
| Control  | 1.00 ± 0.00a | 1.00 ± 0.00a | 1.00 ± 0.00a | 1.00 ± 0.00a | 0.00% (0/153)a |
| BMP      | 11.58 ± 4.34b| 1.36 ± 0.21a | 1.53 ± 0.12a | 1.43 ± 0.17a | 50.32% (78/155)b |
| BMPKY1   | 52.95 ± 19.38c| 2.16 ± 0.36b | 1.60 ± 0.12c | 1.58 ± 0.15b | 53.72% (65/121)c |
| BMPKY3   | 1.81 ± 0.63a | 0.98 ± 0.05a | 1.36 ± 0.31a | 1.26 ± 0.19a | 65.65% (86/131)c |
| BMPKY5   | 1.55 ± 0.04a | 0.86 ± 0.07a | 3.32 ± 0.51b | 3.27 ± 0.37b | 55.13% (86/156)c |

Average gene expression was calculated for the three cell lines and repeated in triplicate. *c*Significant difference (P<0.05). TNMD: Tenomodulin; SCX: Scleraxis; DCN: Decorin; TNC: Tenascin-C; COL1: type I collagen.

Table 3. Comparison of differentiation scores for tenogenic marker expression among treatments

|          | TNMD | SCX  | DCN  | TNC  | COL1 | Total |
|----------|------|------|------|------|------|-------|
| Control  | 1    | 2    | 2    | 2    | 0    | 9     |
| BMP      | 2    | 2    | 2    | 2    | 2    | 10    |
| BMPKY1   | 3    | 3    | 2    | 2    | 3    | 13    |
| BMPKY3   | 1    | 2    | 2    | 3    | 3    | 8     |
| BMPKY5   | 1    | 2    | 3    | 3    | 3    | 12    |

The highest expression received 3 points, medium expression received 2 points, the lowest expression received 1 point, and no expression received 0 points. TNMD: Tenomodulin; SCX: Scleraxis; DCN: Decorin; TNC: Tenascin-C; COL1: type I collagen.
the determination of the optimal concentrations of KY02111 and BMP12, investigation of the effect of diverse cell lines, and comparison of KY02111 with other molecules.

Acknowledgments

This project was not financially funded by any agency. All materials were provided by the lab of Tuempeng Wongtawan in the Faculty of Veterinary Science and the lab of Aungkura Supokawej in the Faculty of Medical Science, Mahidol University.

References

1. Ahrberg, A.B., Horstmeier, C., Berner, D., Brehm, W., Gittel, C., Hillmann, A., Josten, C., Rossi, G., Schubert, S., Winter, K., and Burk, J. 2018. Effects of mesenchymal stromal cells versus serum on tendon healing in a controlled experimental trial in an equine model. BMC Musculoskelet. Disord. 19: 230. [Medline] [CrossRef]

2. Alves, A.G., Stewart, A.A., Dudhia, J., Kasashima, Y., Godship, A.E., and Smith, R.K. 2011. Cell-based therapies for tendon and ligament injuries. Vet. Clin. North Am. Equine Pract. 27: 315–333. [Medline] [CrossRef]

3. Arai, H.N., Sato, F., Yamamoto, T., Woljtjen, K., Kiyonari, H., Yoshimoto, Y., Shukunami, C., Akiyama, H., Kist, R., and Sehara-Fujisawa, A. 2019. Metalloprotease-dependent attenuation of BMP signaling restricts cardiac neural crest cell fate. Cell Rep. 29: 603–616.e5. [Medline] [CrossRef]

4. Barfian, A., Judavisastra, H., Alfarafisa, N.M., Wibowo, U.A., and Rosadi, I. 2018. Chondrogenic differentiation of adipose-derived mesenchymal stem cells induced by L-ascorbic acid and platelet rich plasma on silk fibroin scaffold. PeerJ 6: e5809. [Medline] [CrossRef]

5. Basson, M.A. 2012. Signaling in cell differentiation and morphogenesis. Cold Spring Harb. Perspect. Biol. 4: a008151. [Medline] [CrossRef]

6. Beerts, C., Suls, M., Broeckx, S.Y., Seys, B., Vandenberghe, A., Declercq, J., Duchateau, L., Vidal, M.A., and Spaas, J.H. 2017. Tenogenically induced allogeneic peripheral blood mesenchymal stem cells in allogeneic platelet-rich plasma: 2-year follow-up after tendon or ligament treatment in horses. Front. Vet. Sci. 4: 158. [Medline] [CrossRef]

7. Blake, J.A., and Ziman, M.R. 2014. Pax genes: regulators of lineage specification and progenitor cell maintenance. Development 141: 737–751. [Medline] [CrossRef]

8. Broeckx, S.Y., Spaas, J.H., Chiers, K., Duchateau, L., Van Hecke, I., Van Brantegem, L., Dumoulin, M., Martens, A.M., and Pille, F. 2018. Equine allogeneic chondrogenic induced mesenchymal stem cells: a GCP target animal safety and biodistribution study. Res. Vet. Sci. 117: 246–254. [Medline] [CrossRef]

9. Costa-Almeida, R., Calejo, I., and Gomes, M.E. 2019. Mesenchymal stem cells empowering tendon regenerative therapies. Int. J. Mol. Sci. 20: 3002. [Medline] [CrossRef]

10. Derfoul, A., Perkins, G.L., Hall, D.J., and Tuan, R.S. 2006. Glucocorticoids promote chondrogenic differentiation of adult human mesenchymal stem cells by enhancing expression of cartilage extracellular matrix genes. Stem Cells 24: 1487–1495. [Medline] [CrossRef]

11. Deshmukh, V., Seo, T., O’Green, A.L.L., Ibanez, M., Hofliena, B., Kc, S., Stewart, J., Dellamary, L., Chiu, K., Ghias, A., Barroga, C., Kennedy, S., Tambiah, J., Hood, J., and Yazici, Y. 2021. SM04755, a small-molecule inhibitor of the Wnt pathway, as a potential topical treatment for tendinopathy. J. Orthop. Res. 39: 2048–2061. [Medline] [CrossRef]

12. Godwin, E.E., Young, N.J., Dudhia, J., Beamish, I.C., and Smith, R.K.W. 2012. 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. 44: 25–32. [Medline] [CrossRef]

13. Gugjoo, M.B., Amarpal, Makhdoomi, D.M., and Sharma, G.T. 2019. Equine mesenchymal stem cells: properties, sources, characterization, and potential therapeutic applications. J. Equine Vet. Sci. 72: 16–27. [Medline] [CrossRef]

14. Huang, X., Zhong, L., Hendriks, J., and Karperien, M. 2018. The effects of the WNT-signaling modulators BIO and PKF118-310 on the chondrogenic differentiation of human mesenchymal stem cells. Int. J. Mol. Sci. 19: 561. [Medline] [CrossRef]

15. Jelinsky, S.A., Li, L., Ellis, D., Archambault, J., Li, J., St Andre, M., Morris, C., and Seeherman, H. 2011. Treatment with rhBMP12 or rhBMP13 increase the rate and the quality of rat Achilles tendon repair. J. Orthop. Res. 29: 1604–1612. [Medline] [CrossRef]

16. Kim, J.H., Liu, X., Wang, J., Chen, X., Zhang, H., Kim, S.H., Cui, J., Li, R., Zhang, W., Kong, Y., Zhang, J., Shui, W., Lamplot, J., Rogers, M.R., Zhao, C., Wang, N., Rajan, P., Tomal, J., Statz, J., Wu, N., Luu, H.H., Haydon, R.C., and He, T.C. 2013. Wnt signaling in bone formation and its therapeutic potential for bone diseases. Ther. Adv. Musculoskelet. Dis. 5: 13–31. [Medline] [CrossRef]

17. Kishimoto, Y., Ohkawara, B., Sakai, T., Ito, M., Masuda, A., Ishiguro, N., Shukunami, C., Docheva, D., and Ohno, K. 2017. Wnt/β-catenin signaling suppresses expressions of Scx, Mkx, and Tnmd in tendon-derived cells. J. Orthop. Res. 39: e0182051. [Medline] [CrossRef]

18. Korchunjit, W., Laikul, A., Taylor, J., Watchrarat, K., Korchunjit, W., Korochunjit and T. Wongtawan, A. SUPOKAWEJ, W. KORCHUNJIT AND T. WONGTAWAN

19. Kusuma, G.D., Menicanin, D., Gronthos, S., Manuelpillai,
Norelli, J.B., Plaza, D.P., Stal, D.N., Varghese, A.M., Liang, H., and Grande, D.A. 2018. Tenogenically differentiated adipose-derived stem cells are effective in Achilles tendon repair in vivo. J. Tissue Eng. 9: 2041731418811183. [Medline] [CrossRef]

Patterson-Kane, J.C., and Rich, T. 2014. Achilles tendon injuries in elite athletes: lessons in pathophysiology from their equine counterparts. ILAR J. 55: 86–99. [Medline] [CrossRef]

Shen, H., Gelberman, R.H., Silva, M.J., Sakiyama-Elbert, S.E., and Thomopoulos, S. 2013. BMP12 induces tenogenic differentiation of adipose-derivedstromal cells. PLoS One 8: e77613. [Medline] [CrossRef]

Shojaei, A., and Parham, A. 2019. Strategies of tenogenic differentiation of equine stem cells for tendon repair: current status and challenges. Stem Cell Res. Ther. 10: 181. [Medline] [CrossRef]

Smith, R.K.W., Werling, N.J., Dakin, S.G., Alam, R., Goodship, A.E., and Dudhia, J. 2013. Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. PLoS One 8: e75697. [Medline] [CrossRef]

Sun, C., Lan, W., Li, B., Zuo, R., Xing, H., Liu, M., Li, J., Yao, Y., Wu, J., Tang, Y., Liu, H., and Zhou, Y. 2019. Glucose regulates tissue-specific chondro-osteoogenic differentiation of human cartilage endplate stem cells via O-GlcNAcylation of Sox9 and Runx2. Stem Cell Res. Ther. 10: 357. [Medline] [CrossRef]

Tani, S., Chung, U.I., Obha, S., and Hojo, H. 2020. Understanding paraxial mesodermal development and sclerotome specification for skeletal repair. Exp. Mol. Med. 52: 1166–1177. [Medline] [CrossRef]

Tarfider, S., Ricupero, C., Minhas, S., Yu, R.J., Alex, A.D., and Lee, C.H. 2019. A combination of Oxo-M and 4-PPBP as a potential regenerative therapeutics for tendon injury. Theranostics 9: 4241–4254. [Medline] [CrossRef]

Tesena, P., Korchunjit, W., Taylor, J., and Wongtawan, T. 2017. Comparison of commercial RNA extraction kits and qPCR master mixes for studying gene expression in small biopsy tissue samples from the equine gastric epithelium. J. Equine Sci. 28: 135–141. [Medline] [CrossRef]

Thorpe, C.T., Clegg, P.D., and Birch, H.L. 2010. A review of tendon injury: why is the equine superficial digital flexor tendon most at risk? Equine Vet. J. 42: 174–180. [Medline] [CrossRef]

Vandenberghe, A., Broeckx, S.Y., Beerts, C., Seys, B., Zimmerman, M., Verweire, I., Suls, M., and Spas, J.H. 2015. Tenogenically induced allogeneic mesenchymal stem cells for the treatment of proximal suspensory ligation desmitis in a horse. Front. Vet. Sci. 2: 49. [Medline]
41. Wang, Y., Tang, H., He, G., Shi, Y., Kang, X., Lyu, J., Zhou, M., Zhu, M., Zhang, J., and Tang, K. 2018. High concentration of aspirin induces apoptosis in rat tendon stem cells via inhibition of the Wnt/β-catenin pathway. Cell. Physiol. Biochem. 50: 2046–2059. [Medline] [CrossRef]

42. Watchrarat, K., Korchunjit, W., Buranasinsup, S., Taylor, J., Ritruechai, P., and Wongtawan, T. 2017. MEM α promotes cell proliferation and expression of bone marrow derived equine mesenchymal stem cell gene markers but depresses differentiation gene markers. J. Equine Vet. Sci. 50: 8–14. [CrossRef]

43. Yoshimoto, Y., Takimoto, A., Watanabe, H., Hiraki, Y., Kondoh, G., and Shukunami, C. 2017. Scleraxis is required for maturation of tissue domains for proper integration of the musculoskeletal system. Sci. Rep. 7: 45010. [Medline] [CrossRef]

44. Yu, Y., Bliss, J.P., Bruce, W.J.M., and Walsh, W.R. 2007. Bone morphogenetic proteins and Smad expression in ovine tendon-bone healing. Arthroscopy 23: 205–210. [Medline] [CrossRef]

45. Yuasa, M., Yamada, T., Taniyama, T., Masaoka, T., Xuetao, W., Yoshii, T., Horie, M., Yasuda, H., Uemura, T., Okawa, A., and Sotome, S. 2015. Dexamethasone enhances osteogenic differentiation of bone marrow- and muscle-derived stromal cells and augments ectopic bone formation induced by bone morphogenetic protein-2. PLoS One 10: e0116462. [Medline] [CrossRef]