Distinct Effects of Platelet-Rich Plasma and BMP13 on Rotator Cuff Tendon Injury Healing in a Rat Model

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Distinct Effects of Platelet-Rich Plasma and BMP13 on Rotator Cuff Tendon Injury Healing in a Rat Model


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Background: Although platelet-rich plasma (PRP) is used clinically to augment tendon healing, bone morphogenetic protein–13 (BMP13) may provide a better therapeutic avenue to improve early tendon healing and repair.

Hypothesis: Exogenous expression of BMP13 in tenocytes will up-regulate genes involved in tendon healing. Direct delivery of adenovirus-mediated BMP13 (AdBMP13) into the injured rat supraspinatus tendon will increase biomechanical properties.

Study Design: Controlled laboratory study.

Methods: Exogenous expression of BMP13 and the major growth factors in PRP (transforming growth factor–β1 [TGF–β1], vascular endothelial growth factor–A [VEGF–A], and platelet-derived growth factor–BB [PDGF–BB]) was accomplished by using recombinant adenoviral vectors. The expression of tendon- and matrix-associated genes in growth factor–treated tenocytes was analyzed by use of semiquantitative reverse-transcription polymerase chain reaction. A total of 32 rats with supraspinatus defect were divided into 4 groups and injected with adenovirus-containing green fluorescent protein (AdGFP; negative control), PRP, AdBMP13, or PRP + AdBMP13. All rats were sacrificed at 2 weeks after surgery, and tendons were harvested for biomechanical testing and histologic analysis.

Results: BMP13 up-regulated type III collagen expression compared with AdGFP control and PRP growth factors (P < .01). BMP13 and PRP growth factors each up-regulated fibronectin expression (P < .01). There was an increase in stress to failure in each of the 3 treatment groups (P < .05 for PRP; P < .01 for AdBMP13 or PRP + AdBMP13) compared with AdGFP control. AdBMP13 demonstrated higher stress to failure than did the PRPs (P < .01). The addition of PRP did not increase the BMP13-enhanced stress to failure or stiffness. The biomechanical results were further supported by histologic analysis of the retrieved samples.

Conclusion: Exogenous expression of BMP13 enhances tendon healing more effectively than PRP as assessed by tendon- and matrix-associated gene expression, biomechanical testing, and histologic analysis.

Clinical Relevance: While PRP is used in the clinical setting, BMP13 may be explored as a superior biofactor to improve rotator cuff tendon healing and reduce the incidence of retears.

Keywords: platelet-rich plasma; PRP; BMP13; bone morphogenetic protein; rotator cuff

Rotator cuff tears are a common cause of pain and disability. While surgical repair has been shown to improve pain, strength, and function, there are high rates of recurrent defects after repair. A recent study by Iannotti et al demonstrated that the majority of retears after arthroscopic rotator cuff repair occur between 6 and 26 weeks. Tissue engineering strategies may enhance the biological environment of rotator cuff healing and reduce the incidence of retears after surgical repair.

Tendon healing occurs via a scar-mediated pathway and does not regenerate the native tendon-bone interface that was formed during prenatal development. Instead, the fibrovascular scar formed at the repair interface is mechanically weaker than the native insertion site and more prone to failure. This scarring occurs via a...
3-phase process of inflammation, repair, and remodeling.22
Kobayashi et al17 and Wurrgler-Hauri et al35 examined the expression of growth factors during the healing of acute rotator cuff tears, and these studies suggest that the cytokines involved in tendon healing play major roles in cell proliferation, chemotaxis, differentiation, and matrix synthesis. By manipulating the critical signaling pathways of tendon healing, it may be possible to more closely recreate the native tendon insertion site that was formed during embryologic development and thereby improve rotator cuff tendon healing.

Platelet-rich plasma (PRP) is an autologous concentration of platelets and growth factors including transforming growth factor–β (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor–2 (FGF-2), and insulin-like growth factor–1 (IGF-1).1 The increased concentration and release of growth factors within PRP may enhance the recruitment and proliferation of tenocytes, stem cells, and endothelial cells.10,18,23,51 Platelet-rich plasma has been used for orthopaedic indications including intraoperative augmentation of rotator cuff repairs. However, several recent studies have demonstrated varying results, and definitive functional improvement has not been consistently proven.31,48 Furthermore, a recent study by Beck et al4 demonstrated that PRP augmentation of rotator cuff repairs in a rat model decreased tendon tissue stiffness acutely and failed to enhance tendon-to-bone healing. These findings suggest that alternative strategies for biological augmentation of rotator cuff repairs should be pursued.

Bone morphogenetic proteins (BMPs) are members of the TGF-β superfamily of more than 20 proteins with critical roles in bone, cartilage, tendon, and ligament development and regeneration.23,34,41 BMP12, BMP13, and BMP14 have each demonstrated the ability to promote formation of tendon after ectopic injection in rats, and several studies have demonstrated that these proteins may improve tendon healing in animal models.24,25,17 Studies have shown that BMP13 is the most tenogenic and least osteogenic among the group.12,17 To our knowledge, no studies have compared the in vivo and in vitro effects of BMP13 and PRP on rotator cuff healing. The purpose of this study was to examine the molecular effects of exogenous delivery of adenovirus-mediated BMP13 (AdBMP13) compared with the growth factors found in highest concentrations in PRP (TGF-β1, VEGF, and PDGF) in tenocytes. We also compared the differential effects of PRP and AdBMP13 on the biomechanical properties of injured rat supraspinatus tendons. We hypothesized that exogenous expression of AdBMP13 in tenocytes will up-regulate genes involved in tendon healing and that direct delivery of AdBMP13 into the injured rat supraspinatus tendon will increase biomechanical and histologic properties.

MATERIALS AND METHODS

Cell Culture and Chemicals

The HEK293 cells were purchased from ATCC. Cells were maintained in complete Dulbecco’s modified Eagle’s medium (DMEM) at 37°C. All chemicals were purchased from Fisher Scientific or Sigma-Aldrich unless otherwise noted.

Construction of Recombinant Adenoviruses

Recombinant adenoviruses were generated using the AdEasy system.12,13,27,29,39,39 The coding regions of human BMP13, TGF-β1, VEGF-A, and PDGF-BB were amplified by polymerase chain reaction (PCR) and cloned into an adenoviral shuttle vector to generate recombinant adenoviruses. These recombinant adenoviruses were used to transfect HEK293 cells.11,26 The resulting adenoviruses were designated AdBMP13, AdTGF-β1, AdVEGF-A, and AdPDGF-BB. Adenovirus-containing green fluorescent protein (AdGFP) was used as a negative control, as previously described.3 AdBMP13 also expresses GFP, while AdTGF-β1, AdVEGF-A, and AdPDGF-BB also express red fluorescent protein. Cloning details are available on request.

Isolation and Treatment of Tenocytes

Tenocytes were isolated from the Achilles tendon of 4-week-old CD-1 mice (Figure 1A).3 Briefly, the Achilles tendon was first carefully transected distal to the musculo-tendinous junction and then transected proximal to its bony insertion at the calcaneus. The tendon sheath and surrounding paratenon were stripped off. The tendons were minced into small pieces and digested in phosphate-buffered saline (PBS) with 1% penicillin-streptomycin solution with 3 mg/mL collagenase type I (Roche) and 4 mg/mL dispase (Roche) for 1 hour. Isolated cells were plated in 6-well plates in complete DMEM supplemented with 10% fetal bovine serum (FBS) and incubated at 37°C, 95% humidified air, and 5% CO2. Cell colonies were visible at 7 days, and at 14 days tenocytes were passaged by enzymatic digestion (0.1% trypsin) to 25-cm2 flasks containing DMEM supplemented with 10% FBS (Figure 1B). Cells were plated at equal confluency and treated with AdBMP13, AdTGF-β1, AdVEGF-A, AdPDGF-BB, or AdGFP. Equal efficiency of adenoviral transduction for each treatment was achieved by dose titration and direct visualization under fluorescence imaging (Figure 1C).

RNA Isolation and Semiquantitative Reverse-Transcription PCR Analysis

The analysis using semiquantitative reverse-transcription PCR (sqPCR) was performed as previously described.6,9,34,40,49,51,52,61 Briefly, total RNA was isolated from tenocytes at 5 days after infection with the indicated adenovirus using TRIZOL reagents (Invitrogen). The complementary DNA (cDNA) products were generated for sqPCR analysis. Gene expression levels were normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and amplified with mouse gene-specific primers.

References

1. References 6, 12, 24, 25, 29, 33, 45, 49, 52, 60, 61.
2. References 6, 11, 26-29, 33, 34, 46, 49, 52, 61.
(Table 1). Products of the PCR were resolved and visualized on 1.5% agarose gels. The sqPCR data were analyzed by densitometry of gel bands using ImageJ software (National Institutes of Health; http://rsbweb.nih.gov/ij/download.html) by a second independent technician blinded to sample groups using random number assignment.

In Vitro AdBMP13 Transduction in Rat Supraspinatus Tendon

Two donor Sprague-Dawley rats were used to demonstrate continuing BMP13 or GFP expression in vitro. The supraspinatus tendon was exposed through a deltoid-splitting approach followed by division of the acromioclavicular joint. Each tendon was meticulously dissected from surrounding tissues and first removed from the humeral insertion under magnification. The supraspinatus muscle was then removed from the supraspinatus tendon under magnification. Harvested tendons were cultured in vitro in complete DMEM with 10% FBS after direct injection with 10 μL (10^8 plaque-forming units [pfu] per injection) of AdBMP13 or AdGFP.

PRP Preparation

The protocol for the preparation of platelet concentrate was adapted from Aspenberg and Virchenko. Briefly, a single donor rat was used to harvest PRP for 3 recipient rats. Blood was harvested by an open thoracotomy and intracardiac puncture into the left ventricle of the anesthetized Sprague-Dawley rats. Approximately 5 to 8 mL of whole blood was withdrawn from each specimen with a 22-gauge needle into a syringe containing 0.25 mL of
TABLE 1
Primers for Reverse-Transcription Polymerase Chain Reaction of Tenocyte Gene Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5'-3')</th>
<th>Reverse Primer (5'-3')</th>
<th>Amplicon Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen type III</td>
<td>GCACACGCAGCTCAAGATGAGA</td>
<td>TCTCCAAATGGGATCTTGG</td>
<td>185bp</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>AATGAAAAGGGATGGAC</td>
<td>CTGGTGTCCCTTCGGTCCT</td>
<td>244bp</td>
</tr>
<tr>
<td>Scleraxis</td>
<td>CTGGCCTCCAGTCAATTCTT</td>
<td>GTCACGGCTCTGGCTCAACTT</td>
<td>237bp</td>
</tr>
<tr>
<td>Tenascin C</td>
<td>CCAATGAAAGGATCGGAAGAA</td>
<td>TCATGACGTCTGACTCCAC</td>
<td>226bp</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)</td>
<td>ACCCGAGAGACTGTTGGATGG</td>
<td>CACATTGGGGGTAGGAACAC</td>
<td>171bp</td>
</tr>
</tbody>
</table>

Surgical Procedure

All animal-related experiments in this study followed the animal use and care guidelines approved by the Institutional Animal Care and Use Committee. A total of 32 Sprague-Dawley rats (weight, 250-300 g) were divided into 4 groups of 8 rats: AdGFP control, PRP, AdBMP13, and PRP+AdBMP13. Animals were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) and maintained with 2% isoflurane (Baxter). Each rat underwent right-sided shoulder surgery by use of sterile technique in the lateral decubitus position. Left shoulders were not operated on, and the intact tendons served as additional comparisons. The supraspinatus tendon was exposed through a deltoid-splitting approach followed by division of the acromioclavicular joint. A 2 × 2-mm defect was created using a sterile punch (Miltex) at the insertion of the supraspinatus tendon (Figure 2). Under direct visualization, the supraspinatus tendon at the defect was injected with either 10 μL of AdGFP (10⁸ pfu), 10 μL of PRP (10⁹ platelets/mL), 10 μL of AdBMP13 (10⁸ pfu), or 10 μL of PRP (10⁹ platelets/mL) + 10 μL of AdBMP13 (10⁸ pfu). The dose of each adenovirus was based on pilot studies using this same rat rotator cuff model and previously published studies using AdBMP13 and AdGFP that demonstrated a negligible inflammatory response and sufficient, sustained adenoviral-mediated growth factor overexpression for at least 2 weeks. Dosing of PRP was based on previous studies using PRP. The wound was closed in standard layered fashion. One surgeon created the supraspinatus tendon defect, a laboratory assistant prepared all injectables, and a second surgeon injected all rats and was blinded to the substance being injected. Buprenorphine (50 μg/kg) was administered subcutaneously during the postoperative period for analgesia. The rats were allowed to walk freely in their cages with equal access to food and water and were monitored on a daily basis until they were sacrificed.

Biomechanical Testing

All rats were sacrificed at 2 weeks, and all supraspinatus tendons (6 rats from each group) were harvested for biomechanical testing, including 8 untreated, intact tendons from the contralateral (left) shoulder. The technician harvesting the tendons was blinded as to which experimental groups the rats belonged. The humeral insertion of each tendon was left intact and meticulously dissected from surrounding tissues. The supraspinatus muscle was then removed from the supraspinatus tendon under magnification. The humerus was embedded in polymethylmethacrylate (PMMA) to prevent fracture through the humeral physis and placed in a custom-designed testing system with a 500-N load cell for uniaxial tensile testing on the Instron 6500 plus (Instron). The end of each tendon and humerus was secured with sandpaper-lined clamps. Grip-to-grip distance was standardized across all specimens. Each specimen was preloaded to 0.10 N, preconditioned for 10 cycles at a rate 0.03 Hz, and held for 300 seconds. Ramp to failure was then applied at a rate of 0.003 Hz. Maximum stress-to-failure data were recorded for each specimen on an Apple Power Macintosh 8600 computer with an IEEE 488 SCSI interface (MacSCSI 488 IO Tech; IOTech) and stiffness for each specimen calculated from load-deformation curves with Sigma Plot 8.0 (SPSS Inc).

Histologic Evaluation

All rats were sacrificed at 2 weeks, and the supraspinatus tendons and attached humeral head tissues were
harvested from 2 rats in each group. The retrieved samples were fixed in formalin, decalcified, and paraffin embedded. The embedded samples were sectioned and subjected to hematoxylin and eosin (H&E) staining. The histologic analysis of the tendon defect repair sites was performed by 2 blinded pathologists independently.

Statistical Analysis

A power analysis was performed with a primary outcome of biomechanical testing of tendon attachment strength. Our power analysis was based on a pilot study evaluating rotator cuff tendon healing with AdBMP13. In this pilot study, the average ultimate tensile stress to failure was 10.2 ± 2.0 MPa at 2 weeks. A strength increase of 20% between groups was considered clinically significant. With these estimations, a power of 0.80 is achieved using 8 specimens per group with α = 0.05 for biomechanical testing. The Sigma-Stat program (Jandel Scientific) was used to perform the power calculation. All biomechanical outcomes were expressed as mean ± standard deviation. Nonparametric statistical methods (Mann-Whitney U tests) were used for the analysis of the biomechanical data between the experimental (PRP, AdBMP13, PRP+AdBMP13), control (AdGFP), and intact tendon groups because of the non-normal distribution of the data in the groups being compared. SAS statistical software (version 9.1; SAS Corp) was used to perform the analyses. Statistical significance was set with P = .05. Microsoft Excel was used to calculate standard deviations and statistically significant differences of band densitometric analysis of tendon matrix- and gene-associated genes between the experimental (AdBMP13, AdTGF-β1, AdVEGF-A, and AdPDGF-BB) and control (AdGFP) groups using 2-tailed Student t tests as previously described. All in vitro experiments were performed in at least 3 independent experiments.

RESULTS

AdBMP13 Increases Expression of Genes Associated With Tendon Healing

We analyzed the effects of adenoviral delivery of BMP13 and each of the growth factors found in high concentrations in PRP (TGF-β1, VEGF-A, or PDGF-BB) on the expression of 4 major genes involved in the process of tendon healing using sqPCR analysis and densitometric quantification compared with AdGFP control. We first standardized cDNA levels to GAPDH, which demonstrated no quantitative difference between treatment groups and control (P > .20) (Figure 3E).

Only treatment with AdBMP13 up-regulated type III collagen expression compared with AdGFP control (P < .01) (Figure 3B). Furthermore, type III collagen was up-regulated when treated with AdBMP13 compared with each of the PRP growth factors tested (P < .01). Treatment with AdBMP13, AdTGF-β1, AdVEGF-A, or AdPDGF-BB up-regulated fibronectin expression compared with AdGFP control (P < .01) (Figure 3A). Furthermore, fibronectin was more up-regulated when treated with AdBMP13 compared with each of the growth factors of PRP tested (P < .05). Treatment with AdBMP13, AdTGF-β1, or AdVEGF-A significantly up-regulated both tenasin C and scleraxis expression compared with AdPDGF-BB or AdGFP (P < .05) (Figure 3, C and D).}

In AdBMP13-treated tenocytes, we observed 3-, 3.4-, 1.8-, and 2.4-fold increases in type III collagen, fibronectin, tenasin C, and scleraxis, respectively, compared with AdGFP control. Compared with AdPDGF-BB, AdBMP13-treated tenocytes demonstrated 2.4-, 2.2-, 1.8-, and 2.0-fold increases in type III collagen, fibronectin, tenasin C, and scleraxis, respectively.
AdBMP13 and GFP Overexpression Persists at 2 Weeks After Virus Transduction

Tendons were visualized under fluorescence microscopy at 7 and 14 days after harvest and demonstrated GFP signal consistent with continuing gene expression at each time point (Figure 4). These findings suggest that when used in vivo in the rat supraspinatus tendon, the infection and gene overexpression persisted at least until the rats were sacrificed for tendon harvesting at 2 weeks after injection.

Biomechanical Testing and Histologic Evaluation

All tendons were found to fail at the bone-tendon interface, and none failed by intrasubstance tear. No harvested specimens were discarded. The mean stress to failure was higher in the intact, untreated tendons (23.5 ± 1.3 MPa) compared with GFP control (10.1 ± 3.1 MPa; P = .0034), PRP (13.9 ± 1.76 MPa; P = .0034), and BMP13 (18.1 ± 2.8 MPa; P = .013) but not PRP + BMP13 (20.0 ± 4.7 MPa; P = .30) (Figure 5A). Compared with the GFP control, the
mean stress to failure was higher in PRP \( (P = .030) \), BMP13 \( (P = .0034) \), and PRP + BMP13 \( (P = .0053) \). Compared with PRP alone, the mean stress to failure was higher in BMP13 \( (P = .0053) \) and PRP + BMP13 \( (P = .018) \). There was no difference in stress to failure between BMP13 and PRP + BMP13 \( (P = .38) \).

Biomechanical stiffness, or the Young modulus, is defined as the length of tissue deformation (strain) for a given stress; a higher stiffness indicates the ability to withstand a greater stress with the same deformation and is beneficial after tendon healing. The mean stiffness was higher in the intact, untreated tendon \( (27.6 \pm 5.2 \text{ MPa}) \) compared with GFP control \( (10.7 \pm 3.5 \text{ MPa}; P = .0051) \), PRP \( (14.4 \pm 2.9 \text{ MPa}; P = .0034) \), and PRP + BMP13 \( (19.3 \pm 7.2 \text{ MPa}; P = .045) \) but not BMP13 \( (22.7 \pm 7.7 \text{ MPa}; P = .30) \) (Figure 5B). The mean stiffness was higher in the BMP13 and PRP + BMP13 groups compared
with GFP control ($P = .020$ and $0.013$, respectively), but not for the PRP group compared with GFP control ($P = .054$). There was no significant difference in mean stiffness between PRP, BMP13, or PRP+BMP13 treatment groups.

We further examined the histologic characteristics at the tendon injury repair sites. When the retrieved samples were subjected to H&E staining, we found that BMP13 alone promoted effective tendon healing, while GFP control and PRP-only groups showed little or no healing at 2 weeks after injury (Figure 6). Significant healing was observed in the BMP13+PRP group, which may be attributable to the activity of BMP13, although further investigations may be warranted. Nonetheless, the histologic data are largely consistent with and supportive of the biomechanical testing results.

**DISCUSSION**

In this study, we initially used tenocyte gene expression analysis to compare the effects of the major growth factors in PRP and AdBMP13 on tenocytes isolated from the Achilles tendons of CD-1 mice. We observed that in comparison to 3 major growth factors found in PRP, BMP13 up-regulates the expression of tendon cell- and matrix-associated genes known to be critical in the early tendon healing process. With these in vitro findings, we sought to explore the in vivo effects of AdBMP13 compared with PRP in a more clinically translational study. We decided to investigate supraspinatus tendon healing after injury in a small animal injury model that closely resembles the human shoulder joint, selecting a rat model based on previous work by Carpenter and colleagues. To demonstrate effective delivery of growth factor using adenovirus before biomechanical testing, we first showed in vitro that AdBMP13 (and AdGFP control) expression continues for up to 2 weeks after direct injection into the supraspinatus tendon. This provided visual confirmation and validation of successful, sustained adenoviral-mediated delivery of BMP13. Our subsequent biomechanical analysis demonstrated that treatment with PRP and AdBMP13, in combination or alone, increases the stress to failure and stiffness of the supraspinatus tendon during the first 2 weeks after injury compared with GFP control. Furthermore, AdBMP13 increased stress to failure significantly more than PRP alone. Finally, histologic analysis supported our biomechanical findings.

During the early rotator cuff healing response, there is an increase in type III collagen, while in later stages of healing there is an increase in type I collagen. Although type I collagen is the major component of mature tendon extracellular matrix (ECM), type III collagen is found in high concentrations at the insertion sites of highly stressed tendons, including the supraspinatus. Type III collagen has a critical function in providing tensile strength during tendon healing after injury. We demonstrated a significant increase in type III collagen...
production in tenocytes treated with AdBMP13 after 5 days of in vitro incubation compared with control and compared with each of the major growth factors in PRP tested. We also demonstrated a significantly greater stress to failure in AdBMP13-treated and AdBMP13 + PRP-treated tendons in vivo at 2 weeks after treatment compared with control as well as PRP. Compared with injured control tendons, PRP treatment resulted in a 37.6% increase in stress to failure, while AdBMP13 treatment resulted in a 79.2% increase and PRP + AdBMP13 a 98% increase in stress to failure. Furthermore, compared with PRP treatment, AdBMP13 and PRP + AdBMP13 treatment resulted in a 30.2% and a 43.9% increase in stress to failure, respectively. This increase in tensile strength is presumably due in large part to an increase in type III collagen content in AdBMP13-treated tendons. While we found no significant differences in stress to failure or stiffness between AdBMP13 and AdBMP13 + PRP treatment groups, we did find significant differences in stress to failure when comparing AdBMP13 or AdBMP13 + PRP to PRP alone. Thus, the enhanced tensile strength appears to be attributable to AdBMP13 and not PRP. Finally, while AdBMP13 alone demonstrated a more significant increase in stiffness than AdBMP13 + PRP compared with control (112% and 80.4%, respectively), there was no difference in stiffness between AdBMP13 and AdBMP13 + PRP. We attribute these findings to the inherent statistical error involved in biomechanical testing; a larger sample size may have eliminated this statistical difference. Furthermore, histologic analysis demonstrated significantly enhanced healing in BMP13-containing treatment groups and thus was corroborative with our biomechanical findings.

Previous biochemical studies have demonstrated increased expression of the ECM glycoproteins tenascin C and fibronectin after supraspinatus rupture and subsequent tendon healing. 47, 54 We showed significant up-regulation of fibronectin after AdBMP13 treatment compared with control as well as compared with treatment with PRP growth factors after 5 days of in vitro incubation. We also demonstrated significant up-regulation of tenasin C by AdBMP13, AdTGF-β1, and AdVEGF-A. The increased production of these ECM components may have played a role in enhancing the biomechanical properties of the supraspinatus tendons in our 3 treatment groups.

Scleraxis is essential for tenocyte differentiation and tendon development. 8, 21 It is also up-regulated in response to injury during the repair process of tendon 35 and is known to regulate expression of ECM genes by tenocytes. 16, 38 Gulotta et al 21 demonstrated increased stiffness and load-to-failure in a rat rotator cuff repair model treated with adenovirally delivered scleraxis compared with control with a microstructure more closely resembling the native tendon-bone insertion site. We have demonstrated that AdBMP13, AdTGF-β1, and AdVEGF-A increase scleraxis expression compared with control. Consistent with the results reported by Gulotta et al, the in vivo increase in tensile strength and stiffness in our 3 treatment groups may be partially attributable to increased expression of scleraxis mediated by AdBMP13 and at least 2 of the growth factors of PRP (AdTGF-β1 and AdVEGF-A).

There are several limitations to our study. For in vitro experiments of tenocyte gene expression, we chose to use adenovirus-mediated delivery of growth factors, while we used PRP for in vivo biomechanical studies. For in vitro studies, this allowed for better intergroup reliability of results as assessed by imaging under direct fluorescence while also allowing us to determine which individual growth factor component of PRP may contribute most to its potential tendon-healing properties. For in vivo biomechanical studies, we decided to use PRP instead of adenoviral delivery of the individual growth factors found in PRP for several reasons. First, whole PRP, and not the individual growth factors, is already widely used clinically with a well-established delivery vehicle, 11-14 whereas BMP13 is not FDA-approved and does not have a well-established delivery vehicle. Although BMP13 does not have a well-established delivery vehicle, our in vitro experiment using GFP-labeled BMP13 adenovirus allowed us to visualize and confirm delivery and continued expression of BMP13 (and GFP control) within the supraspinatus tendon for 2 weeks, allowing us to then proceed with in vivo biomechanical and histologic studies at 2 weeks after treatment. Second, for in vivo biomechanical and histologic studies, there would have been an impractical number of treatment groups if we had used adenovirus-mediated delivery of the individual growth factors of PRP and combinations of PRP growth factors with AdBMP13. While adenovirus-mediated delivery of BMP13 may not be directly applicable to human use, this study serves as a proof of concept that BMP13 likely enhances tendon healing and growth factor delivery must be optimized for human therapies. Although we demonstrated adenovirus as an effective delivery vehicle for BMP13, comparing continuous overexpression of BMP13 to a single dose of PRP may serve as a less than ideal comparison. We additionally acknowledge that thrombin activation of PRP may affect growth factor release based on our preparation protocol. Future studies should investigate single-dose applications of BMP13 using other delivery mechanisms or multiple-dose applications of both BMP13 and PRP. Additionally, a punch defect at the supraspinatus tendon was used instead of tendon transection followed by surgical fixation due to improved reproducibility between subjects and treatment groups. The rat supraspinatus muscle measures approximately 4 mm in width and 1.5 mm in height, and we believe that transosseous suture fixation is less reproducible than a punch defect, which has been validated by Carpenter et al. 9

In summary, adenoviral delivery of BMP13 significantly increases supraspinatus tendon stress to failure during the early stages of healing after injury. While we demonstrated beneficial effects of PRP on tendon healing, AdBMP13 was superior to PRP. Our molecular, biomechanical, and histologic results provide strong evidence that BMP13 may be a better therapeutic alternative for intraoperative surgical augmentation than PRP. Further research is required to determine the optimal delivery mechanism, dose concentration, and frequency for BMP13 therapy.
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