Mechanotransduction of Bovine Articular Cartilage Superficial Zone Protein by Transforming Growth Factor β Signaling

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Objective. Mechanical signals are key determinants in tissue morphogenesis, maintenance, and restoration strategies in regenerative medicine, although molecular mechanisms of mechanotransduction remain to be elucidated. This study was undertaken to investigate the mechanotransduction process of expression of superficial zone protein (SZP), a critical joint lubricant.

Methods. Regional expression of SZP was first quantified in cartilage obtained from the femoral condyles of immature bovines, using immunoblotting, and visualized by immunohistochemistry. Contact pressure mapping in whole joints was accomplished using pressure-sensitive film and a load application system for joint testing. Friction measurements on cartilage plugs were acquired under boundary lubrication conditions using a pin-on-disk tribometer modified for reciprocating sliding. Direct mechanical stimulation by shear loading of articular cartilage explants was performed with and without inhibition of transforming growth factor β (TGFβ) signaling, and SZP content in media was quantified by enzyme-linked immunosorbent assay.

Results. An unexpected pattern of SZP localization in knee cartilage was initially identified, with anterior regions exhibiting high levels of SZP expression. Regional SZP patterns were regulated by mechanical signals and correlated with tribological behavior. Direct relationships were demonstrated between high levels of SZP expression, maximum contact pressures, and low friction coefficients. Levels of SZP expression and accumulation were increased by applying shear stress, depending on location within the knee, and were decreased to control levels with the use of a specific inhibitor of TGFβ receptor type I kinase and subsequent phospho-Smad2/3 activity.

Conclusion. These findings indicate a new role for TGFβ signaling in the mechanism of cellular mechanotransduction that is especially significant for joint lubrication.

Osteoarthritis (OA) is the most common form of arthritis, affecting 12.1% of US adults (1). Treatment of OA is a critical unmet need in biotechnology and medicine for the regeneration of damaged joints and articular cartilage in the elderly. During locomotion, animal joints allow for normal function by minimizing friction and wear (2,3). Superficial zone protein (SZP), a glycoprotein secreted by chondrocytes in the superficial layer of articular cartilage (4,5), is thought to be a key surface molecule or lubricant involved in boundary lubrication. SZP is also known as lubricin (6), megakaryocyte-stimulating factor precursor (7), and PRG4 (5). In addition to its function as a boundary lubricant, SZP inhibits synovial cell overgrowth (7). Down-regulation of SZP has been associated with the pathogenesis of OA (8).
Mechanical loading is an important factor in tissue homeostasis (9). In articular cartilage, tissue performs the essential biomechanical functions of load support and lubrication, with minimal wear or damage during the human lifespan (10). Chondrocytes respond to mechanical loading, and in particular, shear loading influences the expression of SZP (11). However, the molecular basis of mechanotransduction in articular cartilage remains unknown.

In this study, mechanotransduction of SZP in articular cartilage was investigated using biomechanical and biochemical assays. This work was motivated by an earlier study that revealed a mechanism by which growth factor shedding into a dynamically regulated extracellular volume gives rise to mechanotransduction (12). Based on this finding and the regional localization of growth factors to the superficial zone of articular cartilage (13), it was hypothesized that mechanotransduction of SZP expression in articular cartilage occurred through growth factor–mediated signaling pathways.

The focus in the present study was on the signaling pathway for transforming growth factor β (TGFβ), a potent growth factor in articular cartilage and SZP expression (14). The results showed that regional patterns of SZP expression were driven by mechanical signals. SZP expression patterns also correlated with mechanical function as determined by tribological assays. There was a differential regional response of SZP expression to shear loading, and increased SZP expression levels were reduced to control levels in the presence of transforming growth factor–mediated signaling, and SZP subsequently influences joint tribology.

MATERIALS AND METHODS

SZP localization. SZP expression patterns were characterized across the surface of 3-month-old bovine calf femoral condyle cartilage, by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with subsequent immunoblotting in 8 separate locations on the lateral femoral condyles (labeled L1 through L4) and medial femoral condyles (labeled M1 through M4) (Figure 1A). Bovine stifle joints were obtained from a local abattoir within 6 hours of slaughter. Joints were dissected under aseptic conditions to expose the femoral condyles. Osteochondral plugs were removed using a coring reamer with a 5-mm diameter (Fisher Scientific, Fair Lawn, NJ). The superficial 0.5 mm was removed from each plug using a custom cutting jig.

For immunoblotting, protein was extracted from superficial zone sections of 7 joints using a tissue homogenizer (Brinkmann, Westbury, NY) in 1 ml of 4M guanidine HCl (pH 7.2) supplemented with 0.2% CHAPS, 10 mM EDTA, 0.05M Tris, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Homogenized tissue was frozen and thawed once in liquid nitrogen and then centrifuged at 14,000g for 10 minutes. The supernatant was buffer-exchanged with 6M urea containing 0.05M Tris (pH 7.4) using Centricron filters (Millipore, Bedford, MA). Standardized protein extracts (0.2 µg) were determined by the Lowry method (15), and then separated using SDS-PAGE. Immunostaining was performed by a standard method with S6.79 (1:5,000) as the primary antibody (16) and anti-mouse horseradish peroxidase–conjugated goat antibody as the secondary antibody (1:3,000; Vector, Burlingame, CA). Immunoblots were quantified using background subtraction and integrated density algorithms in a standard software package (ImageJ [NIH Image, National Institutes of Health, Bethesda, MD; online at: http://rsbweb.nih.gov/ij/]). Relative integrated densities were computed as the mean ± SEM density of the joints normalized to that of location L1. For enzyme-linked immunosorbent assay (ELISA), protein was quantified in media collected from cartilage cultures following shear loading. The SZP content in samples that were serially diluted and reacted with the primary and secondary antibodies described above was determined according to a standard method.

For immunohistochemistry, tissue sections from 6 joints were fixed in Bouin’s solution for 1 hour, followed by paraffin embedding and sectioning. Immunostaining was performed by a standard method with S6.79 (1:5,000) as the primary antibody (16) and an ABC kit (Vector) with mouse IgG secondary antibody for signal detection.

Contact pressure distributions. Regional SZP expression was examined in terms of mechanical parameters that are known to vary as a function of spatial position in the tibiofemoral joint (17). Mechanical stresses approach 18 MPa in cartilage during simple daily activities (18). Contact areas and pressure in bovine knee joints (n = 3) under physiologically relevant loading were measured with ultra–low-range pressure-sensitive film (Fuji Photo Film Company, Kanagawa, Japan) within 12 hours after slaughter. The joint capsule and collateral ligaments were transected while leaving the cruciate ligaments intact, to allow film access to the joint space. Knees were then anatomically aligned in a load application system for joint testing (19). Pressure-sensitive film packets were created to match both the size and the shape of the femoral condyles. The load application system constrained flexion at a neutral posture (135° flexion; n = 3) (20) and flexed posture (115° flexion; n = 1) during application of compressive loads. Relative tibiofemoral motions were unconstrained in all other degrees of freedom. For each test, film packets were inserted between each condyle and corresponding meniscus. Orientation of the film was recorded by placing registration marks on the film and joint in unloaded regions. Specimens were first preloaded with 100N for 5 seconds, followed by compressive loads of 1, 2, or 3 times body weight (400, 800, and 1,200N, respectively) for 5 seconds. Following testing, cruciate ligaments were transected to expose the condyles, and images of articular surfaces were acquired with and without registered film packets. A representative diagram of contact pressure distributions was created from images using a software pack-
age (Adobe) and color calibration tables provided by the manufacturer (Fuji Photo Film Company).

Mechanotransduction and shear loading. Mechanotransduction of SZP was investigated using direct mechanical stimulation of articular cartilage explants with and without inhibition of TGFβ signaling. The present study was motivated by the results of previous studies, which revealed a mechanism by which growth factor shedding into a dynamically regulated extracellular volume gives rise to mechanotransduction (12), the regional localization of growth factors to the superficial zone of articular cartilage (13), and the potent influence of TGFβ in SZP expression (14).

Direct stimulation of SZP expression by mechanical forces was accomplished using shear loading of explants from locations M1 (anterior) and M4 (posterior) (Figures 2B and C). Full-thickness sections (4 mm thick) were removed from plugs and placed in serum-free, chemically defined low-glucose Dulbecco’s modified Eagle’s medium—Ham’s F-12 (1:1; Invitrogen, San Diego, CA) supplemented with 0.1% bovine serum albumin, 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml ascorbate 2-phosphate, for 3 hours. Medium was supplemented with SB431542 (Tocris, Ellisville, MO) for plugs used to determine SZP expression during shear loading with TGFβ pathway inhibition. Plugs were then affixed to acrylic pins using ethyl cyanoacrylate and placed in a custom pin-on-disk tribometer modified for reciprocating sliding (21). The articular surface was brought into contact with a polished glass disk under a normal load of 1.8N, producing an average Hertzian pressure of 0.1 MPa. Contact was established at a radial distance of 5 mm from the disk center, resulting in a linear sliding speed of 0.5 mm/second. The duration of each friction experiment was 300 seconds. Prior to the initiation of each test, the sample was allowed to equilibrate for 2 minutes. Following testing, the superficial 0.5 mm was removed and replaced in 1 ml of medium. SZP was allowed to accumulate in the medium during 48 hours of tissue incubation (37°C, 5% CO2). The SZP content was quantified by ELISA in media collected from cartilage cultures following shear loading.

Additional experiments were conducted to demonstrate the specificity of TGFβ signaling in chondrocyte mechanotransduction. To eliminate the possibility that cytotoxic effects introduced in the process of using the vehicle (DMSO) might inhibit TGFβ signaling, the influence of the vehicle or the vehicle and inhibitor on SZP expression was examined using SZP expression in untreated controls as a reference. Plugs were harvested from additional animal joints (24 plugs total; n = 8) and were separated into 3 groups: control plugs (untreated), plugs treated with DMSO (1 μl/ml medium), and

Figure 1. Dependence of immunolocalization of superficial zone protein (SZP) on the geometry of femoral condyles. A, Femoral condyle locations where explants were harvested for friction testing and analysis. B, Dependence of SZP expression on condyle location. Significantly higher SZP expression levels were measured in anterior locations L1, L2, and L3 than in posterior location L4 (P < 0.002). Similarly, significantly higher SZP expression levels were measured in anterior location M1 than in locations M2, M3, and M4 (P < 0.001). Values are the mean and SEM, normalized to L1. C, Results of sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting of explants harvested from different articular locations. D, Results of toluidine blue staining of superficial zone sections (0.5 mm), revealing similar cell and proteoglycan distribution patterns at all femoral condyle locations. SZP staining expression decreased from anterior to posterior regions (original magnification × 200). Ab = antibody.
Figure 2. Determination of SZP expression by contact stress distribution on femoral condyles through transforming growth factor β (TGFβ)-mediated signaling pathways. A, Representative pressure distributions during compressive loading and simulated in vivo postures, determined using pressure-sensitive film and a load application system for joint testing. For a normal load equal to 1, 2, and 3 times body weight in the 135° neutral posture and 115° flexed posture, regions of contact stress were consistently located in the anterior regions of the joint, corresponding to SZP expression patterns. B, Significant increase in SZP expression levels during shear loading of explants from location M1, but not from location M4 (P = 0.759 versus unloaded controls). Values are the mean ± SEM of 6 samples from each location. * = P = 0.021. C, Decrease in SZP expression levels at location M1 to control levels during shear loading of explants in the presence of SB431542, a specific inhibitor of TGFβ receptor type I kinase activity. Values are the mean and SEM of 17 samples. * = P = 0.024; ** = P = 0.005. D, SZP expression levels at location M1 in unloaded control samples, samples treated with DMSO (the carrier vehicle for SB431542), and samples treated with DMSO plus SB431542. Values are the mean and SEM. E, Immunostaining for phospho-Smad2/3. Staining was particularly enhanced in superficial zone chondrocytes from location M1 compared with untreated controls or explants treated with SB431542. See Figure 1 for other definitions.
plugs treated with DMSO (1 μl/ml medium) plus SB431542 (10 ng/ml). SZP was allowed to accumulate in the medium during 48 hours of tissue incubation (37°C, 5% CO₂). SZP content was quantified by ELISA, as described above.

Specificity of TGFβ signaling was investigated by visualization of phospho-Smad2/3 following shear loading in the presence or absence of SB431542 (10 ng/ml). Twenty-four plugs from locations M1 and M4 were harvested from different animals (n = 4) and tested as described above. Following testing, the plugs were first placed in a chemically defined medium for 1 hour to allow for phospho-Smad2/3 activation and then in Bouin’s solution for tissue fixation. The plugs were embedded in paraffin and sectioned according to a standard protocol. Tissue sections were immunostained using a primary antibody for phospho-Smad2/3 (Ser-433/435; Santa Cruz Biotechnology, Santa Cruz, CA).

**Boundary lubrication.** To further test whether the SZP expression pattern was related to mechanical function, friction tests were performed under boundary lubrication conditions with a pin-on-disk tribometer operated in reciprocating mode (21) using plugs from different cartilage locations. Plugs from lateral locations (labeled L1 through L4) and medial locations (labeled M1 through M4) were harvested as described above from additional separate bovine joints (n = 7). Superficial zone sections (4 mm thick) were removed from plugs, immediately affixed to acrylic pins using ethyl cyanoacrylate, and then used in the friction experiments. The articular surface was brought into contact with a polished glass disk while totally immersed in phosphate buffered saline. The applied average contact pressure of 0.1 MPa in these tests was within the physiologic range during walking (22). Prior to initiation of sliding, the sample was allowed to equilibrate for 2 minutes under the applied normal load to minimize fluid effects during testing. Data processing was performed with a standard software package (Microsoft Excel). Since linear trends between the friction coefficient and time were observed for all samples (Figure 3A), only data from the first 60 seconds were used to compute mean ± SEM friction coefficients for each femoral condyle location and subsequently correlate the average friction coefficients with immunoblotting data (R² = 0.478 for lateral condyles and R² = 0.994 for medial condyles) (Figures 3C and D). The friction coefficient was also measured in the middle zone of 7 joints that were used as negative controls (locations L2 or L3) because they yielded similar levels of SZP expression (Figure 3B).

Explant testing under boundary lubrication conditions was verified using hydrodynamic theory (23) and equilibrium experiments. Explants from both locations L2 and L3 were tested at an average contact pressure of 0.1 and 0.5 MPa and sliding velocity fixed at 0.5 mm/second for 1 minute after equilibration times of 0, 2, 5, and 10 minutes. Additionally, explants from location M1 were tested at sliding velocities of 0.5 and 1.6 mm/second with average contact pressure of 0.1 MPa.

**Statistical analysis.** Differences in SZP expression levels at femoral condyle locations and following shear loading were determined by one-way analysis of variance with Fisher’s protected least significant difference post hoc test, using a standard software package (SAS Institute, Cary, NC). Differences in the friction coefficient following equilibration times of 2 or 10 minutes were determined using Student’s paired 2-tailed t-test. P values less than 0.05 were considered significant. Results are presented as the mean ± SEM.

**RESULTS**

In situ SZP expression patterns were found to depend on the geometry of the femoral condyle surface and location in articulating knee joints. Significantly higher levels of SZP expression were found in anterior locations L1, L2, and L3 than in posterior location L4 (P < 0.002). Significantly higher levels of SZP expression were also observed in anterior location M1 than in locations M2, M3, and M4 (P < 0.001) (Figure 1B). Overall, lateral condyles exhibited higher levels of SZP expression than did medial condyles (P < 0.001).

In situ SZP expression patterns were confirmed and visualized using immunohistochemistry. Toluidine blue staining revealed similar cell and proteoglycan distribution patterns throughout the cartilage depth at different femoral condyle locations (Figure 1D). However, staining with SZP antibody (S6.79) (16) revealed striking patterns of SZP immunolocalization (Figure 1D). Levels of SZP expression decreased from anterior to posterior regions, with L1 and M1 exhibiting staining into the depth of the cartilage, while L4 and M4 exhibited staining only on the most superficial tissue layer (lamina splendens). Middle sections (1.25 mm deep) and deep sections (2.50 mm deep) from location L2 were used as negative controls and exhibited no immunostaining for SZP (results not shown).

Maximum contact pressure distributions (Figure 2A) corresponded to regions with the highest levels of SZP expression. Regions of high contact pressure were consistently located in the anterior femoral condyles near L1 and M1 for compressive loads of 1, 2, and 3 times body weight in the 135° neutral flexion posture of the bovine knee (20). To simulate natural walking posture, compressive loading was applied in the 115° flexion posture, resulting in the shift of high contact pressure regions to the posterior; however, these regions were still located in the anterior femoral condyles near L1 and L2 (lateral) and M2 (medial).

Shear loading of explants from location M1 (P = 0.021) but not M4 (P = 0.759) increased SZP expression levels above those in unloaded controls (Figure 2B). SZP expression decreased to control levels during shear loading in the presence of SB431542, a specific inhibitor of TGFβRI kinase activity (24) (Figure 2C). Shear-loaded explants exhibited significantly higher levels of SZP expression than shear-loaded, SB431542-treated explants (P = 0.024) or control (unloaded) explants (P =
Figure 3. Effects of femoral condyle location and superficial zone protein (SZP) expression levels on friction coefficient. A, Linear increase in the friction coefficient of all explants after removal of SZP from the articular surface. B, Increase in the friction coefficient after removal of superficial cartilage in explants. C and D, Nonlinear dependence of the friction coefficient on SZP expression levels in lateral condyles ($R^2 = 0.478$) (C) and medial condyles ($R^2 = 0.994$) (D). E, Sufficiency of an equilibration time of 2 minutes for the loading protocols used. $\sigma =$ contact pressure; $v =$ velocity. F, Lack of effect of changes in the sliding velocity on the friction coefficient. These data suggest that SZP plays a key role in boundary lubrication of synovial joints. Values are the mean $\pm$ SEM.
friction coefficients (formed at different sliding speeds yielded similar average measurements (of 2–10 minutes did not affect friction coefficient measurements (P = 0.05)). Treatment of explants with DMSO alone or with SB431542 plus DMSO did not produce any differences compared with untreated controls (P > 0.470) (Figure 2D). Following shear loading, immunostaining for phospho-Smad2/3 was particularly enhanced in the superficial zone chondrocytes at location M1 compared with untreated controls or explants treated with SB431542 (Figure 2E).

The friction coefficient increased linearly as a function of sliding time or distance (Figure 3A). The removal of superficial cartilage (1.25 mm) from 6 samples (negative controls) resulted in a higher friction coefficient and a weak time or distance dependence (Figure 3B). A striking relationship was observed between the friction coefficient on the articular surface and the level of SZP expression. The friction coefficient was inversely correlated with SZP expression levels in a nonlinear trend for locations on the lateral condyles (R² = 0.478) (Figure 3C) and medial condyles (R² = 0.994) (Figure 3D). With mean contact pressure fixed at 0.1 MPa, variation of the equilibration time in the range 0.994) (Figure 3D). With mean contact pressure fixed at 0.1 MPa, variation of the equilibration time in the range of 2–10 minutes did not affect friction coefficient measurements (P > 0.05). Additionally, experiments performed at different sliding speeds yielded similar average friction coefficients (P > 0.05).

DISCUSSION

TGFβ signaling controls a wide range of cellular processes including cell division, differentiation, adhesion, and apoptosis (25). The results of this study show that TGFβ signaling mediates the transduction of mechanical signals to cellular protein expression. In particular, the TGFβ pathway mediates the expression of superficial zone protein, a critical joint lubricant (4). Loss of SZP has been implicated in the pathogenesis of OA (8). Mechanical signals are critical determinants of tissue morphogenesis and maintenance (9). It is known that shear loading up-regulates SZP expression (11,26). Dynamic shear stimulation can produce PRG4 expression levels 3–4 times higher than those in unloaded controls, depending on the loading conditions (11).

The results of this study are consistent with those of previous studies that have shown up-regulation of SZP by shear loading. In contrast to 24-hour continuous loading methods for studies of PRG4 synthesis (11), the present study demonstrated a 2–3-fold up-regulation of the protein (Figures 2B and C) with a mechanical stimulation of only 5 minutes. Although the reduced duration of mechanical stimulation may account for the diminished up-regulation found in the present study compared with that reported by Nugent et al (11), sufficient SZP accumulation was measured to enable the performance of short-term mechanotransduction experiments. The present study also demonstrated a differential and regional response of cartilage explants to shear loading (Figure 2B), suggesting that chondrocyte subpopulations or exogenous TGFβ content may vary regionally across the articular surface.

Molecular mechanisms of mechanotransduction remain largely unexplored. Growth factor pathways provide one potential mechanism of mechanotransduction, as evidenced in epithelial cells in a previous study, in which cellular signaling was triggered via autocrine binding of epidermal growth factor family ligands to the epidermal growth factor receptor in a decreased lateral intercellular space (12). This mechanism did not require induction of force-dependent biochemical processes within the cell or cell membrane. SZP expression may be mediated in this way through TGFβ signaling pathways. SB431542 prevents TGFβ-induced Smad translocation into the nucleus (27,28).

In the present study, shear loading at anterior location M1 resulted in activation of phospho-Smad2/3. While the influence of growth factor pathways in articular cartilage was demonstrated, transduction and regulation of mechanical forces may occur through many types of signaling pathways. At this point it is unclear to what extent alternative pathways (29) mediate SZP mechanotransduction. Further, since TGFβ signaling leads to changes in the expression of several hundred genes (25), it remains to be determined if specific transcription coactivators or corepressors are involved with Smad proteins in targeted gene expression. For the articular cartilage system examined in this study, it is unknown whether preferential binding of TGFβ with its receptor is a result of constant growth factor shedding in a dynamically regulated extracellular volume or up-regulation of TGFβ synthesis by mechanical loading.

Animal joints are complex biomechanical and biochemical systems in which mixed modes of lubrication (including hydrodynamic and boundary lubrication) (2,30,31) allow for normal activities such as locomotion. In hydrodynamic lubrication, the mechanical load is transmitted via a thin layer of fluid lubricant pressurized between 2 articulating surfaces. In boundary lubrication, the mechanical load is transmitted through asperity solid–solid contacts established between contacting surfaces that are separated by a molecularly thin lubricant layer. While hydrodynamic effects in cartilage lubri-
tion are certainly essential to normal functioning of the joint (30), in the absence of an effective boundary lubricant, joint degeneration may occur rapidly (32).

The results of the present study indicated that SZP played a profound role in boundary lubrication of synovial joints. However, the role of SZP in boundary lubrication remains controversial (6,33,34). SZP is believed to contribute to the unique tribological properties of cartilage (31,35,36), with the mucin-like domains of the protein contributing to boundary lubrication through extensive O-linked oligosaccharides (7,36). An alternative theory has been suggested that surface-active phospholipid provides lubrication (33). However, the role of phospholipids has been challenged (6). It has been reported that the removal of the superficial zone of bovine articular cartilage does not increase the friction coefficient in reciprocating sliding (34). The present study demonstrated that SZP may be removed from the surface within a short time period after testing (<600 seconds). Furthermore, the removal of the superficial zone resulted in a higher friction coefficient (Figure 3B). Although these results do not rule out contributions to boundary lubrication from other proteins, e.g., surface-active phospholipid (33), they provide strong evidence of the principal role of SZP in joint tribology.

The tribological behavior of the cartilage demonstrated a strong dependence on SZP expression. It is likely that the higher gradient of SZP expression in medial condyle locations compared with that in lateral condyle locations contributed to the higher correlation coefficient in medial condyles. Also, it is possible that the removal of protein from the surface by the rubbing process contributed to the linear trends shown in Figures 3A and B, and that protein removal followed by mechanically stimulated replenishment contributed to the evolution of a sacrificial boundary lubrication mechanism. Alternatively, exuded fluid may have contributed to this phenomenon, although the weak time or distance dependence seen in Figure 3B suggests that a fluid exudation did not occur during testing. Fluid effects were minimized using an equilibration period prior to testing (Figure 3E) to ensure that friction experiments were conducted under boundary lubrication conditions. In addition, fluid shear rate effects did not influence friction behavior during sliding (Figure 3F).

The main findings of this study were that in situ SZP expression patterns depended on the geometry of the femoral condyle surface and location in articulating knee joints as determined by immunoblotting and immunohistochemistry, anterior regions of the joint demonstrated significantly higher SZP expression levels than did posterior regions, regions with the highest levels of SZP expression corresponded to maximum contact pressure distributions and exhibited the lowest friction coefficients, and SZP expression levels increased after shear loading, depending on the location within the knee, and decreased to control levels in the presence of a specific inhibitor of TGFβRI kinase activity. These findings suggest that joint mechanics (physical forces) induce SZP signaling that is mediated by TGFβ, and SZP subsequently influences joint mechanics (tribology). Knowledge of SZP regulatory pathways will provide insight into the progression of cartilage degeneration and enable the development of regenerative strategies aimed at restoration of the surface lubrication characteristics of articular cartilage.

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AUTHOR CONTRIBUTIONS

Dr. Neu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Neu, Khalafi, Reddi.

Acquisition of data. Neu, Khalafi, Komvopoulos.

Analysis and interpretation of data. Neu, Khalafi, Komvopoulos, Schmid, Reddi.


Statistical analysis. Neu.

Correlation of mechanotransduction and cartilage lubrication mechanisms. Neu, Komvopoulos.

Antibody development. Schmid.

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