Distribution of Lubricin in the Ruptured Human Rotator Cuff and Biceps Tendon

A Pilot Study

Tadanao Funakoshi MD, PhD, Scott D. Martin MD,
Thomas M. Schmid PhD, Myron Spector PhD

Received: 13 February 2009 / Accepted: 14 September 2009 / Published online: 2 October 2009
© The Association of Bone and Joint Surgeons® 2009

Abstract

Background Lubricin is a lubricant for diarthrodial joint tissues and has antiadhesion properties; its presence in the (caprine) rotator cuff suggests it may have a role in intra-fascicular lubrication.

Questions/Purposes To preliminarily address this role, we asked: (1) What is the distribution of lubricin in human ruptured supraspinatus and biceps tendons? (2) What are the potential cellular sources of lubricin?

Methods We obtained seven torn rotator cuff samples and four torn biceps tendon samples from 10 patients; as

control tissues, we obtained the right and left supraspinatus tendons from each of six cadavers. Specimens were fixed in formalin and processed for immunohistochemical evaluation using a monoclonal antibody for lubricin.

Results We found lubricin as a discrete layer on the torn edges of all of the ruptured supraspinatus and biceps tendon samples. None of the transected edges of the tissues produced during excision of the tissues showed the presence of lubricin. Lubricin was found in 3% to 10% of the tendon cells in the cadaveric controls and in 1% to 29% of the tendon cells in the torn supraspinatus and biceps tendon samples.

Clinical Relevance The lubricin layer on the torn edges of ruptured human supraspinatus and biceps tendons may interfere with the integrative bonding of the torn edges necessary for repair.

This material is based on work supported by the Office of Research and Development, Rehabilitation Research and Development Service, Department of Veterans Affairs (MS). MS is a VA Research Career Scientist.

The research-related use of excess human material/tissue and related health/medical information was approved by the Brigham and Women's Hospital Institutional Review Board Protocol Number 2007-P-000924/1; BWH.

This work was performed at the VA Boston Healthcare System and the Brigham and Women's Hospital, Boston, MA, USA.

T. Funakoshi, S. D. Martin, M. Spector
Department of Orthopaedic Surgery, Brigham and Women's
Hospital, Harvard Medical School, Boston, MA, USA

T. Funakoshi, M. Spector (✉)
Tissue Engineering, VA Boston Healthcare System,
150 S Huntington Avenue, MS 151, Boston, MA 02130, USA
e-mail: mspector@rics.bwh.harvard.edu

T. Funakoshi
Department of Orthopaedic Surgery, Hokkaido University
Graduate School of Medicine, Sapporo, Japan

T. M. Schmid
Department of Biochemistry, Rush University Medical Center,
Chicago, IL, USA

Introduction

The limited healing of the ruptured rotator cuff has been documented in animal models [2, 32] and in followup studies of repairs in human subjects [5, 12, 13, 20] designed to identify factors associated with an inadequate reparative process. Several histologic studies of the ruptured supraspinatus tendon have focused on cellular features and the condition of the extracellular matrix that serve as impediments to healing [22] and affect the degree to which the torn edge needs to be débrided before a repair procedure [9, 19].

Lubricin initially was discovered in joint fluid [14, 30]. It is synthesized by synovial cells [1, 24] and plays an important role in joint lubrication [15, 31]. Subsequent reports indicate another protein, superficial zone protein synthesized by superficial zone chondrocytes [26], is

homologous to megakaryocyte stimulating factor [7] and lubricin [14] and that these proteins are products of the megakaryocyte stimulating factor gene [14]. Proteoglycan 4 is another name collectively given to these homologous glycoproteins encoded by 12 exons of the gene, also known as *Prg4* [6].

Lubricin is a lubricating and antiadhesive glycoprotein that has been reported in canine [29] and caprine tendons [8] in fascicles and in fascicular sheaths suggesting it may facilitate the relative movement of collagen bundles [29] and play a role in normal interfascicular lubrication [8]. In a previous study [8], some caprine supraspinatus tendon cells contained lubricin, thus suggesting endogenous synthesis of this glycoprotein. These findings raise questions regarding where lubricin is located in the ruptured rotator cuff tendons, whether it is upregulated in the ruptured tissue in which cytokines that increase its synthesis are elevated, and what its effect may be on the reparative process.

Lubricin occurs in the caprine humeral joint synovial lining [8]; the edges of torn supraspinatus and biceps tendons are likely exposed to synovial fluid, and likely lubricin, by way of the communication between the subacromial bursa and humeral joint space. Given lubricin is an antiadhesive protein, it may interfere with cell adhesion to the natural or torn tendon surface [23] and interfere with integrative repair processes [6] required for complete healing.

We therefore determined (1) if a discrete lubricin layer was on the torn edges of the ruptured tendons; (2) the percentage of tendon cells that contained lubricin and if lubricin also was present in the extracellular matrix; (3) if lubricin occurred on the surfaces of the tendons and in the fascicular sheaths; and (4) the cell number density at the torn and transected edges of the tendons.

Materials and Methods

We obtained tissues from 10 patients: seven ruptured rotator cuff samples and four biceps tendon samples (Table 1). All patients met the following criteria: a painful shoulder and a supraspinatus tendon of medium size (2–3 cm) with a tear that could be treated by a single- or double-row arthroscopic repair. At arthroscopic surgery, we harvested the edge of the full-thickness supraspinatus tendon with the approximate dimensions of 15 × 3 mm. One of the shoulders had an articular-side partial rotator cuff tear and the others were full-thickness tears (Table 1). One patient had a history of traumatic dislocation 6 weeks before surgery (Table 1, SSP 6). The other patients had no obvious traumatic episodes as the cause of the shoulder pain. We obtained four ruptured biceps tendon specimens for study, one at an open biceps tenodesis (BT 1) and the others from an arthroscopic

Table 1. Patient data*

SSP number	Age at index (years)	Gender	Rotator cuff condition	Biceps tendon condition
1	61	F	Full-thickness tear	Intact
2	49	M	Full-thickness tear	Complete rupture
3	50	M	Full-thickness tear	Intact
4	62	M	Partial tear APRCT	Intact
5	61	F	Full-thickness tear	Hypertrophy
6	63	F	Full-thickness tear	Intact
7	59	M	Full-thickness tear	Laceration
BT number				
1	59	M	Full-thickness tear	Laceration
2	46	M	Intact	Laceration
3	58	M	Intact	Laceration
4	58	M	Partial tear APRCT	Laceration

* All of the supraspinatus samples were from the right side except Number 4; all of the biceps tendon samples were from the right side except Number 3; SSP = supraspinatus; BT = biceps tendon; F = female; M = male; APRCT = articular-side partial rotator cuff tear.

procedure. One of the biceps tendon samples (Table 1, BT1) was from the same patient from whom a torn supraspinatus sample was resected (Table 1, SSP 7).

The sample size power analysis was based on the following. The principal outcome variable was the presence or absence of a lubricin layer on the torn edge of the ruptured supraspinatus tendon. The internal control was the transected edge, produced during excision of the tissue sample, which was at least 2 mm away from the torn edge. We expected all of the torn edges would display the presence of a lubricin layer and none of the transected control edges would have a lubricin layer. Using Fisher's exact test, a sample size of four was necessary to yield $p < 0.05$. We obtained seven supraspinatus tendon samples to allow for the loss of samples resulting from inadequate tissue processing and to achieve a lower value of α . The collection of supraspinatus tendon specimens stopped after we analyzed seven samples, all of which displayed a lubricin layer on the torn edge and none on the transected edge (a difference of $p = 0.0006$ using Fisher's exact test). The collection of the torn biceps tendon samples stopped after four based on the finding of the lubricin layer on all of the torn edges and none on the transected border ($p = 0.03$, Fisher's exact test).

After the supraspinatus and biceps tendon samples were excised, the torn and transected edges were distinguished by labeling with Pyocyanine (methylrosanilinium chloride) in the operating room. Tissues then were immediately

immersed in 4% paraformaldehyde in the operating room and fixed for at least 3 days.

For intact control tissue, we obtained the right and left supraspinatus tendons from each of four male and two female cadavers, which were available at the time of the study with no specific selection criteria other than the fact that they did not display obvious trauma or signs of surgical procedures around the shoulder. No information was available regarding the age, cause and time of death, or how the bodies were preserved. The tendon samples were removed from the surrounding tissue and were fixed in formalin for at least 3 days.

After formalin fixation, the samples were dehydrated in a tissue processor (Hypercenter XP Model; Thermo Electron Corp, Waltham, MA), embedded in paraffin, and stored at -20°C . The samples were sectioned as 6- μm thick slices using a microtome (Finesse Model; Thermo Electron Corp).

We performed immunohistochemical staining of lubricin using a previously published protocol [35] and the LSAB-2 System (DakoCytomation, Carpinteria, CA) with a Dako Autostainer with the program for lubricin. After deparaffinization with xylene, we rehydrated the microtomed sections in ethanol. We made a final wash with tris-buffered saline (S3001; DakoCytomation) to reduce enzymatic activity. Sections then were treated with 0.1% protease XIV (P5174; Sigma, St Louis, MO) for 45 minutes to facilitate antibody penetration of the tissue. The sections then were treated with peroxidase-blocking reagent (S2001; DakoCytomation) for 10 minutes and 5% goat serum (Sigma) for 30 minutes before incubation with the primary antibody. A purified monoclonal antibody (#S6.79; Rush University Medical Center, Chicago, IL) was used at 1:1000 dilution (1 $\mu\text{g}/\text{mL}$ protein concentration) for 30 minutes. The antilubricin monoclonal antibody was made in the mouse against human superficial zone protein [27]. This antibody reacts with various mammalian lubricin molecules, including human [27, 35], goat [8], dog [27, 29], bovine [33, 35], guinea pig [27], and rabbit [27]. The S6.79 antibody recognizes the amino terminus of lubricin. Studies (T. Schmid, unpublished observations) suggest the epitope for the antibody is in the region of the protein coded by exon 3. Therefore, we presumed the antibody would recognize all of the four splice variants found in human tissue, because they all include the region coded by exon 3 [29].

One of the serial sections from paraffin blocks was allocated as a negative immunohistochemical control and treated with nonspecific mouse myeloma immunoglobulin IgG2a (Catalog 02-6200; Zymed Laboratories, South San Francisco, CA) instead of the lubricin antibody at the same concentration. Observation was achieved using biotinylated link as a secondary reagent, streptavidin–horseradish

peroxidase as a tertiary reagent (K0675; DakoCytomation), and substrate (AEC substrate chromogen; K3464; DakoCytomation). At the end of the immunohistochemical procedures, the slides were counterstained with hematoxylin and coverslips applied with Faramount (S3025; DakoCytomation).

We also stained one serial microtomed section from each sample with hematoxylin and eosin for general cell identification and examination of the structure of the extracellular matrix. One section from each sample was stained with Masson's trichrome stain for collagen. Microscope sections were viewed under normal and polarized light to reveal the birefringence characteristic of the crimp of the collagen bundles comprising the fascicles of the tendons. Only regions of the cadaveric specimens that displayed a normal histologic appearance were used as controls; eg, areas that displayed a fibrillated surface were excluded from the following analysis. No analyses were performed to determine differences in the lubricin layer or histologic makeup on the bursal and joint sides of the tendon.

When lubricin occurred on the torn edge of the supraspinatus and biceps tendon samples, it was present as a discrete layer less than approximately 10 μm in thickness. The area percentage of the torn edge displaying the presence of a lubricin layer, assuming a thickness of a surface layer of 5 μm , was estimated as a percentage of the edge displaying the layer using 10% increments. These measurements and those that follow were recorded by one evaluator (TF) with selected concurrence by a second evaluator (MS). No assessment of interobserver variability was made for any of the measurements. Because of the unknown interobserver variability, we did not base differences among groups on fine discriminations of the data. To determine the amount of lubricin staining in fascicular sheaths of the ruptured and cadaveric supraspinatus and biceps tendons, we graded one section from each tissue sample from 0 to ++ as follows: 0 = none; + = 20% or less of the sheath area; and ++ = greater than 20% of the sheath area. The value of 20% was an arbitrary dividing point between grades of + and ++ to provide a coarse discrimination of the relative amount of lubricin staining of the fascicular sheaths. Fascicles comprising the tendon were identified on the basis of the crimp pattern revealed by polarized microscopy.

We graded the lubricin staining of the extracellular matrix of the fascicles (ie, intrafascicular staining) on one section from each tissue sample with 0 to ++++ with the arbitrary cutoffs: 0 = none; + = up to 25% of the area; ++ = 26% to 50% of the area; +++ = 51% to 75% of the area; and ++++ = 76% to 100% of the area. In the ruptured supraspinatus and biceps tendon samples, we performed these analyses of the interfascicular and

intrafascicular staining separately on fascicles bordering the torn edge and those near the transected edge of the specimens.

We determined the percentage of lubricin-positive cells and the cell number density for the intact cadaveric and ruptured supraspinatus tendon samples and for the ruptured biceps tendon samples. Intracellular deposits of lubricin were identified on the basis of the following features: the red chromogen was adjacent to the hematoxylin-stained nucleus and the chromogen displayed a defined shape consistent with an intracellular morphology.

Five random fields of view (FOVs) in one microscope slide from each tissue sample were selected with an objective lens magnification of 20 $\times$ using a 10 $\times$ magnification eyepiece. The FOV was a square with 0.6 mm on a side. The total number of cells and the number of lubricin-staining cells in the area of the FOVs were counted. In the case of the ruptured tendons, we performed separate analyses on regions bordering the torn and transected edges of the tissue.

We assumed the percentage of lubricin-containing cells and the cell number density near the transected edge greater than 2 mm away from the torn edge would represent intact tissue at this site based on a prior study of the cell type and density at select locations away from the torn edge [9]. For the supraspinatus tendon samples, the percent lubricin-staining cells and the cell density at the transected site were compared with the corresponding values in the cadaveric controls. We also determined the percentage of lubricin-expressing cells and cell density in the supraspinatus and biceps tendon regions bordering the torn and transected edges. These analyses involved counting several hundred cells per location in each tendon sample. Because these data were not normally distributed, nonparametric statistical tests of significance were used. Unpaired comparisons of the data from the transected site of the supraspinatus tendon samples with the cadaveric specimens were made using the Mann-Whitney U test. Paired values from the torn and transected edges of the same ruptured supraspinatus specimens were compared using the Wilcoxon signed rank test.

Results

The torn edges of all of the ruptured supraspinatus and biceps tendons displayed a discrete layer of lubricin, less than 10 μ m thick (Figs. 1, 2; Table 2). None of the immunohistochemical negative control sections showed the red chromogen. No lubricin staining was observed on the edges of the tendons clearly identifiable as the location of the transection (Figs. 1A, 2A–B). Several torn edges displayed delaminations and folds, the surfaces of which

displayed the presence of lubricin as did fibrillated areas (Figs. 1A–F, 2A–B). Some edges displayed relatively few cells in the vicinity of the tear (Figs. 1A–C, 2B) and others had high cell densities (Figs. 1D–E, 2A).

The percentages of lubricin-containing cells (Figs. 1G–J; 2E–F) near the torn and transected edges (Table 2) were similar for the ruptured supraspinatus and biceps tendons: 16% $\pm$ 9% (mean $\pm$ SD) and 11% $\pm$ 9%, respectively, for the ruptured supraspinatus and 12% $\pm$ 7% and 13% $\pm$ 8% for the biceps tendon. We observed no difference in the percentage of the lubricin-containing cells at the torn versus transected edge for either tendon type, the torn edges of the supraspinatus and biceps tendons, or the transected edge of either ruptured tendon versus the cadaveric samples (Table 3; Fig. 3). Lubricin-containing cells in the tissues were generally of fibroblast morphology (Figs. 1H, 3E–F), but occasional rounded cells in lacunae, characteristic of chondrocytes, stained for lubricin. In some regions of the tissue, lubricin-expressing cells were of crimped morphology in register with the crimp of the extracellular matrix (Fig. 2F). Lubricin staining of the extracellular matrix was evident in the ruptured tendon samples (Table 2; Figs. 1E, 2B) and in the cadaveric specimens (Table 3; Fig. 3D).

The intact cadaveric tendons displayed lubricin layers on the joint- and bursal-facing surfaces (and the superior aspect and humeral head side; Fig. 3A–E). The cadaveric samples and the ruptured tendons also displayed the presence of lubricin in fascicular sheaths (Fig. 3D; Table 3) and on the surfaces of separations in the tendon (Figs. 1F, 2C–D, 3B), which may have existed *in vivo* or been caused during the histologic processing of the tissue. Lubricin staining also could be seen in planes that appeared to separate bundles of collagen fibers (Fig. 2C, white arrow), across which the collagen crimp remained in registry (Fig. 2D).

The cell number densities at the torn and transected edges of the supraspinatus tendons were 498 $\pm$ 225 and 317 $\pm$ 181 cells/mm², respectively, and for the biceps tendons 423 $\pm$ 191 and 265 $\pm$ 99 cells/mm² (Table 2). The cell density in the cadaveric supraspinatus tendon was 311 $\pm$ 99 cells/mm² (Table 3). We observed a difference in cell density between the torn and transected edges for the supraspinatus tendon (p = 0.018), but not for the biceps tendon (p = 0.068). There was no difference in cell density at the transected edge of the ruptured supraspinatus tendon and in the cadaveric samples.

Discussion

The recent finding of lubricin in caprine infraspinatus tendon [8], suggesting that it may facilitate intrafascicular

Fig. 1A–J continued

lubrication, has raised the question of the role of this lubricating and antiadhesion glycoprotein in healing of the ruptured rotator cuff. Our primary purpose was to determine if a layer of lubricin was on the torn edges of the ruptured human supraspinatus and biceps tendons to consider whether lubricin potentially may interfere with tendon repair. Secondary purposes were to determine the percentage of tendon cells that contained lubricin to ascertain if these cells were potential sources of the lubricin on the torn edges and to assess the presence of lubricin on the surfaces of the tendons and in the fascicular sheaths and the cell density at select locations in the tendons.

Our study had several limitations. First is the sample size. That a lubricin layer was found on the torn edge of every ruptured sample and no such layer was seen on the surgically transected internal control edge resulted in the

statistical differences even with the small sample size. We did not have enough samples, however, to stratify the data according to other factors, including age, gender, and time after rupture. Second, the phenotype of the cells was not determined other than by morphology and no analysis of the extracellular matrix molecules was performed. Future studies are needed to determine if the trauma associated with the rupture of the tendon induced a chondrogenic or fibrochondrogenic differentiation and to determine the makeup of the extracellular matrix. Third, we used a single monoclonal antibody to lubricin. Although this antibody has been used in immunohistochemical studies of a wide array of tissues with consistent findings, future work should use other lubricin antibodies for comparison. Fourth, the study did not include cadaveric controls matched for age, gender, and other factors. A fifth

Fig. 1A–J Sections through the remnants of ruptured supraspinatus tendon specimens stained for lubricin. **(A)** A section through the end of a ruptured supraspinatus tendon (SSP 3; Table 2) shows the absence of lubricin staining on the transected edge of the samples (arrows). Most of the convoluted surfaces of the torn edge of the sample display the presence of a discrete lubricin layer. Two low-magnification images were stitched together to produce the figure. **(B)** An immunohistochemical micrograph of the torn edge of a ruptured supraspinatus tendon shows the lubricin layer (SSP 3; Table 2). In this region of the tear, most of the tissue underlying the torn edge displays a low cell number density. Two low-magnification images were stitched together to produce the figure. **(C)** Fragments and frayed edges of the tendon are covered with a lubricin layer (SSP 3; Table 2). **(D)** A finger-like process of the torn edge of the tendon is surfaced with lubricin. The processes in this micrograph display high

cell density (SSP 5; Table 2). **(E)** The torn edge of the ruptured tendon shows a lubricin surface layer and lubricin distributed throughout at the underlying extracellular matrix (SSP 5; Table 2). **(F)** Delaminations in a ruptured supraspinatus tendon are seen (SSP 7; Table 2). The inset is the image viewed under polarized light. **(G)** Lubricin-expressing cells are observed underlying a lubricin surface layer (SSP 6; Table 2). **(H)** Lubricin in cells and extracellular matrix are seen (SSP 5; Table 2). **(I)** A region of high cell density in which most of the cells contain lubricin (SSP 2; Table 2) is shown. The boxed area is shown at higher magnification **(J)**. **(J)** The intracellular presence of lubricin is seen in this illustration (arrows indicate representative examples). (Stain, immunohistochemically stained for lubricin with hematoxylin counterstain; original magnification: **(A)** 4×; **(B)** 4×; **(C)** 4×; **(D)** 10×; **(E)** 10×; **(F)** 10×; **(G)** 1000×; **(H)** 1000×; **(I)** 400×; **(J)** 1000×.)

limitation relating to our supposition regarding the potential role of lubricin in interfering with tendon repair is lubricin may have other functions, not yet discovered, that may favor tissue repair.

The notable finding of our study is the observation of a discrete lubricin layer on the torn edges of supraspinatus

and biceps tendons. The fact that transected edges, produced during trimming of the samples after resection, were not surfaced with lubricin suggests the lubricin layer on the separated surfaces was formed in vivo. The principal source of the lubricin found on the torn edge was likely the joint fluid. Prior study in the caprine humeral joint [8]

Fig. 2A–F Immunohistochemical analysis of ruptured biceps tendon specimens showed the distribution of lubricin. **(A)** A section through the end of a ruptured biceps tendon (BT 3; Table 2) shows the absence of a lubricin layer on the transected edge of the samples (arrows). Most of the surfaces of the torn edge of the sample display the presence of a discrete lubricin layer. Most of the tissue processes and projections at the torn edge show a high cell number density. Two low magnification images were stitched together to produce the figure. **(B)** A convoluted surface of the torn edge of a ruptured biceps shows the presence of a lubricin layer (BT 2; Table 2). No lubricin is seen on the transected edge of the samples (arrows). Most of the sample has low cell density, and no cells are seen in selected fragments (lower left). **(C)** A lubricin-coated fissure (arrow) can be seen through a biceps sample (BT 2; Table 2). Lubricin also can be seen in the extracellular matrix. **(D)** A polarized light micrograph of the area in C is shown. **(E)** Lubricin-containing cells near a lubricin surface layer are seen in this sample (BT 2; Table 2). **(F)** A crimped portion of a ruptured biceps tendon sample shows the presence of lubricin adapting to the crimp pattern (BT 2; Table 2). (Stain, immunohistochemically stained for lubricin with hematoxylin counterstain; original magnification: **(A)** 4×; **(B)** 4×; **(C)** 400×; **(D)** 400×; **(E)** 1000×; **(F)** 400×.)

Table 2. Lubricin distribution in the torn rotator cuff and biceps tendon samples

Location and parameter	Supraspinatus tendon							Biceps tendon			
	1	2	3	4	5	6	7	1	2	3	4
Width (mm)*	2.5	2.9	3.7	2.8	2.1	3.2	4.4	12.7	4.8	8.1	5.2
Torn edge											
Percent of surface with a lubricin layer [†]	90	80	80	20	90	20	80	90	80	60	100
Fascicular sheath [‡]	++	+	++	0	+	+	+	+	+	+	+
Intrafascicular ECM [§]	++	+	+	+	+	+	+	+	++	+	++
Percent lubricin-positive cells, mean $\pm$ SD	19 $\pm$ 8.0	24 $\pm$ 8.0	6 $\pm$ 11.3	29 $\pm$ 2.9	12 $\pm$ 9.2	7 $\pm$ 7.4	16 $\pm$ 7.3	6 $\pm$ 2.7	12 $\pm$ 3.4	8 $\pm$ 3.7	22 $\pm$ 8.9
Cell number density (cells/mm ²), mean $\pm$ SD	407 $\pm$ 248	860 $\pm$ 56	452 $\pm$ 199	124 $\pm$ 80	487 $\pm$ 131	647 $\pm$ 189	512 $\pm$ 89	226 $\pm$ 79	685 $\pm$ 339	391 $\pm$ 168	388 $\pm$ 63
Transected edge											
Fascicular sheath	++	+	+	0	+	0	0	+	+	0	+
Intrafascicular ECM	++	+	+	0	+	0	+	0	+	0	+
Percent lubricin-positive cells, mean $\pm$ SD	19 $\pm$ 2.8	27 $\pm$ 10.4	10 $\pm$ 6.0	5 $\pm$ 2.8	5 $\pm$ 4.0	1 $\pm$ 0.4	11 $\pm$ 5.1	9 $\pm$ 3.7	8 $\pm$ 1.6	10 $\pm$ 4.5	24 $\pm$ 8.9
Cell number density (cells/mm ²), mean $\pm$ SD	337 $\pm$ 172	466 $\pm$ 80	308 $\pm$ 136	88 $\pm$ 18	127 $\pm$ 44	608 $\pm$ 186	288 $\pm$ 72	118 $\pm$ 47	309 $\pm$ 83	295 $\pm$ 140	337 $\pm$ 100

* Width of resected tissue sample measured from the torn surface to the transected surface; [†] area percentage of the torn edge displaying the presence of a lubricin layer, assuming a thickness of a surface layer of 5 μ m; there was no lubricin staining on the transected edge of the tissue; [‡] Grade, 0-++ based on the lubricin staining in the fascicular sheaths: 0 = none; + = $\leq$ 20% of the sheath area; ++ = $>$ 20% of the sheath area; [§] Grade, 0-++++ based on the areal percentage of lubricin staining within the fascicles: 0 = none; + = up to 25%; ++ = 26%-50%; +++ = 51%-75%; ++++ = 76%-100%; ECM = extracellular matrix.

Table 3. Lubricin distribution in intact cadaveric rotator cuff specimens and cell number density

Parameter	1 (M)*		2 (M)		3 (M)		4 (M)		5 (F)		6 (F)	
	L	R	L	R	L	R	L	R	L	R	L	R
Fascicular sheath [†]	++	+	+	+	+	+	+	+	+	+	0	+
Intrafascicular ECM [‡]	+	+	+	+	+	+	+	+	+	+	+	+
Percent lubricin-positive cells, mean $\pm$ SD	10 $\pm$ 3.4	8 $\pm$ 2.1	10 $\pm$ 2.4	3 $\pm$ 1.5	6 $\pm$ 1.0	5 $\pm$ 2.6	9 $\pm$ 0.9	4 $\pm$ 1.7	7 $\pm$ 2.9	5 $\pm$ 1.0	4 $\pm$ 0.8	8 $\pm$ 3.9
Cell number density (cells/mm ²), mean $\pm$ SD	277 $\pm$ 98	413 $\pm$ 54	157 $\pm$ 43	238 $\pm$ 24	327 $\pm$ 57	213 $\pm$ 59	384 $\pm$ 62	287 $\pm$ 60	416 $\pm$ 105	499 $\pm$ 127	247 $\pm$ 32	268 $\pm$ 17

* M = male; F = female; L = left; R = right; [†]Grade, 0-++ = none; + = $\leq$ 20% of the sheath area; ++ = $>$ 20% of the sheath area; [‡]Grade 0-+++ = 76%-100%; ECM = extracellular matrix. ++ = 26%-50%; +++ = 51%-75%; ++++ = 76%-100%; 0 = none; + = 1%-25% of the area; ++ = 26%-50%; +++ = 51%-75%; ++++ = 76%-100%; ECM = extracellular matrix.

found that the synovial cells and superficial zone chondrocytes of the humeral head cartilage expressed lubricin. Likely the same cells in the human humeral joint synthesize lubricin, which accumulates in the joint fluid. The finding of a lubricin layer on the torn edge of the tendons provides a speculative supposition for why healing of these tissues is at times incomplete. A prior study investigated the effects of a surface layer of lubricin on integrative binding using articular cartilage explants [6]. Apposing surfaces to which lubricin was bound displayed less binding in mechanical tests compared with control surfaces with no lubricin. Restoration of the contiguity of the ends of a torn tendon requires adhesion of cells and extracellular matrix molecules, which fill the gap, with the torn edges. The presence of an antiadhesion protein on the surfaces would likely interfere with this process.

We found 3% to 10% of cells in the cadaveric tendons and 1% to 29% of the cells in the ruptured tendons contained lubricin. The presence of lubricin in the extracellular matrix suggested the lubricin released from the tendon cells may be diffusing through the tissue, perhaps to reach the surface. Evidence of the constitutive production of lubricin by cells in the tendons is important in light of some studies suggesting mechanical stimuli regulate lubricin expression [10, 23, 26, 29]. Jones and Flannery [16] also investigated the effects of various cytokines on lubricin expression and suggested transforming growth factor (TGF)- β increased lubricin synthesis, secretion, and cartilage boundary association. Among the cytokines found in animal models [17, 18] and human subjects with ruptured rotator cuffs [21, 25, 33] is TGF- β [17, 25]. Moreover, of importance is a recent finding [34] that contraction of myofibroblasts (ie, α -SMA expressing fibroblast) in the torn rotator cuff [22] activates latent TGF- β 1 from extracellular matrix. It would be expected that the mechanical trauma and TGF- β release in the ruptured rotator cuff would upregulate the amount of lubricin in the environment of the torn edges. Additional studies are needed to test this hypothesis.

We found lubricin layers on the bursal and humeral joint surfaces of and in tissue planes in the the supraspinatus and biceps tendons. Other investigators using the same antibody observed lubricin on the surface of canine digitorum profundus [28, 29] and on the surface and in the fascicular sheaths of the caprine infraspinatus tendon [8]. The same properties of lubricin that underlie its physiologic function on the surface of tendons and in fascicular sheaths may disfavor the healing of ruptured tendons when lubricin forms on the torn edges.

One of the critical questions related to the repair of the ruptured rotator cuff is the degree to which the torn ends should be débrided, ie, freshened up before reattachment [3, 4, 9, 11, 12, 19]. One issue relates to the vascularity and cell type and density [9] at the torn ends, which would

Fig. 3A–F Immunohistochemical micrographs show the distribution of lubricin (red chromogen) in the cadaveric (A–D) supraspinatus and (E–F) biceps tendons. (A) A discrete layer of lubricin can be seen on the bursal side (B) of the tendon (top). Lubricin also can be seen distributed through the extracellular matrix of the tissue in selected regions (5L; Table 2). (B) A lubricin layer is shown on the joint side (J) of the cadaveric supraspinatus tendon (bottom). The edges of some fissures in the supraspinatus tendon also were covered with lubricin (arrow; 4L; Table 2). (C) A higher magnification micrograph shows a lubricin layer (approximately 10 μm in thickness) on the joint side (J) of the supraspinatus tendon (5L; Table 2). (D) Thin lubricin-containing planes can be seen separating collagen bundles (white

arrows). Lubricin also can be seen in elongated fibroblasts (black arrows) and in the extracellular matrix (5R; Table 2). (E) A lubricin layer can be seen on the superior aspect (SA) of the cadaveric biceps tendon (top). Elongated cells are dispersed through the lubricin-staining surface. Fissures in the tissue, which do not stain for lubricin, may be tears produced during microtoming (2R; Table 2). (F) The humeral head side (HHS) of the biceps tendon (bottom) is surfaced with a layer of lubricin. Lubricin also can be seen intracellularly (arrow; 2R; Table 2). (Stain, immunohistochemically stained for lubricin with hematoxylin counterstain; original magnification: (A) 200×; (B) 400×; (C) 1000×; (D) 400×; (E) 400×; (F) 400×.)

favor the reparative process. Previously no differences were observed in nuclear distribution (reflecting cell number) in sites near and away from the tear [9]. In contrast, in our current work, we found the cell number density at the torn edge was 50% greater than that at the transected edge, from 2 to 4.5 mm away from the tear; the cell density

at the transected edge was comparable with the cadaveric control samples. This is consistent with the speculation that, when removing the antiadhesion lubricin layer, which may interfere with integrative healing, as much of the torn edge as possible should be preserved to access the reparative potential of the underlying hypercellular zone.

Our findings may prove useful in raising questions regarding the potential benefits of therapeutic agents to be injected in the ruptured rotator cuff, but which increase the lubricin layer on the torn edges and might impair healing. Moreover, the results may inform future studies of other ruptured intraarticular tissues (meniscus, cruciate ligaments, and articular cartilage) with respect to the presence and role of lubricin on the torn edges.

References

- Berthelot JM. Could rheumatoid arthritis result from an abnormal T cell response towards lubricin/superficial zone protein? *Med Hypotheses*. 2004;62:894–897.
- Carpenter JE, Thomopoulos S, Flanagan CL, DeBano CM, Soslowky LJ. Rotator cuff defect healing: a biomechanical and histologic analysis in an animal model. *J Shoulder Elbow Surg*. 1998;7:599–605.
- Codman EA. *The Shoulder*. Boston, MA: Thomas Todd; 1934.
- Craig E. Open anterior acromioplasty for full thickness rotator cuff tears. In: Craig E, ed. *The Shoulder*. New York, NY: Rowan Press; 1995:21–27.
- DeFranco MJ, Bershadsky B, Ciccone J, Yum JK, Iannotti JP. Functional outcome of arthroscopic rotator cuff repairs: a correlation of anatomic and clinical results. *J Shoulder Elbow Surg*. 2007;16:759–765.
- Englert C, McGowan KB, Klein TJ, Giurea A, Schumacher BL, Sah RL. Inhibition of integrative cartilage repair by proteoglycan 4 in synovial fluid. *Arthritis Rheum*. 2005;52:1091–1099.
- Flannery CR, Hughes CE, Schumacher BL, Tudor D, Aydelotte MB, Kuettner KE, Caterson B. Articular cartilage superficial zone protein (SZP) is homologous to megakaryocyte stimulating factor precursor and is a multifunctional proteoglycan with potential growth-promoting, cytoprotective, and lubricating properties in cartilage metabolism. *Biochem Biophys Res Commun*. 1999;254:535–541.
- Funakoshi T, Schmid T, Hsu HP, Spector M. Lubricin distribution in the goat infraspinatus tendon: a basis for interfascicular lubrication. *J Bone Joint Surg Am*. 2008;90:803–814.
- Goodmurphy CW, Osborn J, Akesson EJ, Johnson S, Stanesco V, Regan WD. An immunocytochemical analysis of torn rotator cuff tendon taken at the time of repair. *J Shoulder Elbow Surg*. 2003;12:368–374.
- Grad S, Lee CR, Gorna K, Gogolewski S, Wimmer MA, Alini M. Surface motion upregulates superficial zone protein and hyaluronan production in chondrocyte-seeded three-dimensional scaffolds. *Tissue Eng*. 2005;11:249–256.
- Hawkins RH. The rotator cuff and biceps tendon. In: Evarts M, ed. *Surgery of the Musculoskeletal System*. New York, NY: Churchill Livingstone; 1983.
- Hawkins RJ, Misamore GW, Hobeika PE. Surgery for full-thickness rotator-cuff tears. *J Bone Joint Surg Am*. 1985;67:1349–1355.
- Iannotti JP. Full-thickness rotator cuff tears: factors affecting surgical outcome. *J Am Acad Orthop Surg*. 1994;2:87–95.
- Jay GD, Tantravahi U, Britt DE, Barrach HJ, Cha CJ. Homology of lubricin and superficial zone protein (SZP): products of megakaryocyte stimulating factor (MSF) gene expression by human synovial fibroblasts and articular chondrocytes localized to chromosome 1q25. *J Orthop Res*. 2001;19:677–687.
- Jay GD, Torres JR, Warman ML, Laderer MC, Breuer KS. The role of lubricin in the mechanical behavior of synovial fluid. *Proc Natl Acad Sci USA*. 2007;104:6194–6199.
- Jones ARC, Flannery CR. Bioregulation of lubricin expression by growth factors and cytokines. *Eur Cell Mater*. 2007;13:40–45.
- Kobayashi M, Itoi E, Minagawa H, Miyakoshi N, Takahashi S, Tuoheti Y, Okada K, Shimada Y. Expression of growth factors in the early phase of supraspinatus tendon healing in rabbits. *J Shoulder Elbow Surg*. 2006;15:371–377.
- Koshima H, Kondo S, Mishima S, Choi HR, Shimpō H, Sakai T, Ishiguro N. Expression of interleukin-1 β , cyclooxygenase-2, and prostaglandin E2 in a rotator cuff tear in rabbits. *J Orthop Res*. 2007;25:92–97.
- Longo UG, Franceschi F, Ruzzini L, Rabitti C, Morini S, Maffulli N, Denaro V. Histopathology of the supraspinatus tendon in rotator cuff tears. *Am J Sports Med*. 2008;36:533–538.
- Matthews TJ, Hand GC, Rees JL, Athanasou NA, Carr AJ. Pathology of the torn rotator cuff tendon. Reduction in potential for repair as tear size increases. *J Bone Joint Surg Br*. 2006;88:489–495.
- Osawa T, Shinozaki T, Takagishi K. Multivariate analysis of biochemical markers in synovial fluid from the shoulder joint for diagnosis of rotator cuff tears. *Rheumatology Int*. 2005;25:436–441.
- Premdas J, Tang J-B, Warner JP, Meaney Murray M, Spector M. The presence of smooth muscle actin in fibroblasts in the torn human rotator cuff. *J Orthop Res*. 2001;19:221–228.
- Rees SG, Davies JR, Tudor D, Flannery CR, Hughes CE, Dent CM, Caterson B. Immunolocalisation and expression of proteoglycan 4 (cartilage superficial zone proteoglycan) in tendon. *Matrix Biol*. 2002;21:593–602.
- Rhee DK, Marcelino J, Baker M, Gong Y, Smits P, Lefebvre V, Jay GD, Stewart M, Wang H, Warman ML, Carpten JD. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. *J Clin Invest*. 2005;115:622–631.
- Sakai H, Fujita K, Sakai Y, Mizuno K. Immunolocalization of cytokines and growth factors in subacromial bursa of rotator cuff tear patients. *Kobe J Med Sci*. 2001;47:25–34.
- Schumacher BL, Block JA, Schmid TM, Aydelotte MB, Kuettner KE. A novel proteoglycan synthesized and secreted by chondrocytes of the superficial zone of articular cartilage. *Arch Biochem Biophys*. 1994;311:144–152.
- Su JL, Schumacher BL, Lindley KM, Soloveyichik V, Burkhardt W, Triantafyllou JA, Kuettner K, Schmid T. Detection of superficial zone protein in human and animal body fluids by cross-species monoclonal antibodies specific to superficial zone protein. *Hybridoma*. 2001;20:149–157.
- Sun Y, Berger EJ, Zhao C, An KN, Amadio PC, Jay G. Mapping lubricin in canine musculoskeletal tissues. *Connect Tissue Res*. 2006;47:215–221.
- Sun Y, Berger EJ, Zhao C, Jay GD, An KN, Amadio PC. Expression and mapping of lubricin in canine flexor tendon. *J Orthop Res*. 2006;24:1861–1868.
- Swann DA, Silver FH, Slayter HS, Stafford W, Shore E. The molecular structure and lubricating activity of lubricin isolated from bovine and human synovial fluids. *Biochem J*. 1985;225:195–201.
- Swann DA, Slayter HS, Silver FH. The molecular structure of lubricating glycoprotein-I, the boundary lubricant for articular cartilage. *J Biol Chem*. 1981;256:5921–5925.
- Thomopoulos S, Hattersley G, Rosen V, Mertens M, Galatz L, Williams GR, Soslowky LJ. The localized expression of

- extracellular matrix components in healing tendon insertion sites: an in situ hybridization study. *J Orthop Res.* 2002;20:454–463.
33. Voloshin I, Gelinas J, Maloney MD, O’Keefe RJ, Bigliani LU, Blaine TA. Proinflammatory cytokines and metalloproteases are expressed in the subacromial bursa in patients with rotator cuff disease. *Arthroscopy.* 2005;21:1076.
34. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol.* 2007;179:1311–1323.
35. Zhang D, Johnson LJ, Hsu HP, Spector M. Cartilaginous deposits in subchondral bone in regions of exposed bone in osteoarthritis of the human knee: histomorphometric study of PRG4 distribution in osteoarthritic cartilage. *J Orthop Res.* 2007;25:873–883.