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From Cell Biology to Tissue Engineering

Abundant lubricin expression suggests a link between synoviocytes, synovial tumors, and myxomas

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Summary. Progenitor cell differentiation into fibroblast-like synoviocytes (FLSs) and their ensuing phenotypic changes are incompletely explored. Synovial lining is composed of intimal macrophages and FLSs. FLSs have epithelioid morphology and directionally secrete components of synovial fluid, including lubricin. We stained human tissues and tumors using two anti-lubricin antibodies. Lubricin was found in FLSs in synovium and in tenosynovial giant cell tumors (TSGCTs) and not in the associated monocyte/macrophage cells, which were identified by double immunostaining for CD163. In TSGCTs, giant cells, known to form by fusion of mononuclear cells, were negative for both lubricin and CD163. Occasional mononuclear cells with the same phenotype were also seen, suggesting that the precursors of the giant cells are derived from the minor CD163-negative monocyte subset. Lubricin was also detected in intramuscular myxomas, in early myxoid changes of ganglion cysts, and in one of five low-grade myxofibrosarcomas, but not in other fibroconnective tissues, epithelial tissues, or other tumors tested. This suggests that lubricin expression may typify adaptive and neoplastic changes along a pathway toward FLSs. Further support for this concept comes from ganglion cysts and juxta-articular myxoma tumors, which show a spectrum of myxoid, cystic and synovial differentiation, and in which moderate lubricin staining of myxoid stroma was seen.

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Introduction

Joints, tendon sheaths, and bursae are lined by specialized tissue consisting of intimal macrophages and fibroblast-like synoviocytes (FLSs), which resemble one another histologically. Nevertheless, the former are marrow-derived and related to monocytes, while the latter are stromally derived and secrete lubricating synovial fluid components. FLSs have been shown to express the monocyte attractant macrophage colony-stimulating factor (M-CSF) (Seitz et al., 1994), also known as CSF1, indicating a dynamic relationship between the two synovial lining cell types in maintaining architectural homeostasis. FLSs secrete lubricin (Flannery et al., 1999) and hyaluronan. Lubricin is a secreted, mucinous glycoprotein that is approximately 50% (w/w) carbohydrate (Swann et al., 1981) and functions as a lubricant in synovial fluid (Swann et al.,

Abbreviations. FLSs, fibroblast-like synoviocytes; TSGCTs, tenosynovial giant cell tumors; M-CSF, macrophage colony-stimulating factor; Prg4, proteoglycan 4; SZP, superficial zone protein; DOL54, downstream of the liposarcoma-associated fusion oncoprotein 54; CACP, camptodactyly-arthropathy-coxa vara pericarditis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; BSA, bovine serum albumin; PBS, phosphate-buffered saline; HRP, horseradish peroxidase; H&E, hematoxylin and eosin; TBS, Tris-buffered saline; DAB, 3,3'-diaminobenzidine; GCTTS, giant cell tumor of tendon sheath; PVNS, pigmented villonodular synovitis; RANK-L, receptor activator of nuclear factor kappa-B ligand; GNAS1, stimulatory G-protein alpha subunit

1981; Jay, 1992; Jay et al., 2001). (Lubricin is also known as proteoglycan 4 (Prg4), superficial zone protein (SZP), and downstream of the liposarcoma-associated fusion oncoprotein 54 (DOL54).) The physiological role of lubricin is apparent in rare individuals with autosomal recessive, loss-of-function mutations (Marcelino et al., 1999); they suffer from camptodactyly-arthropathy-coxa vara pericarditis (CACP) syndrome (MIM 208250), a disorder of premature joint contracture caused by a non-inflammatory arthropathy (Marcelino et al., 1999; Faivre et al., 2000). The expression pattern of lubricin mRNA is highly restricted. Of 305 tissues in the human body, the synovial membrane has the highest expression (Hruz et al., 2008). The protein expression pattern of lubricin is incompletely characterized, due in part to lack of robust and specific reagents.

Ganglion cysts, the most common soft tissue masses of the hand and wrist, are thought to be the end result of a degenerative change characterized by excessive mucin secretion by fibroblasts (Goldblum et al., 2014a). Resection specimens of ganglion cysts, in addition to classic fibrous-walled cavities with few lining cells and containing clear viscous material, sometimes have synovial-lined areas and areas with stromal myxoid change with sparse cellularity. These changes are mirrored in the histology of an infrequent tumor, juxta-articular myxoma, which contains hypocellular myxoid areas, synovial-like areas, and cystic areas devoid of lining cells (Graadt van Roggen et al., 1999; Wakely Jr et al., 2005). Intramuscular myxomas, which harbor a stimulatory G-protein alpha subunit (GNAS1) mutation (Delaney et al., 2009; Walther et al., 2014), are benign neoplasms composed of sparse stromal cells in abundant myxoid matrix (Goldblum et al., 2014a), and they bear a resemblance to the much smaller, sparsely cellular myxoid areas near ganglion cysts.

Classification of mesenchymal tumors is based on resemblance to non-neoplastic cell and tissue counterparts and on identification of characteristic chromosomal translocations and other genetic features. Using standard histochemical staining, the lineage of most well-differentiated tumors, such as those of smooth muscle or of cartilage, is easy to identify. However, some mesenchymal tumors have distinctive myxoid, chondromyxoid, or fibromyxoid stroma on routine histological stains, which may have a corresponding biochemical correlate. For example, low-grade fibromyxoid tumors typically express the mucin glycoprotein MUC4 (Doyle et al., 2011), and its intracellular form can be detected by immunohistochemistry. The specific composition of the matrix in these tumors may reflect a normal pathway of mesenchymal differentiation.

The most common neoplasms related to the synovium are TSGCTs. Localized and diffuse TSGCTs, also known as giant cell tumors of tendon sheath (GCTTS) and pigmented villonodular synovitis (PVNS), respectively, are composed of monocytes/macrophages, including siderophages and xanthoma cells, multinucleated osteoclast-like giant cells, and plump FLSs (Goldblum et al., 2014b). The neoplastic cells in TSGCTs are the FLSs—not the marrow derived monocyte/macrophages (O'Connell et al., 1995; West et al., 2006; Boland et al., 2009; Goldblum et al., 2014b). The FLSs in TSGCTs overproduce M-CSF, usually as a result of chromosomal translocation (West et al., 2006; Cupp et al., 2007), thereby recruiting monocyte/macrophages, promoting macrophage differentiation and survival (Motoyoshi, 1998), and triggering receptor activator of nuclear factor kappa-B ligand (RANKL)-induced formation of osteoclast-like giant cells from monocytes (Fujikawa et al., 1996; Quinn et al., 1998). The marrow-derived monocytic cells express lineage-identifying markers such as CD4 (personal observation, IJM), CD14 (Athanasou et al., 1991), CD45, CD68, and CD163 (Goldblum et al., 2014b). The FLSs have an immunoprofile (positive for clusterin, desmin and podoplanin; negative for CD163) identical to normal synovial lining cells (West et al., 2006; Boland et al., 2009).

Our aim was to characterize the expression pattern of lubricin in human tissues and in selected tumors, using two anti-lubricin antibodies. We detected lubricin within FLSs of synovial lining and in tenosynovial giant cell tumors (TSGCTs), distinguishing FLSs from monocyte lineage (CD163-positive) cells by two-color immunostaining. These reagents also detected abundant cytoplasmic lubricin in intramuscular myxoma cells, suggesting relatedness to FLSs.

Materials and methods

Human tissues and fluids

Archival tissues and fresh fluids from existing pathological specimens from the authors' institution were collected with approval from the Institutional Review Board (IRB no. 13052202-IRB01, 2013) of the authors' institution, with waiver of consent. Synovial fluid was taken from a non-inflamed knee of an adult at autopsy without additional processing. Synovial samples were taken from non-inflammatory arthropathy specimens at arthroplasty, from synovium near an intratendinous cyst, and from non-inflammatory synovitis of possible traumatic etiology. The following representative samples from surgical resection specimens were stained with both Ab28484 (polyclonal, hereafter designated pAb28484) and S6.79 (monoclonal) antibodies: synovium (n=2), liver, colon, intact mesothelial-lined hernia sac tissue, gallbladder with acute cholecystitis, fetal and placental tissues from 15 to 16 weeks of gestation (umbilical cord, placenta, heart, small intestine, peripheral nerve, kidney, brain, eye, lung, liver with hematopoietic elements, bone, cartilage, skin and subcutis, and colon), localized (n=11) and diffuse tenosynovial giant cell tumors, ganglion cysts (n=3), intramuscular myxomas (n=4), an atrial myxoma, chordomas (n=2), low-grade myxofibrosarcomas (n=5),

low-grade fibromyxoid sarcomas (n=2), myxoid liposarcomas (n=2), a well-differentiated liposarcoma with myxoid areas, extraskeletal myxoid chondrosarcomas (n=2), a giant cell tumor of soft tissue, a low-grade micropapillary serous ovarian adenocarcinoma, and high-grade papillary serous ovarian carcinomas (n=2). Additional specimens were stained only with the S6.79 monoclonal antibody (after depletion of the active lot of pAb28484, see below): additional synovium (n=4), additional liver (n=5), pericardium, scalp skin, female breast, premenopausal ovary, fallopian tube, uterine corpus with secretory endometrium, ectocervix and endocervix, tail of pancreas, benign reactive lymph nodes, eyelid, thyroid, brain, lung, bronchus, prostate with stromal and glandular hyperplasia, nasopharyngeal mucosa (n=2), testes, epididymis, parotid, duodenum, fundus and antrum of stomach, esophagus, adrenal gland, kidney, parathyroid, ileum, additional localized and diffuse tenosynovial giant cell tumors (one each), additional ganglion cyst, a juxta-articular myxoma, and additional chordoma.

Antibodies

Two primary anti-lubricin antibodies were used: pAb28484, an affinity-purified rabbit polyclonal antibody made against a C-terminal peptide corresponding to amino acids #1356-1374 of human lubricin (Abcam, Cambridge, MA, USA), and S6.79, a purified mouse monoclonal antibody raised against purified, native human lubricin (Su et al., 2001). Due to a limited supply of pAb28484 lot no. GR116636, only a few non-neoplastic tissues could be examined with pAb28484; A subsequent lot of pAb28484 (lot no. GR137897) showed less specific staining on Western blotting and immunohistochemistry and was not used. Secondary antibodies were: horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (catalog no. 31462, Thermo Fisher Scientific, Rockford, IL, USA), HRP-conjugated goat anti-mouse IgG (catalog no. 31432, Thermo Fisher Scientific, Rockford, IL, USA). For two-color immunostaining, the primary anti-CD163 monoclonal antibody used was 10D6 (NCL-L-CD163, Leica Microsystems, Buffalo Grove, IL, USA). HRP-conjugated anti-rabbit IgG (Bond™ Polymer Refine Detection kit, catalog no. DS9800, Leica Microsystems, Buffalo Grove, IL, USA) was used for the double staining blocking step, and the secondary antibody was from the Bond™ Polymer Refine Red Detection kit (catalog no. DS9390, Leica Microsystems, Buffalo Grove, IL, USA), which uses a rabbit anti-mouse IgG and an alkaline phosphatase-conjugated anti-rabbit IgG.

Western blotting

Synovial fluid from one individual was boiled at 95°C for 5 min in non-reducing Laemmli sample buffer (catalog no. 161-0737, Bio-Rad, Hercules, CA, USA). Synovial fluid (0.250 µL for pAb28484 and 0.125 µL for S6.79) was electrophoresed on 4.5-10-20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels, according to Laemmli (1970). Resolved synovial fluid proteins were transferred to a nitrocellulose membrane (0.45 µm, Bio-Rad, Hercules, CA, USA) in transfer buffer (12 mM Tris, 10 mM sodium acetate, 15 mM EDTA, pH 9.5) at 25 V, for 16 h at 4°C. Membranes were blocked in 1% (w/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.4, incubated with primary anti-lubricin antibody (pAb28484 or S6.79; 2 µg/mL) in 0.1% (w/v) BSA in PBS, pH 7.4, for 16 h at 4°C. Membranes were incubated with secondary antibodies (HRP-conjugated goat anti-rabbit IgG or goat anti-mouse IgG; 1:5000) in PBS, 0.05% Tween-20, pH 7.4, for 1 h at room temperature, developed with Super Signal West Pico chemiluminescent substrate (catalog no. 34077, Thermo Fisher Scientific, Rockford, IL, USA), and imaged (90 s for pAb28484, 1.160 s for S6.79) (ChemiDoc™ XRS+, Bio-Rad, Hercules, CA, USA).

Immunohistochemistry and special stains

Representative hematoxylin and eosin (H&E)-stained slides were reviewed. Five-µm-thick sections were cut from formalin-fixed paraffin-embedded tissue, baked at 65°C for 1 h, deparaffinized in xylene, and rehydrated in a graded ethanol series. Antigen retrieval was performed at 95°C for 20 min in 10 mM Tris, 1 mM EDTA, 0.05% Tween-20, pH 9.0. Tissue sections were blocked with 1% (w/v) BSA in PBS, pH 7.4, at 37°C for 30 min. Slides were incubated with primary antibodies (pAb28484 at 1:250 or S6.79; 2 µg/mL) in 0.1% (w/v) BSA in PBS, pH 7.4, for 16 h at 4°C. Each experiment included a negative control of human synovium without primary antibody. Endogenous peroxidase activity was quenched by 0.3% H_2O_2 in Tris-buffered saline (TBS), pH 7.5, for 15 min at room temperature. Slides were incubated with secondary antibodies (HRP-conjugated goat anti-rabbit IgG or goat anti-mouse IgG; 1:1000) in PBS, 0.05% Tween-20, pH 7.4, for 1 h at room temperature, and developed with 3,3'-diaminobenzidine (DAB) (SigmaFAST DAB tablets, catalog no. D4293, Sigma, St. Louis, MO, USA). To distinguish brown DAB reactivity from hemosiderin present in the tissue, slides were stained using the Perls' Prussian blue reaction (0.6 M HCl, 0.06 M potassium ferrocyanide) for 20 min at room temperature. Sections were counterstained with hematoxylin (catalog no. 7211, Thermo Fisher Scientific, Rockford, IL, USA), dehydrated, mounted, and photographed using an Olympus BX41 microscope and camera (Diagnostics Instruments, Inc. model 18.2 Color Mosaic, Olympus, Japan). For double immunostaining, coverslips of pAb28484-stained sections were removed by soaking in xylene, and sections were rehydrated in a graded ethanol series. To preclude antibody cross-recognition, as a blocking step, sections were first incubated with an

HRP-conjugated anti-rabbit secondary antibody and developed in DAB according to the manufacturer's protocol. Double staining for CD163 was performed using the anti-CD163 monoclonal antibody 10D6 (1:200) and the red substrate for alkaline phosphatase development using the Bond™ Polymer Refine Detection kit (catalog no. DS9390, Leica Microsystems, Buffalo Grove, IL, USA) according to the manufacturer's protocol with a Leica Bond III automated stainer (Leica Microsystems, Buffalo Grove, IL, USA). Alcian blue (pH 2.5) stain was performed with a Benchmark Special Stains instrument (Ventana Medical Systems, Tucson, AZ, USA) using the manufacturer's protocol.

Results

Western blot analysis of lubricin in human synovial fluid

On Western blot analysis of synovial fluid, the rabbit polyclonal antibody pAb28484 recognized a distinct band that migrated at a position of 150 kDa on SDS-PAGE (Fig. 1), corresponding to the size of the unglycosylated lubricin core protein. The S6.79 mouse monoclonal antibody recognized an approximately 345-kDa polydisperse signal (Su et al., 2001), corresponding to the heavily glycosylated form of lubricin (Fig. 1). The pAb28484 reagent had much weaker reactivity for the high molecular weight form of lubricin (Fig. 1).

Patterns of lubricin detection in synovium using pAb28484 and S6.79 antibodies

Hematoxylin and eosin-stained synovial tissues from pathology specimens showed surface lining morphology that varied from areas lined by sparse cells to areas with a defined double layer of cells (Fig. 2A), to areas with multi-layered surface and subintimal stromal cell accumulation. In all synovial samples (including normal synovium adjacent to TSGCTs) stained with pAb28484, lubricin expression was limited to the cytoplasm of synoviocytes. In areas showing a double layer of synovial lining cells, staining was found predominantly in the basal layer (Fig. 2B), previously reported to correspond to FLSs (Wilkinson et al., 1992; Smith, 2011). The reactivity of S6.79 was most intense on the surface lining in the extracellular space, with occasional, weaker cytoplasmic synoviocyte staining (Fig. 2C). The underlying stroma showed variable intensity staining, overall much weaker than the luminal staining (Fig. 2C). CD163 is strongly expressed in most monocyte-lineage cells, including synovial macrophages, but not in FLSs (Fonseca et al., 2002; Castagnetta et al., 2003; Gheorghe et al., 2012). Double staining using pAb28484 and anti-CD163 showed essentially no overlap between FLSs and macrophages (Fig. 2D,E). Negative controls omitting the primary antibody showed no staining (Fig. 2F).

Patterns of lubricin detection in tenosynovial giant cell tumors

Localized and diffuse TSGCTs showed typical histology by H&E staining (Fig. 3A), with a mixture of mononuclear cells, including plump FLSs, scattered multinucleated osteoclast-like giant cells, siderophages, and xanthoma cells (Goldblum et al., 2014b). All cases stained with pAb28484 showed strong cytoplasmic expression of lubricin in the plump FLSs, but not in the giant cells or in the smaller mononuclear cells (Fig. 3B). The S6.79 antibody stained lubricin in the extracellular matrix, and some of the plump FLSs showed cytoplasmic staining (Fig. 3C). Rare lubricin-positive cells were binucleated (Fig. 3D). Hemosiderin was seen in clustered histiocytes, sometimes near extravasated red blood cells (Fig. 3A), as well as in occasional plump FLSs, but not in multinucleated giant cells. Prussian blue revealed iron primarily in cells negative for lubricin, but lubricin-positive FLSs occasionally contained iron (Fig. 3D). Two-color immunostaining (Fig. 3D-F) showed that pAb28484-positive cells were essentially negative for CD163 (Fig. 3E-F). Multinucleated giant cells were CD163-negative (Fig. 3D-F), as previously reported (Furlong et al., 2003; Nguyen et al., 2005; Komohara et al., 2006; Boland et al., 2009). Interestingly, scattered mononuclear cells were negative for both CD163 and lubricin (Fig. 3D-F, arrows).

Lubricin immunohistochemistry of myxoid tumors and tissues

In three of the ganglion cyst specimens, myxoid

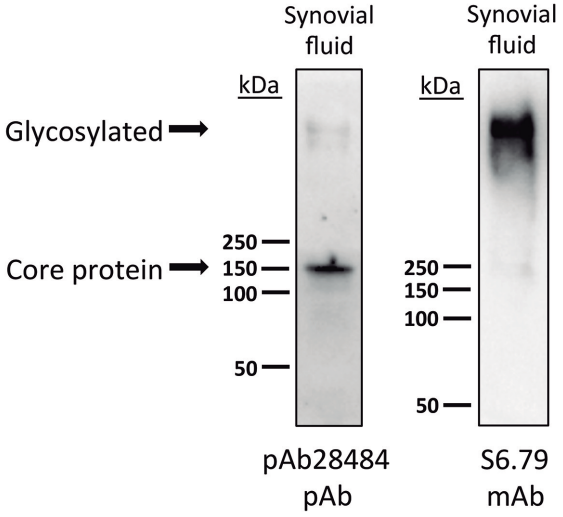

Fig. 1. Western blots of human synovial fluid with anti-lubricin antibodies pAb28484 and S6.79. Arrows mark the positions of the 345-kDa fully glycosylated form and the 150-kDa unglycosylated core protein of lubricin.

stromal degeneration was seen adjacent to the cysts (Fig. 4A), and these areas showed moderate staining with S6.79 (Fig. 4B). In a typical case of juxta-articular myxoma with characteristic synovial, cystic, and transitional areas (Fig. 4C), S6.79 detected lubricin in/around the plump cells and in myxoid stroma (Fig. 4D). In all four intramuscular myxomas studied (Fig. 4E), myxoma cells showed strikingly strong cytoplasmic staining with pAb28484 (Fig. 4F), and S6.79 reactivity was associated with cells and stroma (Fig. 4G). Of five low-grade myxofibrosarcomas, one case (Fig. 5A) showed strong cytoplasmic and extracellular staining with both pAb28484 (Fig. 5B) and S6.79 (Fig. 5C). (This specimen was the third re-excision of a subcutaneous forearm mass in a 63 year-old woman.) Two low-grade fibromyxoid sarcomas were negative with both antibodies. Lubricin was not detected with either antibody in fetal tissues, despite abundant myxoid matrix. We also studied a case of acute cholecystitis as an example of an adaptive myxoid stromal change, with matrix mucopolysaccharides evident by Alcian blue (Fig. 6A). Lubricin was not detected in myofibroblasts or matrix with either pAb28484 (Fig. 6B) or S6.79 (Fig. 6C). Finally, lubricin was not detected in an atrial myxoma with either antibody (data not shown). Results are summarized in Table 1.

All other tumors and tissues tested were negative for staining with both anti-lubricin antibodies, with the following two exceptions: using S6.79, we detected cytoplasmic staining without associated stromal staining

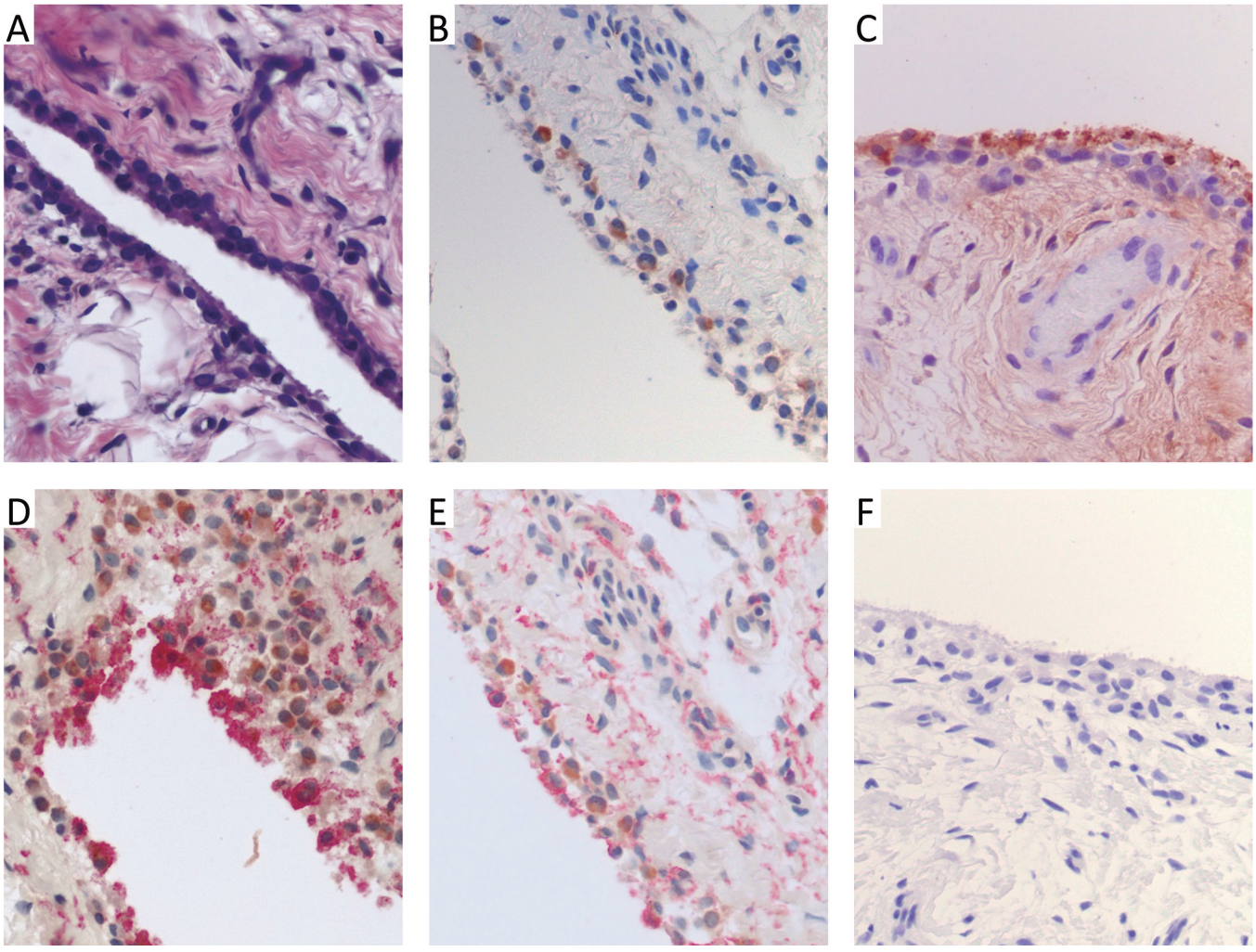

Fig. 2. Lubricin and CD163 expression in human synovium. **A.** H&E-stained synovial lining. **B.** Immunohistochemistry with pAb28484 showed lubricin cytoplasmic expression in FLSs. **C.** Immunohistochemistry with S6.79 showed extracellular reactivity. **D, E.** Dual-color immunostaining for the FLS-marker lubricin (brown) using pAb28484 and the macrophage-marker CD163 (red) demonstrated no overlap between the two synoviocyte types, and comparing the same field **(B vs. E). F.** Negative controls omitting the primary antibody showed no staining. x 325

in infrequent (3% of) cells within islets of Langerhans, and a single sample of pericardium showed weak staining of the mesothelium. However, S6.79 did show strong reactivity in plasma within blood vessels and in extravasated interstitial blood, such as adjacent to a hemorrhagic corpus luteum. Using S6.79 we found extracellular reactivity in lumina of blood vessels and extravasated blood, where it is present at a lower concentration than in synovial fluid (Su et al., 2001; Ai et al., 2015). Although the liver expresses the gene for lubricin (Ikegawa et al., 2000), the intracellular concentration of the glycosylated form in hepatocytes was below the detection threshold with our methods.

Table 1. Human tumors and tissues in which lubricin was detected by immunohistochemistry.

	pAb28484	S6.79
Synovium (n=6)	2/2	6/6
Localized tenosynovial giant cell tumors (n=12)	11/11	12/12
Diffuse tenosynovial giant cell tumors (n=2)	1/1	2/2
Ganglion cysts (n=4)	3/3	4/4
Intramuscular myxoma (n=4)	4/4	4/4
Juxta-articular myxoma (n=1)	N.D.	1/1
Low-grade myxofibrosarcoma (n=5)	1/5	1/5
Pericardium (n=1)	N.D.	1/1

N.D., Not Determined.

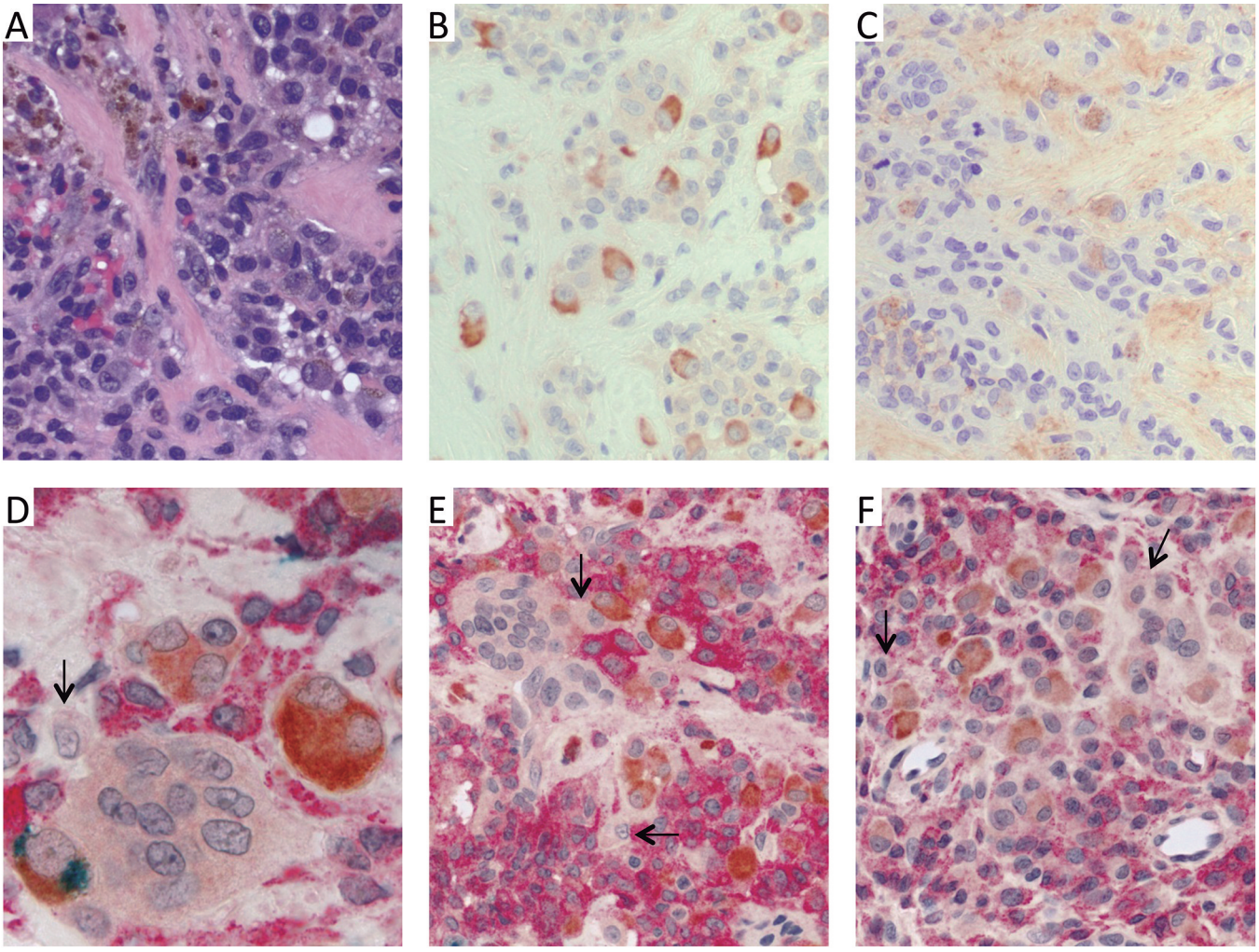

Fig. 3. Lubricin and CD163 expression in localized TSGCT. **A.** H&E-stained localized TSGCT with hemosiderin deposits near extravasated red blood cells. **B.** Immunohistochemistry with pAb28484 showed lubricin cytoplasmic expression in large, plump synoviocyte-like cells. **C.** Immunohistochemistry with S6.79 showed reactivity in the extracellular matrix with occasional lubricin-positive cells. **D-F.** Dual-color immunostaining for the FLS-marker lubricin (brown) using pAb28484 and the macrophage-marker CD163 (red) demonstrated no overlap between the two synoviocyte types. Scattered mononuclear cells (arrows) and multinucleated osteoclast-like giant cells were lubricin-negative and CD163-negative. A-C, E, F, x 325; D, x 800

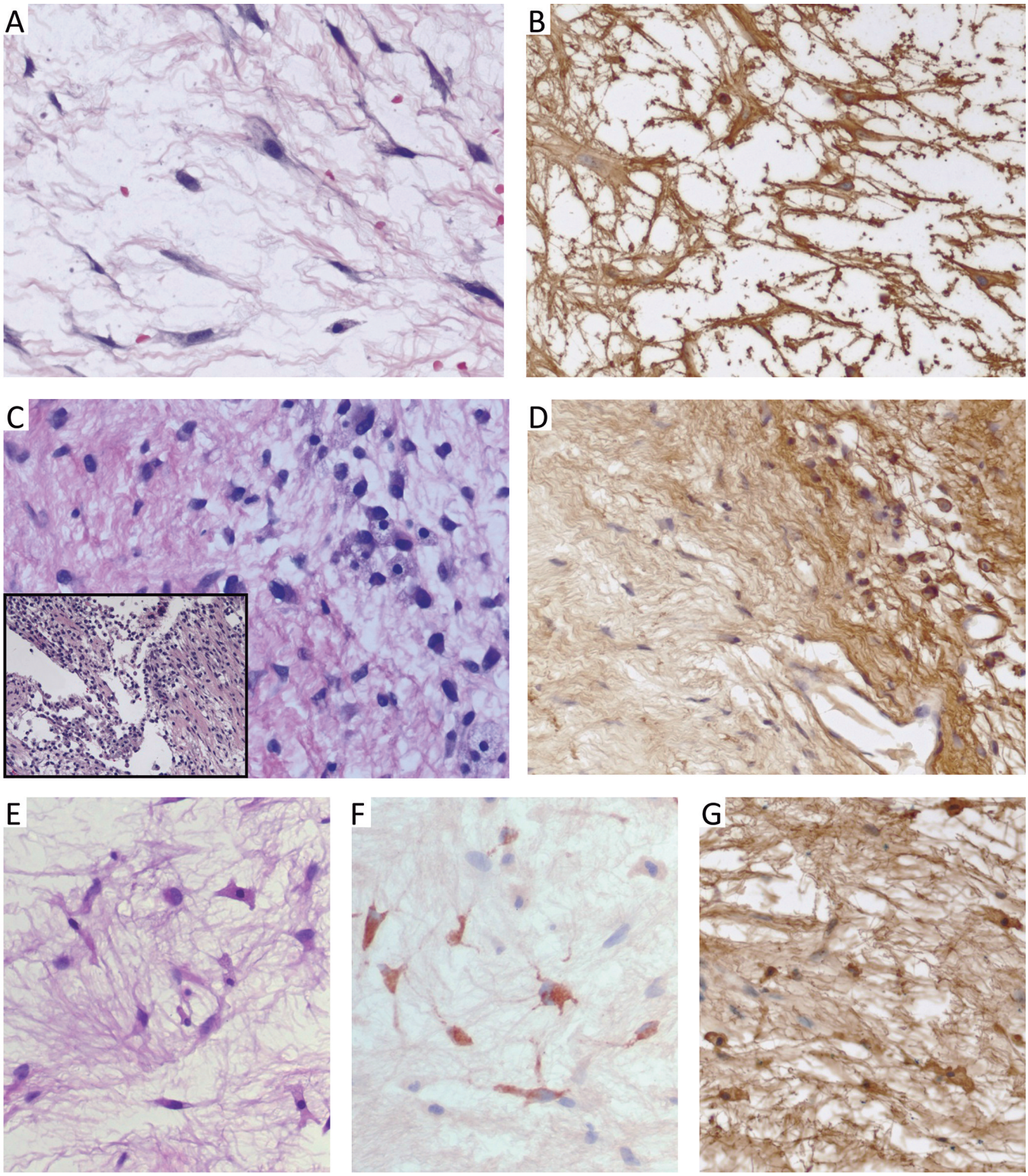

Fig. 4. Lubricin expression in benign myxoid tumors. A ganglion cyst with adjacent myxoid degeneration (**A**, H&E) showed lubricin-positive extracellular matrix with S6.79 **(B)**. A juxta-articular myxoma with myxoid degeneration (**C**, H&E) showed lubricin-positive stroma with S6.79 **(D)**. A representative case of intramuscular myxoma with myxoid stroma (**E**, H&E) showed cytoplasmic staining of myxoma cells with pAb28484 **(F)** and lubricin-positive stroma with S6.79 **(G)**. x 325; inset, x 80

Discussion

In this study, we used two lubricin-specific antibodies, which each recognize a different form of the protein. The affinity purified pAb28484 rabbit polyclonal antibody was generated against a synthetic peptide (amino acids #1356-1374) of human lubricin and has been used by a number of investigators for Western blotting (Antonacci et al., 2012; Schrobback et al., 2012; Musumeci et al., 2014), immunohistochemistry (Ruan et al., 2013; Musumeci et al., 2014), and immunofluorescence (Mrosewski et al., 2014). On Western blots of synovial fluid, pAb28484 recognized a protein migrating at 150-kDa, corresponding to the expected size of the unglycosylated core protein of lubricin, but only weakly reacted with the 345-kDa species, corresponding to the heavily glycosylated form of lubricin. Our findings are consistent with the interpretation that the epitopes recognized by pAb28484 are masked by post-translational modification, because immunohistochemistry showed primarily cytoplasmic rather than extracellular staining. The S6.79 monoclonal antibody

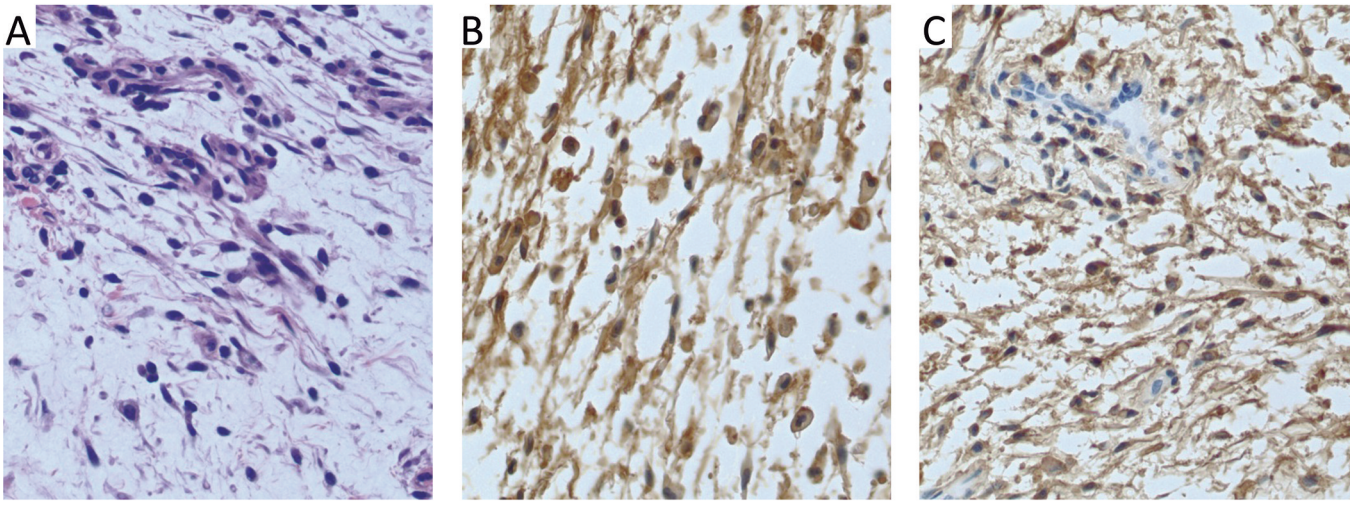

Fig. 5. Lubricin expression in a low-grade myxofibrosarcoma. A low-grade myxofibrosarcoma with myxoid matrix (**A**, H&E) showed lubricin-positive stroma with both pAb28484 **(B)** and S6.79 **(C).** x 325

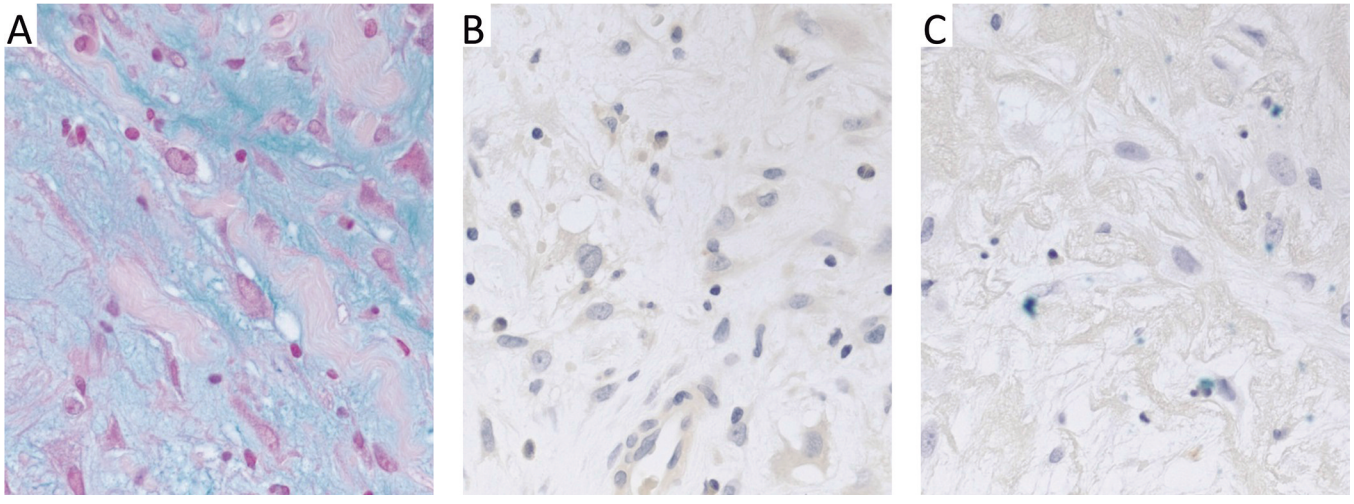

Fig. 6. Lubricin was not observed in an acute cholecystitis. In an example of an adaptive myxoid stromal change, Alcian blue staining of acute cholecystitis showed typical myxoid stroma and matrix mucopolysaccharides by activated myofibroblasts **(A)**, but lubricin was not detected with either pAb28484 **(B)** or S6.79 **(C).** x 325

was generated against native, full-length human lubricin (Su et al., 2001). S6.79 recognizes a glycosylated epitope in the central mucin domain (electron microscopic and biochemical data not shown) and therefore detects the 345-kDa form on Western blots and the secreted, extracellular form by immunohistochemistry. However, S6.79 did stain the cytoplasm of occasional plump FLSs of TSGCTs, consistent with the conclusion that the two antibodies recognize the same protein, but that the glycosylated form of lubricin recognized by S6.79 is only occasionally retained in the cytoplasm. In addition to synovium, we detected intracellular lubricin adjacent to ganglion cysts in stromal cells surrounded by myxoid stroma, and in analogous cells in juxta-articular myxomas. However, we did not detect lubricin in other proteoglycan matrix-rich tissues such as fetal tissue, umbilical cord, or in the myxoid granulation tissue of a case of acute cholecystitis in which activated myofibroblasts were abundant. Thus, abundant lubricin synthesis by stromal cells is not a generalized feature of fibroblasts within myxoid tissue but a phenotype associated with synovium that can also be induced in nearby fibroblasts.

Our study showed strong lubricin expression by normal synoviocytes and by the neoplastic (CD163-negative) cells of TSGCTs, confirming that the latter retain their FLS identity. Our studies also prompted two other observations on the nature of the cell types within TSGCTs. First, we found that the plump, lubricin-expressing FLSs of TSGCTs occasionally contain hemosiderin, which was even more obvious with Prussian blue counterstaining. Thus, like professional phagocytes, FLSs have the capacity to engulf the extravasated red blood cells that are occasionally seen in these tumors. Second, we found that in addition to the giant cells, which have previously been reported to be CD163-negative (Furlong et al., 2003; Nguyen et al., 2005; Komohara et al., 2006; Boland et al., 2009) a small subpopulation of the mononuclear cells with monocyte morphology are negative for both CD163 and lubricin. These monocytes may be the precursor cells that fuse to form the giant cells in TSGCTs (Fig. 7). We note that the frequency of giant cells varies considerably among cases of TSGCTs, as does the fraction of circulating CD163-negative monocytes among individuals (Tippett et al., 2011). Komohara and colleagues (2006) suggested that only a subset of monocytes has the capacity to fuse, so perhaps the frequency of giant cells in these tumors reflects the frequency of CD163-negative monocytes in circulation in a given patient.

Strong lubricin expression was detected in all four of the intramuscular myxomas tested, but we did not detect lubricin in an atrial myxoma, a tumor that arises through a different genetic mechanism (DeMarco et al., 1996; Mantovani et al., 2009; Takahashi et al., 2014). The transcriptome of intramuscular myxomas have not been reported, and it would be interesting to determine if they share any gene expression signatures with FLSs.

Lubricin (Prg4) was previously identified as one of

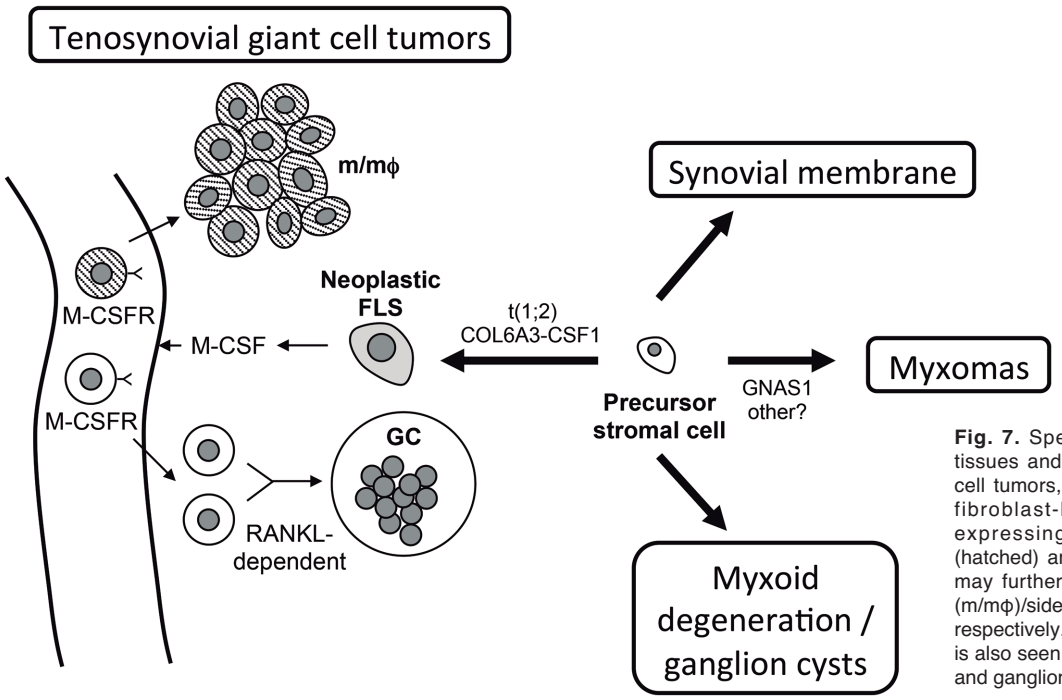

Fig. 7. Spectrum of lubricin-expressing tissues and tumors. In tenosynovial giant cell tumors, neoplastic, lubricin+ (shaded) fibroblast-like synoviocytes (FLSs) expressing M-CSF recruit CD163+ (hatched) and CD163(-) monocytes, which may further differentiate to macrophages (m/mϕ)/siderophages and giant cells (GC), respectively. Significant lubricin expression is also seen in normal synovium, myxomas, and ganglion cyst specimens.

the 30 most abundant proteins in myxofibrosarcoma by mass spectrometry (Willems et al., 2009). In our study, lubricin-positive stromal cells and matrix were observed in one of five cases of myxofibrosarcoma, indicating heterogeneity of these tumors. Interestingly, lubricin was not detected in either of two cases of myxoid liposarcoma, despite the fact that it was identified as a downstream target of the myxoid liposarcoma oncogene (Kuroda et al., 1999). However, Domoto et al. found lubricin mRNA expression in only 50% of myxoid liposarcoma cases (Domoto et al., 2002). Since the histology of myxoid liposarcoma varies considerably in its degree of adipocytic differentiation and matrix composition, our small sample size may not be representative.

In summary, synoviocyte differentiation represents a unique mesenchymal cell phenotype responsible for maintaining a physiological fluid. Since excised ganglion cysts and juxta-articular myxoma specimens show a spectrum ranging from synovial-like to myxoma-like areas, we asked whether they also express lubricin, and we found that they do. Furthermore, using two independent reagents, we found strong and convincing expression of lubricin in the cells and matrix of intramuscular myxomas, which are histologically similar to the small areas of degenerative myxoid change that give rise to ganglion cysts, though the former are neoplastic and bear no proximal relationship to joints.

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