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Expression of lubricin in the human amniotic membrane

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Abstract

Purpose—Lubricin, a boundary lubricant, is the body's unique anti-adhesive, anti-fibrotic, anti-friction and anti-inflammatory glycoprotein. This amphiphile is produced by numerous tissues and acts to regulate a number of processes, such as homeostasis, shear stress, tissue development, innate immunity, inflammation and wound healing. We hypothesize that lubricin is also synthesized and expressed by the amniotic membrane (AM), which also possesses anti-adhesive, anti-fibrotic and anti-inflammatory properties. We also hypothesize that lubricin, at least in part, mediates these AM capabilities. Our goal was to test our hypothesis.

Methods—We obtained multiple samples of fresh, cryopreserved (CP) and freeze-dried (FD) human AMs, as well as fresh placental tissue positive controls, and processed them for light microscopy, immunofluorescence and Western blot analyses. We also evaluated the ability of recombinant human (rh)-lubricin to associate with FD-AM.

Results—Our results demonstrate that all fresh placental, fresh AM and CP-AM samples contained lubricin. Lubricin was expressed in placental chorionic villi, AM epithelial and stromal cells, and CP-AM epithelia. No lubricin could be detected in FD-AMs, but could be restored in FD-AM after overnight incubation with rh-lubricin.

Conclusions—This study supports our hypothesis that lubricin is expressed in human AMs. In addition, our data show that preservation methods influence the extent of this expression. Indeed, the disappearance of lubricin in FD-AMs may explain why dried AM reportedly loses its anti-inflammatory and anti-scarring abilities. It is possible that lubricin may mediate, at least in part, many of the biological properties of AMs.

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Conflict of Interest: Dr. Sullivan is a cofounder of a company, Singularis, that sought to develop a recombinant human lubricin protein. Singularis merged with Lubris BioPharma. A series of patents have been awarded or filed around this technology. The intellectual property for Dr. Sullivan's lubricin applications is owned by the Schepens Eye Research Institute. Drs. Chen, Liu and Sullivan have submitted a provisional patent related to lubricin and amniotic membranes. The intellectual property for this patent is owned by the Schepens Eye Research Institute. Jingyi Wang, Huatao Xie and Ying Li have no conflicts of interest.

Introduction

Lubricin, a boundary lubricant, is the body's unique anti-adhesive, anti-fibrotic, anti-friction and anti-inflammatory glycoprotein.¹⁻⁹ This amphiphile is a product of the proteoglycan 4 gene, contains a 1,404 amino acid core, is extensively O-glycosylated, and is characterized by a long, central mucin-like domain that permits lubricin to adhere and protect tissue surfaces.² Lubricin is produced by numerous tissues, including the cornea, conjunctiva, trabecular meshwork, heart, lung, liver, cartilage, bone, kidney, brain, testis, placenta and small intestine.^{1,10-14} At these locations lubricin may act to regulate a number of processes, such as homeostasis, tissue development, shear stress, innate immunity, inflammation and wound healing.^{1,11-13}

We hypothesize that lubricin is also synthesized and expressed by the amniotic membrane (AM). Our rationale is that this tissue possesses anti-adhesive, anti-fibrotic and anti-inflammatory properties, and promotes ocular surface redevelopment and wound healing.¹⁵⁻¹⁷ We also hypothesize that lubricin, at least in part, mediates these biological AM capabilities.

Material and Methods

To test our hypothesis, we obtained ten samples of human AM, as well as placentas as positive controls,¹⁷ from the Tissue Repository of the Massachusetts General Hospital Pathology Service (Boston). These tissues originated from healthy donors (29 to 38 years old) following Cesarean sections and were de-identified prior to use. We also cryopreserved these AMs (CP-AMs) in glycerine. Our studies were approved by the Human Studies Committee of the Massachusetts Eye and Ear Infirmary (Boston). We also obtained two CP-AMs (gift from Dr. Yukan Huang, Wuhan, China) and six freeze-dried AMs (FD-AM; Ruiji Bio-Engineering Co., Jiangxi, China). One of the FD-AMs was evaluated with or without incubation overnight at 4°C with recombinant human (rh) lubricin (50 µl, 0.675 mg/ml, Lubris BioPharma, Framingham, MA). Samples were processed for immunofluorescence and Western blot analyses.

For histology frozen sections (15 µM) were stained with hematoxylin and eosin (H&E), or were incubated with an aliquot (1:50 dilution) of affinity-purified mouse antibody to human lubricin (Millipore Sigma, Burlington, MA) or the phosphate-buffered saline, pH 7.4 (Boston BioProducts, Ashland, MA) diluent overnight at 4°C. Sections were then exposed to donkey-anti-mouse secondary antibody (1:500, Millipore) for 2 hours at room temperature, and mounted using ProLong Gold antifade reagent with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen, Carlsbad, CA) for nuclear counterstaining. Slides were viewed with Leica SP5 confocal microscope (Buffalo Grove, IL).

For protein determinations Western blots were run as reported,¹ using primary (1:1,000) and secondary (1:5,000) antibody incubation conditions as described above. rhLubricin was included as a positive control.

Results

Our results demonstrate that all fresh placental (n = 10) and fresh AM (n = 10) samples contained lubricin (Figure 1A). Lubricin was expressed in placental chorionic villi (Figures 1 B & C), AM epithelial and stromal cells (Figures 1D&E), and CP-AM epithelia (Figure 1F). All CP-AM samples (n = 12) also contained lubricin, as shown by Western blots. No lubricin could be detected in FD-AMs (n = 6), either by immunofluorescence (Figure 1G) or Western blots (n = 7 experiments). Lubricin expression could be restored in FD-AM after overnight incubation with rhlubricin (Figure 1H).

Discussion

This study supports our hypothesis that lubricin is expressed in human AMs. In addition, our data show that preservation methods influence the extent of this expression.

The earliest reported application of AM in ophthalmic surgery was in 1940 when De Rötth used fetal membranes to reconstruct the ocular surface in patients with symblepharon.¹⁸ Since that time, ophthalmologists have discovered the anti-adhesive, anti-fibrotic and anti-inflammatory features of AMs and used these tissues extensively to facilitate ocular surface reconstruction and wound healing.^{15–17} Because lubricin possesses innate anti-adhesive, anti-fibrotic, anti-friction and anti-inflammatory abilities,^{1–9} and is expressed by AMs, we believe that this glycoprotein may mediate, at least in part, many of the biological properties of AMs.

We could not detect lubricin in FD-AMs. Our finding suggests that the AM drying process leads to a disappearance of lubricin, which can be restored by lubricin exposure. The loss of lubricin may explain why dried, but not cryopreserved, AM loses its anti-adhesive, anti-fibrotic and anti-inflammatory abilities that typically combine to inhibit scar formation.^{19–23} Indeed, this absence of lubricin may account for why the use of dried AM was unable to suppress inflammation, reduce adhesion generation, prevent scar formation and decrease the risk of symblepharon development after strabismus surgery.^{20,22,24}

How would the loss of lubricin in FD-AMs result in such sequelae? To explain, a scar is an area of fibrotic tissue that occurs after an injury, and an adhesion is a band of scar tissue that binds two parts of tissue that are not normally joined together (e.g. symblepharon). Scar and adhesion formations are often caused by fibrotic and inflammatory responses after tissue injury.¹⁰ Fibroblasts are found throughout the body and play an active role in producing the extracellular matrix (ECM). Fibroblasts also participate in the repair process by differentiating into myofibroblasts, which proliferate, migrate to the sites of injury, secrete cytokines, and promote the inflammatory response.²⁵ This myofibroblast activation and associated ECM remodeling are important mechanisms by which mild adhesions transition to dense, fibrous adhesions.^{10,26,27} Proposed mechanisms to block adhesion formation include preventing myofibroblast proliferation and reducing the initial inflammatory response.²⁸

Lubricin, in turn, is known to possess innate anti-fibrotic and anti-inflammatory properties that retard adhesion development following tissue injury. For example, lubricin treatment

suppresses myofibroblast proliferation and ECM remodeling, decreases fibrotic and inflammatory responses, and/or prevents adhesion formation in the pericardial and abdominal cavities, lens, tendon and joints.^{4–10,28,29} Lubricin acts directly on the fibroblasts and immune cells by binding to CD44, TLR2 and TLR4 receptors and suppressing I κ B α phosphorylation and NF κ B translocation.^{2,3,4,9,11,30,31} Lubricin also significantly reduces the IL-1 β -induced increase in IL-6, IL-8, and COX2 expression, as well as that of matrix metalloproteinases (MMPs) –1, –3, –9 and –13, which are involved in fibroblast proliferation and migration.^{32,33}

Of particular interest, lubricin may have other applications related to the eye. We have discovered in a clinical trial that topical rhlubricin significantly reduces the signs and symptoms of dry eye disease.³⁴ This disease is characterized by a vicious cycle of tear film hyperosmolarity and instability, and leads to increased friction, inflammation, eye damage, pain and visual impairment.³⁵ Currently, there is no global cure for dry eye. In addition, others have found that a down regulation of the lubricin gene in human conjunctival fibroblasts correlates with multiple failed glaucoma operations and worse visual acuities in patients.¹¹ Further, binding of lubricin to contact lenses may decrease their friction and improve their comfort.^{12,36,37}

As one additional consideration, the ability of lubricin to prevent adhesion formation could have yet multiple other clinical applications for the eye. Topical lubricin could possibly prevent the development of symblephara that are known to occur in numerous pathological conditions, including pterygia, ocular cicatricial pemphigoid, Stevens-Johnson Syndrome, erythema multiforme, conjunctivitis (i.e. bacterial, viral, vernal and atopic), porphyria cutanea tarda, rosacea, xeroderma pigmentosum, and squamous papilloma of the conjunctiva.^{38,39} If so, this might remove the necessity of surgical approaches, such as amniotic membrane transplants,⁴⁰ to treat these adhesions.

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References

1. Schmidt TA, Sullivan DA, Knop E, et al. Transcription, translation, and function of lubricin, a boundary lubricant, at the ocular surface. *JAMA Ophthalmol* 2013;131:766–776. [PubMed: 23599181]
2. Jay GD, Waller KA. The biology of lubricin: near frictionless joint motion. *Matrix Biol* 2014;39:17–24. [PubMed: 25172828]
3. Alquraini A, Garguilo S, D'Souza G, et al. The interaction of lubricin/proteoglycan 4 (PRG4) with toll-like receptors 2 and 4: an anti-inflammatory role of PRG4 in synovial fluid. *Arthritis Res Ther* 2015;17:353. [PubMed: 26643105]
4. Al-Sharif A, Jamal M, Zhang LX, et al. Lubricin/Proteoglycan 4 Binding to CD44 Receptor: A Mechanism of the Suppression of Proinflammatory Cytokine-Induced Synoviocyte Proliferation by Lubricin. *Arthritis Rheumatol* 2015;67:1503–1513. [PubMed: 25708025]

5. Greenwood-Van Meerveld B, Mohammadi E, Latorre R, et al. Preclinical Animal Studies of Intravesical Recombinant Human Proteoglycan 4 as a Novel Potential Therapy for Diseases Resulting From Increased Bladder Permeability. *Urology* 2018;116:230 e231–230 e237.
6. Alquraini A, Jamal M, Zhang L, et al. The autocrine role of proteoglycan-4 (PRG4) in modulating osteoarthritic synoviocyte proliferation and expression of matrix degrading enzymes. *Arthritis Res Ther* 2017;19:89. [PubMed: 28482921]
7. Waller KA, Chin KE, Jay GD, et al. Intra-articular Recombinant Human Proteoglycan 4 Mitigates Cartilage Damage After Destabilization of the Medial Meniscus in the Yucatan Minipig. *Am J Sports Med* 2017;45:1512–1521. [PubMed: 28129516]
8. Cui Z, Xu C, Li X, et al. Treatment with recombinant lubricin attenuates osteoarthritis by positive feedback loop between articular cartilage and subchondral bone in ovariectomized rats. *Bone* 2015;74:37–47. [PubMed: 25576671]
9. Qadri M, Jay GD, Zhang LX, et al. Recombinant human proteoglycan-4 reduces phagocytosis of urate crystals and downstream nuclear factor kappa B and inflammasome activation and production of cytokines and chemokines in human and murine macrophages. *Arthritis Res Ther* 2018;20:192. [PubMed: 30157934]
10. Park DSJ, Regmi SC, Svystonyuk DA, et al. Human pericardial proteoglycan 4 (lubricin): Implications for postcardiotomy intrathoracic adhesion formation. *J Thorac Cardiovasc Surg* 2018;156:1598–1608 e1591. [PubMed: 29859675]
11. Yu-Wai-Man C, Tagalakis AD, Meng J, et al. Genotype-Phenotype Associations of IL6 and PRG4 With Conjunctival Fibrosis After Glaucoma Surgery. *JAMA Ophthalmol* 2017;135:1147–1155. [PubMed: 28975281]
12. Samsom ML, Morrison S, Masala N, et al. Characterization of full-length recombinant human Proteoglycan 4 as an ocular surface boundary lubricant. *Exp Eye Res* 2014;127:14–19. [PubMed: 24997456]
13. Gill S, Wight TN, Frevert CW. Proteoglycans: key regulators of pulmonary inflammation and the innate immune response to lung infection. *Anat Rec (Hoboken)* 2010;293:968–981. [PubMed: 20503391]
14. <http://www.genecards.org/cgi-bin/carddisp.pl?gene=PRG4>.
15. Tseng SC, Prabhasawat P, Lee SH. Amniotic membrane transplantation for conjunctival surface reconstruction. *Am J Ophthalmol* 1997;124:765–774. [PubMed: 9402822]
16. Sheha H, Casas V, Hayashida CY. The use of amniotic membrane in reducing adhesions after strabismus surgery. *Journal of American Association for Pediatric Ophthalmology and Strabismus* 2009;13:99–101. [PubMed: 18976945]
17. Malhotra C, Jain AK. Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. *World J Transplant* 2014;4:111–121. [PubMed: 25032100]
18. de RÖTTH A Plastic repair of conjunctival defects with fetal membranes. *JAMA Ophthalmol* 1940;23:522–525.
19. Kassem RR, El-Mofty RMA. Amniotic Membrane Transplantation in Strabismus Surgery. *Curr Eye Res* 2019;44:451–464. [PubMed: 30575427]
20. Chun BY, Kim HK, Shin JP. Dried human amniotic membrane does not alleviate inflammation and fibrosis in experimental strabismus surgery. *J Ophthalmol* 2013;2013:369126. [PubMed: 23864935]
21. Kassem RR, Abdel-Hamid MA, Khodeir MM. Effect of lyophilized amniotic membrane on the development of adhesions and fibrosis after extraocular muscle surgery in rabbits. *Curr Eye Res* 2011;36:1020–1027. [PubMed: 21942278]
22. Kassem RR, Gawdat GI, Zedan RH. Severe fibrosis of extraocular muscles after the use of lyophilized amniotic membrane in strabismus surgery. *J AAPOS* 2010;14:548–549. [PubMed: 21168080]
23. Tao H, Fan H. Implantation of amniotic membrane to reduce postlaminectomy epidural adhesions. *Eur Spine J* 2009;18:1202–1212. [PubMed: 19404691]
24. Naxer S, Horn M, Schittkowski M. Processed amniotic membrane for conjunctival reconstruction in complex strabismus surgery. *Strabismus* 20181–7.

25. Baum J, Duffy HS. Fibroblasts and myofibroblasts: what are we talking about? *J Cardiovasc Pharmacol* 2011;57:376–379. [PubMed: 21297493]
26. Ntinioti T, Thomopoulou G-H, Danas E, et al. Myofibroblasts Role in Wound Healing of Eyelid Lesions. *Int J Pathol Clin Res* 2016.
27. Cannata A, Petrella D, Russo CF, et al. Postsurgical intrapericardial adhesions: mechanisms of formation and prevention. *Ann Thorac Surg* 2013;95:1818–1826. [PubMed: 23566646]
28. Oh J, Kuan KG, Tiong LU, et al. Recombinant human lubricin for prevention of postoperative intra-abdominal adhesions in a rat model. *J Surg Res* 2017;208:20–25. [PubMed: 27993210]
29. Bowman SA, Schmidt TA, West-Mays JA. Effect of lubricin on TGF beta-induced EMT of lens epithelial cells. *ARVO* 2015 abstract.
30. Hurtig M, Zaghoul I, Sheardown H, et al. Two compartment pharmacokinetic model describes the intra-articular delivery and retention of rhprg4 following ACL transection in the Yucatan mini pig. *J Orthop Res* 2019;37:386–396. [PubMed: 30488470]
31. Larson KM, Zhang L, Elsaid KA, et al. Reduction of friction by recombinant human proteoglycan 4 in IL-1alpha stimulated bovine cartilage explants. *J Orthop Res* 2017;35:580–589. [PubMed: 27411036]
32. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev* 2010;233:233–255. [PubMed: 20193003]
33. Xue M, McKelvey K, Shen K, et al. Endogenous MMP-9 and not MMP-2 promotes rheumatoid synovial fibroblast survival, inflammation and cartilage degradation. *Rheumatology (Oxford)* 2014;53:2270–2279. [PubMed: 24982240]
34. Lambiase A, Sullivan BD, Schmidt TA, et al. A Two-Week, Randomized, Double-masked Study to Evaluate Safety and Efficacy of Lubricin (150 mug/mL) Eye Drops Versus Sodium Hyaluronate (HA) 0.18% Eye Drops (Vismed(R)) in Patients with Moderate Dry Eye Disease. *Ocul Surf* 2017;15:77–87. [PubMed: 27614318]
35. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf* 2017;15:438–510. [PubMed: 28736340]
36. Korogiannaki M, Samsom M, Schmidt TA, et al. Surface-Functionalized Model Contact Lenses with a Bioinspired Proteoglycan 4 (PRG4)-Grafted Layer. *ACS Appl Mater Interfaces* 2018;10:30125–30136. [PubMed: 30114356]
37. Samsom M, Iwabuchi Y, Sheardown H, et al. Proteoglycan 4 and hyaluronan as boundary lubricants for model contact lens hydrogels. *J Biomed Mater Res B Appl Biomater* 2018;106:1329–1338. [PubMed: 28688149]
38. Liu J, Sheha H, Fu Y, et al. Update on amniotic membrane transplantation. *Expert Rev Ophthalmol* 2010;5:645–661. [PubMed: 21436959]
39. Bierman EO. Symblepharon in pterygium. *Amer J Ophthalmol* 1955;39:894.
40. Gheorghe A, Pop M, Burcea M, et al. New clinical application of amniotic membrane transplant for ocular surface disease. *J Med Life* 2016;9:177–179. [PubMed: 27453750]

Figure 1.

Lubricin expression in human AMs and placental tissues. (A) Identification of lubricin protein in human AMs [A] and placentas [P] by Western blot. Results from 4 different samples are shown, as well as the rh-lubricin control. (B) Staining of placental chorionic villi by H&E. (C) Immunofluorescence staining of lubricin (red) in placental chorionic villi, with DAPI counterstaining of nuclei (blue). (D) Staining of the human AM by H&E. (E) Immunofluorescence staining of lubricin (red) in human AM epithelial and stromal cells, (F) CP-AM epithelia [predominantly] (F) and FD-AM (G) before and (H) after lubricin exposure, with sections counterstained with DAPI (blue). All AMs were positioned with the epithelial side up. All scale bars equal 25 μM.