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Dry Eye and Designer Ophthalmics

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Abstract

EST, proteomic, and antibody capture assays are revealing a level of tear film protein complexity far greater than previously appreciated. A systems biology approach will be needed to fully appreciate function as tear protein doses fluctuate in time through different conditions. Although consensus is growing on what fully constitutes the human tear proteome, questions remain about the source and significance of the ~256 tear proteins designated as 'intracellular'. Many of these may derive from normal cellular turnover and could therefore be informative. A further >183 are designated as 'extracellular'. Surprisingly, only 4 – 5% of these appear to be dysregulated in the three forms of dry eye preliminarily examined to date. Some differ and a couple overlap, suggesting that disease-specific signatures could be identified. Future dry eye treatment might include recombinant tear protein rescue as a personalized ophthalmic approach to ocular surface disease.

Keywords

dry eye; personalized medicine; proteomics; tears; lacritin

The Expanding Tear Proteome

Early study of tears by SDS-polyacrylamide gel electrophoresis (PAGE) identified lysozyme (LYZ), lactotransferrin (LTF; lactoferrin), and lipocalin-1 (LCN1; von Ebner gland protein or tear specific prealbumin) that together constitute 70 to 85% of total tear protein.¹ Later, transferrin (TF), albumin (ALB; serum albumin), secretory IgA (CD79A), and lipophilin (PLP1)¹⁻⁴ were identified, and more recently via immunoassays of increasing sensitivity and reliability⁵: phospholipid transfer protein (PLTP),⁶ growth factors,^{7,8} neurotrophic factors,⁹ cytokines,^{5,10-13} matrix metalloproteinases,^{11,14-16} bradykinins,¹⁷ tachykinins (e.g.,

substance P),¹⁸19 fibronectin (FN1),²⁰ plasminogen activator (PLAU),²¹ defensins,²² aquaporins,²³ phospholipase,²⁴ immunoglobulins,²⁵ lactate dehydrogenase,²⁶ proline-rich 4 (lacrimal; PRR4)²⁷ and insulin (INS).⁸ Continuing efforts are underway to document the complete human tear film proteome by mass spectrometry.^{28–30} De Souza and coworkers identified almost 500 proteins in human closed eye tears using a ultra-high resolution hybrid linear trap – Fourier Transform (LTQ-FT) and a linear ion trap – orbitrap (LTQ-Orbitrap) approach.²⁹ A surprising number (~256) are designated as ‘intracellular’ proteins by Gene Ontology, suggesting that the process of normal epithelial turnover is a significant source.³¹ Others may be contaminants of the method of collection. A further >183 are designated as ‘extracellular’ (Table 1). Preliminary indications primarily by 2-D PAGE are that a surprisingly small fraction (4 – 5%) of these are downregulated in dry eye (*, Δ, † in Table 1).^{28,32,33} This observation could set the stage for assays defining what is healthy, what is diseased and possibly when disease has been initiated.

Functional Checks and Balances

Tear proteins contribute to the anti-microbial and anti-inflammatory defense of the exposed ocular surface. Some form heterocomplexes, and may function in normal epithelial growth, protein/fluid/electrolyte secretion and other aspects of normal ocular physiology. Few have been actually tested on ocular surface cells. Appreciating the true range of functional capacity and synergies will require a systems biology approach that integrates tear protein doses in time through different conditions. Following is a brief commentary on a selection of constituents from each Table 1 functional category with attention to ocular surface biology and dry eye when known.

The capacity for ‘Angiogenesis’ is highly regulated. Normal human tears contain the angiogenic promoters angiogenin (ANG) and endothelial growth factor 1 (ECGF1). Tears also contain the angiogenic inhibitors serpin peptidase inhibitor SERPINF1, brain-specific angiogenesis inhibitor 3 (BAI3) and the multifunctional cell adhesion protein thrombospondin 1 (THBS1; ‘CellAdhesion/Motility/Structure/’). Lactotransferrin (LTF; ‘Proteinase/Inhibitor/Antimicrobial’) is anti-angiogenic when fragmented. The primary inhibitor of corneal angiogenesis is soluble fms-related tyrosine kinase 1 (FLT1) that competes with VEGF for angiogenic VEGF. FLT1 is expressed throughout the corneal epithelium.³⁴

Most tear proteins in the ‘Biosynthetic’ category are primarily intracellular. For example, mitochondrial ATP synthase, H⁺ transporting, mitochondrial F1 complex, beta polypeptide (ATP5B) is a subunit of mitochondrial ATP synthase. The suggestion that ATP5B has an alternative plasmalemmal/extracellular location is interesting because it implicates a potential source of tear ATP. ATP and UTP target ocular surface P2Y purinergic receptors to stimulate the production and release of tear mucins and noninflammatory heparanase.³⁵36 Beta1,4-galactosyltransferase (B4GALT1) is primarily associated with the Golgi apparatus, but its alternative plasmalemmal location is well-established. Here it promotes cell adhesion by binding galactosylated proteins such as laminins.³⁷ Numerous tear proteins are heavily galactosylated (ie. mucins and proteoglycans) and are capable of binding B4GALT1. Peptidylprolyl isomerase (PPIC; cyclophilin C) is most commonly associated with the endoplasmic reticulum where it binds cyclosporine A for immunosuppressive signaling. Its alternative extracellular location in tears may have relevance to the topical application of cyclosporine A for dry eye.

Intracellular ‘Calcium’ signaling is fundamental to the normal function of ocular surface epithelia. Corneal or conjunctival calcification is respectively associated with phosphate in artificial tears³⁸ and elevated serum calcium in chronic renal failure.³⁹ Tear alpha-2-HS-glycoprotein (AHSG) is an inhibitor of calcification. Annexin A5 (ANXA5) is a calcium-

dependent phospholipid binding protein that promotes corneal wound healing.⁴⁰ Although primarily cytoplasmic, ANXA5 has an alternative plasmalemmal/extracellular location. Also in tears is the calcium binding protein calreticulin (CALR). CALR ligation of Sjogren's syndrome autoantigen Ro60 kD enhances anti-Ro60 kD autoantibody recognition.⁴¹ CALR is alternatively plasmalemmal/extracellular, and primarily in the endoplasmic reticulum.

A number of 'Carbohydrate Modifying Proteins' are found in tears. Almost all are listed as primarily intracellular and alternatively extracellular or plasmalemmal. Two exceptions are the extracellular proteins chitinase 3-like 2 (CHI3L2) and lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has a newly identified extracellular role in the eye as a binding partner for transferrin (TF). Interestingly GAPDH expression is iron regulated.⁴² Galectin-3 (LGALS3) is a beta-galactoside binding protein that plays a key role in corneal re-epithelialization after wounding, as revealed by use of galectin-3 null mice.⁴³ Enolase 1 (alpha; ENO1) is strongly expressed in basal cells of the limbus⁴⁴ and is upregulated in keratoconus. AGL has hydrolase and transferase activity. CHI3L2 has a particular affinity for glycans but has no apparent chitinase activity. ENO1 is glycolytic. Carbohydrate modification or binding can substantially alter or regulate function. One example is tear heparanase cleavage of heparan sulfate on cell surface syndecan-1 for lacritin binding and signaling.⁴⁵ The extent to which carbohydrate modifying proteins regulate ocular surface physiology is not fully explored.

Tears are rich in 'Carrier/Binding Protein/Steroid Assoc.' proteins. Albumin (ALB) is abundant in tears as a binding protein for free fatty acids and other elements of the tear film. Alpha-2-glycoprotein 1 (AZGP1) functions in cell adhesion, antigen processing/presentation, immune response, and binds polyunsaturated fatty acids.⁴⁶ CD14 is an important cell surface co-receptor for bacterial lipopolysaccharide in innate immunity. CD14 on macrophages helps clear apoptotic cells. Deleted in Malignant Brain Tumors (DMBT1) is a secreted scavenger receptor cysteine-rich protein that plays a key role in terminal epithelial differentiation and when deleted in mice is embryonic lethal.⁴⁷ Two forms of the predicted twelve different DMBT1 isoforms have been detected in human tears.⁴⁸ Heparan sulfate proteoglycan 2 (HSPG2 or perlecan) is a binding partner for FGF's and several extracellular matrix molecules. HSPG2 mutations in Schwartz-Jampel Syndrome are associated with myopia.⁴⁹ Lipocalin 1 (LCN1) scavenges a wide variety of lipids and potentially other hydrophobic compounds from tears. Some of this lipid content may derive from exudated ocular surface epithelia as part of the normal course of ocular surface epithelial renewal. LCN1 also has magnesium-dependent endonuclease activity.⁵⁰ Transferrin (TF) delivers iron to cells via transferrin receptor uptake. By depleting extracellular iron, transferrin can limit bacterial growth. Thus these proteins contribute to the health and turnover of ocular surface epithelia.

Tear 'Cell Adhesion/Motility/Structure' proteins are surprisingly diverse. Presence of the ubiquitous extracellular matrix/serum constituent fibronectin 1 (FN1; 'Cell Adhesion/Motility/Structure') brings a considerable cell adhesive capacity to tear film reflected in FN1's contribution to corneal wound healing.⁵¹ Gesolin (GSN) is a calcium-regulated protein more commonly associated with amyloidosis. Although its ocular surface role is unknown, it is differentially increased in keratoconus.⁵² Laminin alpha 3 (LAMA3) chain is part of heterotrimeric laminin-5. Mutation in LAMA3 is associated with scarring of the conjunctiva. Laminin mediates corneal epithelial cell adhesion⁵³ and is primarily concentrated in basement membranes. Another tear constituent shared by extracellular matrices is the previously noted thrombospondin 1 (THBS1). THBS1 is highly expressed by corneal epithelial cells, particularly basal cells, is anti-angiogenic, and binds FN1 and heparan sulfate. Thus, the tear film has the compositional attributes of a highly disperse extracellular matrix.

The tear film is enriched by mitogens ('Cell Growth'), some with prosecretory activity. Angiopoietin-1 (ANGPT1), of the VEGF family, signals through Tie2 in bone marrow to help develop the hemopoietic stem cell niche.⁵⁴ Neovascularization following removal of limbal epithelia in mice involves ANGPT1 expression.⁵⁵ Epidermal growth factor (EGF) promotes tear secretion⁵⁶ and cell growth, and is expressed by both lacrimal acinar cells and basal cells of the corneal epithelium. Corneal epithelial cells respond to hepatocyte growth factor (HGF) in cell proliferation assays suggesting partially shared signaling pathways with EGF.⁵⁷ Lacritin (LACRT)⁵⁸ promotes human corneal epithelial cell growth⁵⁹ and MUC16 production (Wang and Laurie, unpublished), and appears to auto-stimulate tear protein secretion⁶⁰ by lacrimal acinar cells via ERK1/2-independent signaling. Lacritin employs a previously noted heparanase 'on-switch' to bind syndecan-1 as a precursor to mitogenic signaling.⁴⁵ Lacritin is largely ocular-specific. Heparanase is constitutively expressed by all layers of the normal corneal epithelium in mice, and has been implicated in glandular morphogenesis, epidermal stem cell migration and cell survival. The transmembrane mucin 4 (MUC4) signals via its EGF domains and interacts with ERBB2 to promote cell differentiation and block apoptosis.⁶¹ Several tear proteins promote neuronal survival including: glial cell derived neurotrophic factor (GDNF), neurotrophin 3 (NTF3) and neurotrophin 5 (NTF5). This mitogenic/prosecretory capacity compensates for the lack of corneal blood supply and may help serve as a master growth regulator of the ocular surface and associated accessory organs.

The level of tear 'Cytoprotective/Antiapoptotic' activity appears to be substantial. In addition to MUC4, GDNF, NTF3 and NTF5 noted above, EGF (porcine photoreceptor)⁶², HGF (human corneal epithelial)⁶³ and lacritin (human corneal epithelial; Wang et al, unpublished) are each cytoprotective. Clusterin (CLU) may help maintain the cornea as a non-keratinizing epithelium,⁶⁴ and is thought to act as an extracellular chaperone. CLU deficiency in the anterior segment of the eye is associated with pseudoexfoliation syndrome/glaucoma.⁶⁵ Mucin 16 (MUC16) on the surface of corneal epithelial cells is protective against *S. aureus* bacterial binding.⁶⁶ Gel forming mucin 5AC (MUC5AC) is thought to help sweep the ocular surface of pathogens during blinking. Very little is known about putative protection from prolactin-inducible protein (PIP), proline-rich 1 (PROL1) and proline rich 4 (PRR4). A peptide from the N-terminus of PROL1 is an analgesic⁶⁷. PROL1 binds the cell growth/maintenance protein statherin.

A number of tear proteins common to 'Extracellular Matrix' have been noted above including: HSPG2, FN1, LAMA3 and THBS1. Others are: the collagen type VI alpha 1 chain (COL6A1), mucin-like 1 (MUCL1) and SPARC-like 1 (SPARCL1). COL6A1 polymerizes with COL6A2 and COL6A2 to form the microfibrillar collagen VI triple helix. Little is known about MUCL1. SPARCL1 influences extracellular matrix formation and is a negative regulator of cell proliferation.⁶⁸

Normal human tear chemokines and cytokines ('Immune') are extensive and varied. Chemokines include: chemokine (C-C motif) ligand 2, 4, 8, 11, 22 and 24 (CCL2 – 24), chemokine (CXC motif) ligand 5, 9, 10 (CXCL5, 9, 10) and pro-platelet basic protein ligand 7 (PPBP). Cytokines include: interleukin 6, 8, 10, 12, 15, 16 (IL6 – 16), interferon gamma (IFNG) and tumor necrosis factor (TNF). How the eye handles this normal chemokine/cytokine load is an important question. TNF in the presence of IFNG is for example inflammatory for the ocular surface and promotes epithelial death. Both IL6 and TNF are increased in tears of Sjögren's syndrome dry eye patients.⁶⁹ A full answer will require attention to dose and time, and the level of cytoprotection from cell growth and cytoprotective/anti-apoptotic factors. Helping in innate defense is complement component 3 (C3), a key player in activation of the complement activation pathways. Individuals deficient in C3 have increased risk of bacterial infection. C3 polymorphism is associated with age-related macular degeneration. Knockout of the C3 gene in C57BL/6.NOD-Aec1Aec2 mice reduced the onset of primary Sjögren's syndrome.⁷⁰ Complement factor H (CFH) regulates the alternative complement pathway. CFH

polymorphism leading to decreased CFH activity is associated with increased risk of age-related macular degeneration. Oxidative stress decreases retinal CFH expression.⁷¹ Oxidative stress is a consequence of dry eye.⁷² The ocular innate ‘immune’ defense mechanism is therefore primed for action.

The inclusion of ‘Lipid/Cholesterol’ associated proteins is in line with the lipid nature of the tear film. Tears contain a wide selection of apolipoproteins (A-I [APOA1], A-II [APOA2], A-IV [APOA4], B [APOB], H [APOH]). Tear APOA1 is reportedly upregulated in diabetic retinopathy.⁷³ Phospholipid transfer protein (PLTP) binds heparin, is catalytically active in tears for phospholipid transfer and is thought to be involved in lipid clearance from the ocular surface, much like LCN1.⁷⁴ Prosaposin (PSAP) is a widely expressed lipid binding glycoprotein found in extracellular and lysosomal locations that is cleaved into four saposins involved in antigen presentation.

Among ‘Other/Unknown’ proteins are angiotensinogen (AGT), Beta-2-microglobulin (B2M), three intriguing proteins with no known function and several others. B2M is associated with the MHC class-I heavy chain on cell surfaces. Mice lacking beta-2 microglobulin reject orthotopic corneal allografts like their C57BL/6 controls.⁷⁵

Several enzymes are highlighted in the ‘Phosphatase/Kinase/GTPase/Other Enzyme’ category. Ceruloplasmin (CP) is a copper binding protein involved in peroxidation of the iron metalloprotein transferrin. Deficiency is associated with retinal pigment degeneration linked with increase iron. Matrix metalloproteinase 9 (MMP9) is upregulated in dry eye tears leading to increased corneal epithelial permeability and turnover. MMP9^{-/-} dry eye mice lack increased permeability and turnover.⁷⁶ Transglutaminase 2 (TGM2) is upregulated in UVB stimulated apoptosis of human corneal epithelial cells. Inhibition of TGM2 suppresses apoptosis.⁷⁷

Tears contain a wide variety of ‘Proteinase/Inhibitor/Antimicrobial’ proteins that play a key role in innate immune defense. Alpha-2-macroglobulin (A2M) is a broad-acting protease inhibitor. It also binds tear proteins B2M, cathepsin B (CTSB; lysosomal cysteine protease) and IL10; and the growth factors nerve growth factor beta polypeptide, platelet-derived growth factor beta polypeptide and transforming growth factor beta 1.⁷⁸ This latter property, via interaction of A2M with its receptor LPR1, is thought to be involved in inhibiting proliferation of retinal glial cells.⁷⁹ Cystatin 4 (CST4) is a cysteine proteinase inhibitor, likely serving a protective role on the ocular surface. Lactotransferrin (LTF) appears to have multiple roles. It reduces UV-B oxidative damage of human corneal epithelial cells,⁸⁰ it may be a general regulator of epithelial cell growth, it is antimicrobial and as previously noted when fragmented has anti-angiogenic activity. A Glu561Asp polymorphism is associated with corneal amyloidosis with trichiasis.⁸¹ LTF binds tear proteins CD14, CP, lipocalin-1 (LCN1), and lysozyme (LYZ). Also anti-bacterial are LCN1 (binds microbial iron chelating siderophores),⁸² lactoperoxidase (LPO), LYZ (a hydrolase that cleaves bacterial cell wall beta(1-4) glycosidic linkages)), and the polymeric immunoglobulin receptor (PIGR) that binds to *Streptococcus pneumoniae*. This overlapping mechanism of proteinases and inhibitors appears to become unbalanced in dry eye.

Chloride intracellular channel 2 (CLIC2; ‘Receptor/Channel/Transport’) regulates intracellular Cl⁻ concentration in the trabecular meshwork cells. Its role on the ocular surface and in tears appears to be unknown.

Selective Tear Protein Loss in Dry Eye

Although >183 ‘extracellular’ and ~256 ‘intracellular’ proteins populate the tear proteome surprisingly few appear to change in dry eye (*, Δ, † in Table 1).^{28,32,33} A discovery phase

comparison of dry eye and normal tears is perhaps best done by sensitive silver- or fluorescent-stained 2-D PAGE. This has advantages to an initial mass spectrometry step. Thus using 2-D PAGE, Koo et al²⁸ detected eight distinct proteins downregulated at least 50% in blepharitis vs normal tears. Each downregulated protein was identified by ESI-Q-TOF MS/MS (Table 1 *). Downregulation for some was verified by Western blotting. Similarly using 2-D differential gel electrophoresis of tears from contact lens-related dry eye versus normals, Green-Church et al³³ found only three tear proteins significantly decreased (Table 1 †). Several others were increased.³³ Each was identified by nano-LC-MS/MS. Current problems with the use of mass spectrometry in the first step include differential ionization efficiency of analytes and a relatively narrow dynamic range. However, a SELDI study suggesting differences between Sjögren's syndrome and normal tears⁸³ was recently pursued in more depth by LC-MS/MS. Only seven proteins were downregulated (Table 1 Δ).³² One common link between the three studies is the downregulation of lacritin, that as noted above functions as a prosecretory mitogen.⁵⁸ This intriguing pattern invites further study with larger sample numbers and sensitive immunoassays capable of quantitatively detecting low abundant tear proteins.

Recombinant Tear Protein Rescue?

Is downregulation of some tear proteins disease provoking? If so, tear proteins can be generated by recombinant DNA technology.⁵⁹ When mixed with vehicle, topical application could prove effective. Over the past 34 years since the discovery of recombinant DNA technology, an increasing number of human recombinant proteins have been successfully developed into therapeutics for the treatment of anemia, cancer, and renal, cardiovascular, rheumatologic and neurological diseases. Human recombinants include: erythropoietin, G-CSF, plasminogen activator, interferons, interleukins and humanized monoclonal antibodies. In age-related macular degeneration (AMD) or rubeosis iridis, recombinant Avastin and the ocular-reformulated Lucentis have proven effective. Each is a humanized fragment of recombinant anti-VEGF monoclonal antibody. Similarly, uveitis can be treated by the recombinant Infliximab (humanized anti-TNF monoclonal antibody). All three are administered by intravitreal injection. Topically applied human recombinant interferon-gamma has shown efficacy for herpetic keratitis.

Recombinant human proteins are the most rapidly expanding class of therapeutics in human health care with an expected 2010 market value of \$52 billion⁸⁴. Each demands a strong translational research effort, and only a fraction of recombinant proteins make it to market. Issues include economy of manufacture and stability in an appropriate vehicle, purity/reproducibility and compliance with current Good Manufacturing Practices, thorough preclinical animal testing of dose efficacy in dry eye models, demonstration of minimal topical and systemic adverse reactions or toxic effects, and definition of basic parameters of absorption, distribution and excretion. For an effective recombinant dry eye therapeutic, there should be evidence of active and preferably prolonged induction of basal tears. Tears should be compositionally similar to normal tears and there should be no toxicity to ocular tissues. Administration would be topical and optimally coupled to an immunoassay. With a selection of human recombinant tear proteins or synthetic peptides available for reconstitution, one might have available a 'designer' or personalized ophthalmic treatment regimen for dry eye. Although developing recombinant protein manufacturing processes are time-consuming and much remains to be learned about the cell biology of tear proteins, inducing or reconstituting a natural tear film may be the most satisfactory approach to dry eye.

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TABLE 1

Proteins in the normal human human tear 'proteome' that are predicted to be extracellular according to Gene Ontology (GO). The list is derived from published tear^{22,29-85}, Meibomian gland secretion³⁰, tear capture ELISA or antibody array^{5,86} and lacrimal gland EST⁸⁷ analyses. Not listed are the numerous cytoplasmic proteins that are also detected in tears²⁹.

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
(i) ANGIOGENESIS		
ANG	angiogenin, ribonuclease, RNase A family, 5 ⁵	promotes angiogenesis
BAI3	brain-specific angiogenesis inhibitor 3 ⁸⁵	possible angiogenesis inhibitor
ECGF1	endothelial cell growth factor 1 ²⁹	promotes angiogenesis
SERPINF1	serp. pep. inhib., cl. F (α -2 antiplas., PEDF), mem. 1 ²⁹	promotes neurodifferent. and inhibits angiogenesis
(ii) BIOSYNTHESIS		
ATP5B	ATP synth, H ⁺ transp., mitoch. F1 complex, ppolypep. 29	catalyzes ATP synthesis in mitochondrion
B4GALT1	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase ²⁹	polypep., glycoconjugate and lactose biosynthesis
PDIA6	protein disulfide isomerase family A, member 6 ²⁹	predicted electron transport and protein folding roles
PPIC	peptidylprolyl isomerase C (cyclophilin C) ²⁹	protein folding, binds cyclosporin A
(iii) CALCIUM		
AHSG	alpha-2-HS-glycoprotein ²⁹	calcification inhibitor
ANXA2	annexin A2 Ca ²⁺ depend. phospholip. binding prot. ²⁹	osteoclast formation and bone resorption
ANXA5	annexin A5 Ca ²⁺ depend. phospholip. binding prot. 29,87	promotes Ca ²⁺ channel activity
CALR	calreticulin ²⁹	Ca ²⁺ binding protein in ER and nucleus; SS assoc.
CALU	calumenin ²⁹	Ca ²⁺ binding protein in ER, protein folding/sorting
CANT1	CANT1 ²⁹	Ca ²⁺ activated nucleotidase
NUCB1	nucleobindin 1 ²⁹	Golgi and peripheral membrane Ca ²⁺ binding protein
NUCB2	nucleobindin 2 ²⁹	peripheral membrane Ca ²⁺ binding protein
(iv) CARBOHYDRATE		
AGL	amylase-1, 6-glucosidase, 4-alpha-glucanotransferase ²⁹	glycogen degradation
CHI3L2	chitinase 3-like 2 ²⁹	glycan but not heparin binding
ENO1	enolase 1, (alpha) ²⁹	glycolytic enzyme
GAPDH	glyceraldehyde-3-phosphate dehydrogenase ^{29,87}	carbohydrate metabolism
LGALS3	lectin, galactoside-binding, soluble, 3, galectin ^{29,87}	galactose-specific lectin
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein ²⁹	binds Mac-2 and galectin 1
MANBA	mannosidase, beta A ²⁹	lysosomal N-linked oligosaccharide catabolism

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
PKM2	pyruvate kinase, muscle ²⁹	carbohydrate degradat., binds bacterial Opa protein (v) CARRIER/BINDING PROTEIN STEROID ASSOC.
ALB	<u>albumin</u> * ^{22,29,85}	carrier protein
ARTS-1	type 1 TNFR shedding aminopeptidase regulator ²⁹	binds TNFR1 to promote shedding
AZGP1	alpha-2-glycoprotein 1 ^{22,29,87}	zinc-binding, lipid degrad., cell adhesion
CD14	CD14 molecule ²⁹	binds LPS binding protein (LBP) and apoptotic cells
DMBT1	deleted in malignant brain tumors 1 ^{22,29,85,87}	scavenger receptor, binds surfactant protein D
DSP	desmoplakin ²⁹	key component of desmosomes
GC	group-specific component (vitamin D binding protein) ²⁹	carrier protein for vitamin D and metabolites
HP	haptoglobin ^{22,29}	hemoglobin binding, turnover to diminish iron loss
HPX	hemopexin ^{22,29}	heme binding, turnover
HSPG2	heparan sulfate proteoglycan 2 (perlecan) ^{29,87}	growth factor binding, filtration, matrix polymerization
IGFBP1	insulin-like growth factor binding protein 1 ⁵	slows turnover of IGF's
IGFBP2	insulin-like growth factor binding protein 2 ⁵	slows turnover of IGF's
KPNB1	karyopherin (importin) beta 1 ²⁹	nuclear transport
LCN1	<u>lipocalin 1 (tear prealbumin)</u> ^{22,29-31, 62}	hydrophobic prot. binding, cyst. proteinase. Inhibitor
M6PRBP1	mannose-6-phosphate receptor binding protein 1 ²⁹	endosome-to-Golgi transport
PEBP4	phosphatidylethanolamine-binding protein 4 ²⁹	?
SCGB1D1	<u>secretoglobin, family 1D, member 1</u> ^{22,29,85,87}	in complex that binds steroids, including androgen
SCGB2A1	secretoglobin, family 2A, member 1 ^{22,29,85,87}	possibly binds steroids, including androgen
SCGB2A2	secretoglobin, family 2A, member 2 (mammaglobin 2) ⁸⁸	?
TCN1	transcobalamin I (vitamin B12 binding, R bind. family) ^{29,87}	binds and helps move vitamin B12 into cells
TF	transferrin ^{22,29,85}	iron binding and transport to proliferating cells
TTR	transthyretin (prealbumin, amyloidosis type I) ^{22,29}	thyroxine binding and transport (vi) CELL ADHESION/MOTILITY/STRUCTURE
ACTB	actin, beta ²²	cell structure, motility
ACTA1	actin, alpha 1, skeletal muscle ⁸⁸	formation of filaments
FGA	fibrinogen alpha chain ^{22,29}	cell adhesion, spreading, mitogenic, chemotactic
FGG	fibrinogen gamma chain ²⁹	cell adhesion, spreading, mitogenic, chemotactic
FLRT3	fibronectin leucine rich transmembrane protein 3 ⁸⁵	possibly cell adhesion, receptor signaling
FN1	fibronectin 1 ^{29,87}	cell adhesion, migration, blood coagulation

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
GSN	gelsolin (amyloidosis, Finnish type) ²⁹	blocks actin monom. exchange or promotes nucleat.
LAMA3	laminin, alpha 3 ³⁰	cell adhesion, differentiation
MFGE8	milk fat globule-EGF factor 8 protein ²⁹	cell adhesion, rotavirus binding/inhibition
MSLN	mesothelin ^{29,85}	possible cell adhesion activity
PFN1	profilin 1 ²⁹	regulator of actin polymerization and cytoskeleton
SLIT3	slit homolog 3 (Drosophila) ²⁹	cell migration
THBS1	thrombospondin 1 ²⁹	cell-cell and cell-matrix adhesion
TLN1	talín 1 ²⁹	actin filament assembly and cell spreading
VIM	vimentin ^{29,29}	cytoskeletal intermediate filaments
(vii) CELL GROWTH		
ANGPTL1	angiopoietin-like 1 ^{29,87}	may inhibit cell growth
EGF	epidermal growth factor (beta-urogastrone) ⁵	prosecretory mitogen
GDNF	glial cell derived neurotrophic factor ⁵	dopaminergic neuron survival, differentiation
HGF	hepatocyte growth factor (hepapoietin A; scatter factor) ⁵	serine protease-activated mitogen
LACRT	<u>lacritin</u> *A†22,29,30,85,87	prosecretory mitogen
MUC4	mucin 4 ²⁹	epithelial cell proliferation and differentiation
NTF3	neurotrophin 3 ⁵	sensory neuron survival
NTF5	neurotrophin 5 ⁵	peripheral sensory sympathetic neuron survival
QSOX1	(QSCN6) quiescin Q6 sulfhydryl oxidase 1 ²⁹	growth regulation
SERPINF5	serpin peptidase inhibit., clade B (ovalbum), member 5 ²⁹	blocks mammary tumor growth
(viii) CYTOPROTECTIVE/ANTI-APOPTOTIC		
CLU	clusterin ^{22, 29,85,87}	inhibits apoptosis
MUC16	mucin 16 ²⁹	cytoprotective, hydrophilic
MUC5AC	mucin 5AC ²⁹	mucus/gel-forming, cytoprotective, hydrophilic
PIP*	prolactin-induced protein ^{22, 29,85,87}	inhibitor of T-cell apoptosis, aspartyl proteinase (?)
PRB1	proline-rich protein BstNI subfamily 1 ⁸⁸	?
PROL1	proline rich, lacrimal 1 ^{22, 29,30,85,87}	possible ocular protective function
PRR4	<u>proline rich 4 (lacrimal)</u> †22, 29,30,85,87	possible ocular protective function
(ix) EXTRACELLULAR MATRIX		
COL6A1	collagen, type VI, alpha 1 ²⁹	microfibril component
MUCL1	mucin-like 1 ⁸⁵	?
SPARCL1	SPARC-like 1 (mast9, hevín) ^{29,87}	reg. of collagen assembly and decorin secretion
(x) Immune		

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
ATRN	atractin ²⁹	receptor or clustering of immune cells
C3	complement component 3 ^{22,29,87}	complement activation
C4A	complement component 4A (Rodgers blood group) ²⁹	cleaved to a trimer for complement activation
CCL2	chemokine (C-C motif) ligand 2 ⁵	monocyte, basophil specific chemotaxis
CCL4	chemokine (C-C motif) ligand 4 ⁵	Inflammatory, chemokinetic
CCL8	chemokine (C-C motif) ligand 8 ⁵	monocyte, basophil, eosinophil, lympho. chemotaxis
CCL11	chemokine (C-C motif) ligand 11 ⁵	eosinophil specific chemotaxis
CCL22	chemokine (C-C motif) ligand 22 ⁵	NK cell, dendritic, monocyte chemotaxis
CCL24	chemokine (C-C motif) ligand 24 ⁵	resting T cell chemotaxis
CFB	complement factor B ^{22,29}	CFD cleaved to: prolif. serine protease & antiprolif.
CFH	complement factor H ^{22,29,87}	restricts complement activation to microbial defense
CSF1	colony stimulating factor 1 (macrophage) ⁵	prod'n, different, function of macrophages
CSF2	colony stimulating factor 2 (granulocyte-macrophage) ⁵	prod'n, different, function of granulocytes, macroph.
CSF3	colony stimulating factor 3 (granulocyte) ⁵	prod'n, different, function of granulocytes, macroph.
CXCL5	chemokine (C-X-C motif) ligand 5 ⁵	inflammatory cytokine, neutrophil activation
CXCL10	chemokine (C-X-C motif) ligand 10 ⁵	T cell, monocyte chemotaxis
FCRL5	Fc receptor-like 5 ³⁰	possible mature B cell inhibitory co-receptor
CXCL1	chemokine (C-X-C motif) ligand 1 (MGS activity, alpha) ⁵	neutrophil chemotaxis
CXCL9	chemokine (C-X-C motif) ligand 9 ⁵	T cell chemotaxis
IL6	interleukin 6 (interferon, beta 2) ⁸⁶	B cell, nerve cell differentiation
IL8	interleukin 8 ^{5,86}	inflammatory response mediator, angiogenic
IL10	interleukin 10 ⁵	inhibit activated macrophage cytokine synthesis
IL12	interleukin 12 ⁵	activated T, NK cell mitogen
IL15	interleukin 15 ⁵	T cell proliferation
IL16	interleukin 16 (lymphocyte chemoattractant factor) ⁵	CD4+ lymphocyte, monocyte, eosinophil migration
IFNG	interferon, gamma ⁵	immunoregulatory, antiviral
ORM1	orosomucoid 1 ^{22,29}	apparent modulator of acute-phase immune activity
PPBP	pro-platelet basic prot. (chemokine (C-X-C motif) lig. 7) ⁵	neutrophil chemoattractant, prosecretory
SERPING1	serpin peptid. inhibitor, clade G (C1 inhib), member 1 ²⁹	complement activation regulator
TGFB2	transforming growth factor, beta 2 ⁵	suppress IL-2 T cell growth
TNF	tumor necrosis factor (TNF superfamily, member 2) ⁵	autoimmune, pyrogen, mitogen, differentiation

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
(xi) LIPID/CHOLESTEROL		
ANPEP	alanyl (membrane) aminopeptidase ²⁹	aminopeptidase
ANXA1	annexin A1 ²²	regulate phospholipase a2 activity
APOA1	apolipoprotein A-I ²²	cholesterol transport
APOA2	apolipoprotein A-II ²⁹	in high density lipoprotein particles
APOA4	apolipoprotein A-IV ²²	in HDL and chylomicrons
APOB	apolipoprotein B ²⁹	in chylomicrons and low density lipoproteins
APOH	apolipoprotein H ^{22,29}	lipoprotein metabolism, coagulation
PLA2G2A	phospholipase A2, group IIA ⁸⁵	membrane phospholipid metabolism
PLTP	phospholipid transfer protein ^{29,85}	cholesterol metabolism
PSAP	prosaposin ^{29,87}	enzyme stimulator in glycosphingolipid metabolism
(xii) OTHER/UNKNOWN		
AGT	angiotensinogen ²⁹	cleaved to angiotensin I, blood pressure
B2M	beta-2-microglobulin ^{22,29,85,87}	loss assoc. with hypercatabolic hypoproteinemia
EVC2	Ellis van Creveld syndrome 2 (limbin) ³⁰	mutation assoc. with Ellis-van Creveld syndrome
HSPA4	heat shock 70kDa protein 4 ²⁹	heat shock protein
LRG1	leucine-rich alpha-2-glycoprotein 1 ²⁹	?
SFRP1	secreted frizzled-related protein 1 ²⁹	possible role in polarity of retinal photoreceptor cells
SMR3A	submax. gland androg. reg. prot. 3 homol. A (mouse) 22,85	?
SMR3B	submax. gland androg. reg. prot. 3 hom. B (mouse) 22,85,87	?
(xiii) Phosphatase/Kinase/GTPase/Other Enzyme		
ACPP	acid phosphatase ²⁹	phosphatase
ARHGAP1	Rho GTPase activating protein ²⁹	GTPase activator of Rho, Rac and Cdc42
CA2	carbonic anhydrase II ²⁹	hydration of carbon dioxide
CP	ceruloplasmin (ferroxidase) ^{22,29,87}	peroxidation of Fe(II)transferrin, binds copper
F2	coagulation factor II (thrombin) ²²	fibrinogen to fibrin conversion
F5	coagulation factor V ²⁹	prothrombin/thrombin conversion with coag. factor X
GNPTG	N-acetylglucosamine-1-phosphate transf., γ subunit	targeting of lysosomal hydrolases to lysosomes
HTRA1	HtrA serine peptidase 1 ²⁹	cleaves IGF-binding proteins
MMP9	matrix metalloproteinase 9 ²⁹	matrix collagen IV and V degradation
S100A8	S100 calcium binding protein A8 ^{22,29}	possible cytokine and inhibitor of casein kinase
S100A9	S100 calcium binding protein A9 ^{22,29}	possible inhibitor of casein kinase
TGM2	transglutaminase 2 ²⁹	protein crosslinker

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
TXN	thioredoxin ²⁹	catalyzes dithiol-disulfide exchange and redox rxns
USP5	ubiquitin specific peptidase 5 (isopeptidase T) ²⁹	cellular protein degradation (xiv) PROTEINASE/INHIBITOR/ANTIMICROBIAL
A2M	alpha-2-macroglobulin ^{29,87}	proteinase inhibitor, cytokine transporter
AMBP	alpha-1-microglobulin/bikunin precursor ²⁹	inhibits trypsin, plasmin, elastase
AZU1	azurocidin 1 (cationic antimicrobial protein 37) ²⁹	antibacterial and monocyte chemoattractant
CST1	cystatin SN ^{22,29,85}	cysteine proteinase inhibitor
CST2	cystatin SA ⁸⁵	thiol protease inhibitor
CST3	cystatin C ^{29,85}	abundant cysteine proteinase inhibitor
CST4	cystatin S ^{22,29,85,87}	cysteine proteinase inhibitor
CST5	cystatin D ⁸⁵	cysteine proteinase inhibitor
CTSB	cathepsin B ²⁹	lysosomal cysteine proteinase
CTSD	cathepsin D ²⁹	lysosomal aspartyl proteinase
CTSG	cathepsin G ²⁹	chymotrypsin C-like proteinase, antimicrobial
DCD	dermcidin ²⁹	C-terminal antibacterial, N-terminal prosurvival
DEFA3	defensin, alpha 3, neutrophil-specific ²²	anti-bacterial, -viral, -fungal
DPP4	dipeptidyl-peptidase 4 (CD26) ²⁹	intrinsic membrane serine exopeptidase
ELA2	elastase 2, neutrophil ²²	matrix hydrolysis, antibacterial
IGHA1	immunoglobulin heavy constant alpha 1 ^{22,29,30,85,87}	microbial and foreign antigen defense
IGHA2	immunoglob. heavy constant alpha 2 (A2m marker) ^{31,85}	microbial and foreign antigen defense
IGHM	immunoglobulin heavy constant mu ^{22,29,85}	microbial and foreign antigen defense
IGJ	immunoglobulin J polypeptide ^{22,85}	microbial and foreign antigen defense
IGKC	immunoglobulin kappa constant ⁸⁵	microbial and foreign antigen defense
IGLC2	immunoglobulin lambda constant 2 ⁸⁵	microbial and foreign antigen defense
IGLV1-40	immunoglobulin lambda variable 1-40 ²⁹	microbial and foreign antigen defense
ITIH1	inter-alpha (globulin) inhibitor H1 ²⁹	hyaluronan bp/carrier, pred. serine proteinase inhib.
ITIH2	inter-alpha (globulin) inhibitor H2 ²⁹	hyaluronan bp/carrier, pred. serine proteinase inhib.
ITIH4	inter-alpha (globulin) inhibitor H4 ²⁹	predicted serine proteinase inhibitor
LCN2	lipocalin 2 (oncogene 24p3) ²⁹	MMP9 binding, bacteriostatic, growth factor-like
LPO	lactoperoxidase ^{29,87}	antibacterial
LTF	lactotransferrin ^{22,29,30,85,87}	iron metabolism, antibacterial
LYZ*	lysozyme (renal amyloidosis) ^{*22,29,30,85,87}	hydrolase, antibacterial

Gene Symbol	Protein	Function (as per Locust Link, OMIM or Source)
MPO	myeloperoxidase ²⁹	antimicrobial
MUC7	mucin 7, secreted ²²	antibacterial, antifungal
PIGR	polymeric immunoglobulin receptor ^{22,29,85,87}	antibacterial, polymeric Ig transcellular transport
PLG	plasminogen ²⁹	activates urokin.-type plasmin. activat., collagenas.,
PRB4	proline-rich protein BstNI subfamily 4 ⁸⁵	possible bacterial binding (lost in point mutant)
SERPINA1	<u>serpin peptid. inhibit., clade A (alpha-1), member 1</u> ^{*22,29}	serine proteinase inhibitor, anti-inflammatory
SERPINA3	serpin peptidase inhibit., clade A (alpha-1), member 3 ²⁹	serine proteinase inhibitor
SERPINC1	serpin peptid. inhibit., clade C (antithrom.), member 1 ^{22,29}	blood coagulation cascade regulator
SLPI	secretory leukocyte peptidase inhibitor ^{22,85,87}	acid-stable proteinase inhib., antibacterial
TIMP1	TIMP metalloproteinase inhibitor 1 ^{5,29}	metalloproteinase inhibitor
TIMP2	TIMP metalloproteinase inhibitor 1 ⁵	metalloproteinase inhibitor, inhib. endothelial prolif. (xv) RECEPTOR/CHANNEL/TRANSPORT
CLIC2	chloride intracellular channel 2 ⁸⁵	potential chloride ion channel
SLC7A4	solute carrier family 7, member 4 ⁸⁵	cationic amino acid transport

^{*,Δ,†} suggested to be less than normal in tears from patients suffering from ^{*}blepharitis²⁸ ^ΔSjögren's syndrome³² or [†]contact lens related dry eye³³.