Proteomic and gene expression patterns of keratoconus

Arkasubhra Ghosh, Lei Zhou[1], Anuprita Ghosh, Rohit Shetty[2], Roger Beuerman[1,3,4]

Keratoconus is a progressive corneal thinning disease associated with significant tissue remodeling activities and activation of a variety of signaling networks. However, it is not understood how differential gene and protein expression direct function in keratoconus corneas to drive the underlying pathology, ectasia. Research in the field has focused on discovering differentially expressed genes and proteins and quantifying their levels and activities in keratoconus patient samples. In this study, both microarray analysis of total ribonucleic acid (RNA) and whole proteome analyses are carried out using corneal epithelium and tears from keratoconus patients and compared to healthy controls. A number of structural proteins, signaling molecules, cytokines, proteases, and enzymes have been found to be deregulated in keratoconus corneas. Together, the data provide clues to the complex process of corneal degradation which suggest novel ways to clinically diagnose and manage the disease. This review will focus on discussing these recent advances in the knowledge of keratoconus biology from a gene expression and function point-of-view.

Key words: Deregulation, ectasia, gene expression, keratoconus, mass spectroscopy, proteomics, signaling pathways

Access this article online

Website: www.ijo.in

DOI: 10.4103/0301-4738.116056

Quick Response Code:

Keratoconus (KC) is an asymmetric, progressive ectatic condition that can lead to significant visual impairment.[1] Although the disease has high prevalence, the cellular etiology of the disease is not well understood. Studies from various laboratories across the globe and in varied fields such as genetics, genomics, small biomolecule analyses, and gene expression analysis suggest that the disease may be multifactorial in origin. Furthermore, a variety of genome-wide studies in familial KC implicate differential loci. Therefore, it is even more evident that the disease may be sporadic and dependent on external factors and stimuli that lead to the inception and progression of this complex disease.[2] Although KC was historically thought of as a non-inflammatory condition,[3] recent literature uncovers some compelling evidence of inflammatory molecules being present in patients.[4-7] Allergic history, atopy (eczema, asthma, and hay fever), corneal injury, eye rubbing, and rigid contact lens usage have been shown to be associated with the development of KC. Analysis and quantification of deregulated biomolecules in KC patients or disease models should reveal protein signaling pathways driving the disease. Whole proteome analyses using various technologies like two-dimensional-difference gel electrophoresis mass spectrometry (2D-DIGE/MS) and Liquid chromatography tandem mass spectrometry (LC-MS/MS) have emerged in recent years. A variety of tissues and fluids are analyzed using this technique and the data reveal interesting biomarkers and signaling networks that are useful as clinical biomarkers for disease progression and as potential therapeutic intervention nodes. This review will therefore focus on collating the recent literature on the analysis of proteomic data from KC patients and expression analyses carried out with corneal epithelium and tears from KC patients. We will then discuss the data in the context of probable deregulation of pathways that may thus be the underlying cause of the disease.

Genes, Repair and Regeneration at Ophthalmic Workstation (GROW Research Laboratory), Narayana Nethralaya, Bangalore, Karnataka, India, [2]Vice Chairman, Narayana Nethralaya, Bangalore, Karnataka, India, [1]Singapore Eye Research Institute, [3]SRP Neurosience and Behavioral Disorders, Duke University-National University of Singapore, [4]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Correspondence to: Dr. Arkasubhra Ghosh, Narayana Nethralaya, Bangalore, Karnataka, India. E-mail: arka.s.ghosh@gmail.com

Manuscript received: 14.06.13; Revision accepted: 04.07.13

Proteomic Studies of Keratoconus Reveal a Variety of Differentially Expressed Protein Groups

A proteomic analysis of keratoconus was attempted early by Nielsen *et al*., using 2D-Gel electrophoresis followed by mass spectrometry from patient epithelia.[8] Analysis of differential spots identified gelsolin, S100A4, and cytokeratin 3 to be highly overexpressed in KC epithelium[8] and alpha enolase to be slightly upregulated. However, another study using the same strategy found alpha enolase and beta actin to be poorly expressed in corneal wing and superficial epithelial cells from KC patients.[9] However, cytokeratins and gelsolin proteins have been implicated in other ocular disorders such as vitreoretinopathy as well as in non-ocular diseases like cancer, cystic fibrosis, steatohepatitis, etc.[10] In recent years, the field of tear film proteomics has attracted a lot of attention and has been utilized for analysis of predictive biomarkers for ocular surface diseases. Recent studies have shown that there are more than 1,500 proteins and peptides in the tear film with additional lipids, cytokines, small molecules, and metabolites.[11] These tear film constituents reflect the health of the epithelial cell layer covering the ocular surface and are of intra- and extracellular origin. These proteins have been shown to have functional roles in the epithelial cells or other tissues associated with maintaining the health of the ocular surface. The bulk of these tear components consist of lysozyme, serum albumin, lactoferrin, secretory immunoglobulin A, proline rich proteins, tear lipocalin, and lipophilin.[12] When tear proteome from 44 KC patients were compared to 20 healthy controls by nano-LC tandem

MS/MS, cytokeratins, matrix metalloproteinase 1 (MMP1), and mammoglobin B were found to be increased.[13] Furthermore, they found immunoglobulin alpha and kappa, lipocalin, lysozyme C, and precursors to prolactin to be associated with KC.[13] In another tear film proteomic study using 2-DE/MS method, a few novel proteins, zinc-α2-glycoprotein (ZAG), and immunoglobulin kappa chain (IGKC) as well as lactoferrin were found to have reduced expression in KC patients.[14] Joseph *et al*., identified stromal and epithelial proteins that exhibited differential expression in corneas from KC patients compared to normal human corneas.[15] Epithelial and stromal protein preparations from KC and age-matched normal corneas were separately analyzed by shotgun proteomic approach using Nano-Electrospray Ionization Liquid Chromatography Tandem Mass Spectrometry [Nano-ESI-LC-MS/MS] and 2D-DIGE followed by mass spectrometry. The label-free Nano-ESI-LC-MS/MS method identified six KC epithelial proteins (keratin type I cytoskeletal 14, keratin type I cytoskeletal 16, lamin-A/C, tubulin beta chain, heat shock cognate 71 kDa protein, and S100-A4) to be upregulated. The same experiments revealed five proteins (pyruvate kinase, transketolase, 14-3-3 sigma isoform, nicotinamide adenine dinucleotide phosphate (NADPH) dehydrogenase (quinone) 1, and phosphoglycerate kinase 1) to be down-regulated relative to normal in KC. A similar relative analysis revealed that three KC stromal proteins (vimentin, decorin, keratocan) were up-regulated while Transforming growth factor beta (TGF-beta) ig-h3 (Bigh3), serotransferrin, meprin, A-5 protein, and receptor protein-tyrosine phosphatase μ (MAM) domain-containing protein 2, and isoforms 2C2A of collagen alpha-2(VI) chain) were under-expressed. The 2D-DIGE-mass spectrometry showed that KC corneal epithelium exhibited upregulation of serum albumin, keratin 5, L-lactate dehydrogenase and annexin A8 and downregulation of Ferritin heavy chain protein 1 (FTH 1), calpain small subunit 1, heat shock protein beta 1, and annexin A2. In the stroma, aldehyde dehydrogenase 3A1 (ALDH3A1), keratin 12, apolipoprotein A-IV precursor, haptoglobin precursor, prolipoprotein, and lipoprotein Gln were upregulated in KC corneas.[15] Another recent study demonstrated that KC tears had significantly reduced (~50%) total protein levels.[16] They also found that levels of lactoferrin and secretory IgA were reduced in KC tears and negatively correlated with keratometry readings. The disparity in the results from these studies likely reflects the considerable differences in the instrumental analysis and possibly in the circumstances in obtaining the samples. However, these studies have not been able to produce stable biomarkers useful for clinical stratification of patients.

Differential Gene Expression Occurs in Keratoconus Corneas

Global gene expression analysis was studied by Nielsen *et al*., using microarrays for epithelial ribonucleic acid (RNA) from KC patients and healthy controls.[17] They observed differential expression of 471 genes of which 47 had significantly increased expression and nine had reduced. Of these, two genes, lysyl oxidase (LOX) and TIMP3 have been reported by numerous groups. A number of studies also found tissue inhibitor of metalloproteinase 1 (TIMP1) to be significantly reduced in KC corneas compared to normal.[18,19] The lysyl oxidase group of enzymes has been shown to have lower expression and lesser activity in KC corneas.[20] Katoh *et al*., found human angiopoietin isoform (ANGPTL7) messenger ribonucleic acid (mRNA) to be upregulated in keratoconus[21] cornea indicating the involvement of the WNT/beta-catenin signaling pathway. This is particularly interesting because a more recent study specifically on the Wnt signaling pathway demonstrates that Secreted frizzled-related protein 1 (SFRP1) protein is highly expressed in KC epithelia at both the RNA and protein levels compared to normal.[22] Apart from this, recent literature suggests that inflammatory molecules and abnormal levels of enzymes are present in subjects with KC.[3,6,23] Lema *et al*., have demonstrated that tears from KC patients have higher levels of interleukin 6 (IL6), tumor necrosis factor alpha (TNF-α), and MMP9 compared to healthy controls.[6,7,23] Indeed, Balasubramanium *et al*., found total tear protein levels to be significantly reduced in KC compared with normal or patients who had undergone corneal collagen cross-linking.[4] Tears of KC patients had much higher expression of IL-4, -5, -6, -8, MMP-1, -3, -7, -13, and TNF-α, -β. However, tear IL-6 was increased in keratoconus subjects compared to collagen cross-linked group. Interestingly, while there were no significant differences in tear proteases between normal and cross-linked cohorts, TNF-α expression was significantly increased in the cross-linked group compared with the controls. However, in the KC cohort, much higher gelatinolytic and collagenolytic activities were observed compared to the other two groups.[4]

Discussion

There is a need to understand the expression levels of cellular and secreted proteins in KC patients to elucidate the mechanisms underlying the disease pathogenesis. Recent clinical studies supports the idea that the pathogenesis has an inflammatory component.[4,6,7,24] Studies investigating biochemical and pathologic changes in structural and cellular levels of cornea have also been done,[25] but specific molecular mechanisms of KC or the influence of environment are still not fully understood. Progression of KC could be related to conditions of allergic etiology.[24,26] Tissue degradation in KC involves expression of inflammatory mediators, proinflammatory cytokines, cell adhesion molecules, and MMPs.[4,5,7] The deregulation of cytokeratins and immunoglobulins associated with the disease need to be investigated mechanistically in the light of the degradation process associated with ectasia and tissue remodeling. However, the association with raised oxidative status of the KC cornea evidenced by LOX deregulation[20] and upregulation of the Wnt pathway[21,22] is very interesting since they may prove to be novel intervention nodes for disease management. Furthermore, increased expression of molecules such as S100A4 and alpha enolase, an epithelial cell metabolite, which are associated with inflammation, may have important roles in KC pathogenesis. While the various proteomic and gene expression studies have discovered both common and unique molecules that can serve as biomarkers of KC, additional future studies will reveal the exact molecular mechanisms of its progression that is required to better manage the disease and halt its progression.

References

1. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998;42:297-319.

2. Rabinowitz YS. The genetics of keratoconus. Ophthalmol Clin North Am 2003;16:607-20.
3. Cristina Kenney M, Brown DJ. The cascade hypothesis of keratoconus. Cont Lens Anterior Eye 2003;26:139-46.
4. Balasubramanian SA, Mohan S, Pye DC, Willcox MD. Proteases, proteolysis and inflammatory molecules in the tears of people with keratoconus. Acta Ophthalmol 2012;90:e303-9.
5. Jun AS, Cope L, Speck C, Feng X, Lee S, Meng H. *et al.* Subnormal cytokine profile in the tear fluid of keratoconus patients. PloS One 2011;6:e16437.
6. Lema I, Duran JA. Inflammatory molecules in the tears of patients with keratoconus. Ophthalmology 2005;112:654-9.
7. Lema I, Sobrino T, Duran JA, Brea D, Diez-Feijoo E. Subclinical keratoconus and inflammatory molecules from tears. Br J Ophthalmol 2009;93:820-4.
8. Nielsen K, Vorum H, Fagerholm P, Birkenkamp-Demtroder K, Honore B, Ehlers N, *et al.* Proteome profiling of corneal epithelium and identification of marker proteins for keratoconus, a pilot study. Exp Eye Res 2006;82:201-9.
9. Srivastava, OP, Chandrasekaran D, Pfister RR. Molecular changes in selected epithelial proteins in human keratoconus corneas compared to normal corneas. Mol Vis 2006;12:1615-25.
10. Brouillard F, Fritsch J, Edelman A, Ollero M. Contribution of proteomics to the study of the role of cytokeratins in disease and physiopathology. Proteomics Clin Appl 2008;2:264-85.
11. Zhou L, Zhao SZ, Koh SK, Chen L, Vaz C, Tanavde V, *et al.* In-depth analysis of the human tear proteome. J Proteomics 2012;75:3877-85.
12. de Souza GA, Godoy LM, Mann M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. Genome Biol 2006;7:R72.
13. Pannebaker C, Chandler HL, Nichols JJ. Tear proteomics in keratoconus. Mol Vis 2010;16:1949-57.
14. Lema I, Brea D, Rodriguez-Gonzalez R, Diez-Feijoo E, Sobrino T. Proteomic analysis of the tear film in patients with keratoconus. Mol Vis 2010;16:2055-61.
15. Joseph R, Srivastava OP, Pfister RR. Differential epithelial and stromal protein profiles in keratoconus and normal human corneas. Exp Eye Res 2011;92:282-98.
16. Balasubramanian SA, Pye DC, Willcox MD. Levels of lactoferrin, secretory IgA and serum albumin in the tear film of people with keratoconus. Exp Eye Res 2012;96:132-7.
17. Nielsen K, Birkenkamp-Demtroder K, Ehlers N, Orntoft TF. Identification of differentially expressed genes in keratoconus epithelium analyzed on microarrays. Invest Ophthalmol Vis Sci 2003;44:2466-76.
18. Matthews FJ, Cook SD, Majid MA, Dick AD, Smith VA. Changes in the balance of the tissue inhibitor of matrix metalloproteinases (TIMPs)-1 and -3 may promote keratocyte apoptosis in keratoconus. Exp Eye Re2007;84:1125-34.
19. Kenney MC, Chwa M, Atilano SR, Tran A, Carballo M, Saghizadeh M, *et al.* Increased levels of catalase and cathepsin V/L2 but decreased TIMP-1 in keratoconus corneas: Evidence that oxidative stress plays a role in this disorder. Invest Ophthalmol Vis Sci 2005;46:823-32.
20. Dudakova L, Liskova P, Trojek T, Palos M, Kalasova S, Jirsova K. Changes in lysyl oxidase (LOX) distribution and its decreased activity in keratoconus corneas. Exp Eye Res 2012;104:74-81.
21. Katoh Y, Katoh M. Comparative integromics on angiopoietin family members. Int J Mol Med 2006;17:1145-9.
22. Sutton G, Madigan M, Roufas A, McAvoy J. Secreted frizzled-related protein 1 (SFRP1) is highly upregulated in keratoconus epithelium: A novel finding highlighting a new potential focus for keratoconus research and treatment. Clin Experiment Ophthalmol 2010;38:43-8.
23. Lema I, Duran JA, Ruiz C, Diez-Feijoo E, Acera A, Merayo J. Inflammatory response to contact lenses in patients with keratoconus compared with myopic subjects. Cornea 2008;27:758-63.
24. Nemet AY, Vinker S, Bahar I, Kaiserman I. The association of keratoconus with immune disorders. Cornea 2010;29:1261-4.
25. Yue BY, Sugar J, Schrode K. Histochemical studies of keratoconus. Curr Eye Res 1988;7:81-6.
26. Jacq PL, Sale Y, Cochener B, Lozach P, Colin J. Keratoconus, changes in corneal topography and allergy. Study of 3 groups of patients. J Fr Ophtalmol 1997;20:97-102.

Cite this article as: Ghosh A, Zhou L, Ghosh A, Shetty R, Beuerman R. Proteomic and gene expression patterns of keratoconus. Indian J Ophthalmol 2013;61:389-91.

Source of Support: Nil. **Conflict of Interest:** None declared.