Primary Human Trabecular Meshwork Model for Pseudoexfoliation

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Pseudoexfoliation is an age related fibrillopathy characterized by abnormal fibrillar extracellular material (ECM) in ocular tissues. Pseudoexfoliative aggregate material causing mechanical obstruction of the trabecular meshwork (TM), blood aqueous barrier dysfunction, endothelial cell dysfunction, and abnormal ECM homeostasis cause TM dysfunction/fibrosis eventually leading to glaucoma, if untreated. The pseudoexfoliative material comprises of non-collagenous basement membrane components such as laminin, fibronectin, amyloid P, and vitronectin as well as proteinaceous components of elastic fibres (such as elastin, tropoelastin, fibrillin-1, microfibril-associated glycoprotein-1) and latent TGF-β-binding proteins (LTBP-1 and -2). Transforming growth factor, tissue matrix metalloproteinases (MMPs) and plasminogen activator inhibitor-1 (PAI-1) regulate ECM homeostasis with increased PAI-1 levels causing excessive ECM deposition and reduced degradation in adjoining tissues. While Lysyl oxidase homolog 1 (LOXL1) is deemed necessary for disease pathogenesis, it is now understood that LOXL1 alone does not explain the preferential geographical distribution or the differential role of different genes in disease pathogenesis or glaucoma onset in different ethnic populations. It is well recognized that environmental factors, epigenetics, and their interplay with gene expression is what may hold the key for explaining the disease pathogenesis.

Keywords: pseudoexfoliation ; in vitro cell line model ; human trabecular meshwork ; TGF-β1 ; fibrosis

1. Introduction

These are the challenges to successful consistent replication of the disease in an animal or an in vitro model for pseudoexfoliation. Currently, mice harboring a spontaneous mutation in the lysosomal trafficking regulator (*LYST*) gene have been shown to have closest feature of human disease in the form of iris transillumination defects [1]. Yet, glaucoma was not seen in these mice despite presence of fibrillin positive aggregates in the anterior chamber. Another group reported ocular features consistent with PXF like disorganized ciliary body and accumulation of exfoliation like extracellular material in the anterior segment in mice injected with recombinant adenovirus coding Wnt5a. Yet, presence of glaucoma could not be confirmed in this model [2].

Several groups have shown that the aqueous concentrations of TGF-β1, were higher in eyes with pseudoexfoliation syndrome (PXF) and pseudoexfoliation glaucoma (PXG) than controls or other primary glaucoma [3][4]. Increased levels of few cytokines, namely, interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF) have also been reported in the aqueous humor of glaucomatous eyes [5][6]. These pro-inflammatory cytokines have been shown to favor and create a pro-fibrotic tissue milieu [5][6][7][8] and with oxidative stress they are known to play a role in PXF and PXG [8][9]. In our previous study, we found elevated TGF-β1 levels in the aqueous humor of patients that increased with disease stages, with maximal levels being found in eyes with ocular hypertension and glaucoma [10]. We also found decreasing tear and aqueous MMP9 activity, with increased inflammatory cytokine levels, in severe PXG eyes compared to primary glaucoma [10], suggesting that the elevated TGF levels somehow were important for molecular events that causes the disease. We also found serum levels to parallel the levels in the aqueous and tears with several key miRNA driven by TGF-β1 to be upregulated in eyes with ocular hypertension (OHT) and PXG [11]. This was also associated with downregulation of several unfolded protein response (UPR) genes, a key mechanism of abnormal ECM homeostasis in PXF. This study now explores the molecular effects of sustained TGF-β1 exposure of HTM cell in vitro and to evaluate expression of ECM/elastic fiber components as well as morphological/molecular changes induced thereby.

2. Current Insights

Excessive accumulation of ECM proteins is considered a major feature of glaucomatous TM tissues. The TM cells and surrounding ECM work like a syncytium complex called matrisome that regulates aqueous humor outflow in the anterior chamber [12]. The pathogenic mechanism of increased resistance to aqueous humor outflow in PXF is poorly understood, but excessive ECM deposition, aggravated cell death, and alterations in cytoskeletal organization [13][14][4] apart from mechanical obstruction of the TM spaces by exfoliative material, have been thought to be the key mechanisms. Experimental models to study these mechanisms are lacking with current animal models mimicking certain ocular features of the human disease without evident glaucoma [15][16][17]. One of the key feature of PXF is protein aggregate formation. The exact composition of the pseudoexfoliative material is still not known histological and biochemical approaches have shown that the aggregates in PXF have a protein core surrounded by glycoconjugates that are resistant to enzymatic degradation [16]. The protein core has been shown to be composed of ECM proteins, MMPs and cross-linking enzymes like LOXL1 [15][18][12][16]. Therefore, we tried to create an in vitro model on HTM cells that will mimic PXF conditions in the eye. In this in vitro model, TGF-β1 (10 ng/mL) exposure caused upregulated expression of ECM proteins, EMT morphological changes, paralleled by deregulated enzymatic activity of MMP9 and protein aggregate formation at 48–72 h of exposure, all of which mimic features of PXF disease in the human eye. We visualized the protein aggregates using antibodies that bind to amyloid fibrils, key component of misfolded proteins. Though transmission electron microscope (TEM) and a 3D model of HTM cell culture would have been ideal, nanometer size of the aggregates and disruption during processing prevented visualization of these fibrils. Here we found that chronic TGF-β1 (10 ng/mL) exposure induces EMT in TM cells at 48 h, which was also confirmed by increased α-SMA and vimentin expression. This paralleled increased IL-6 and IL-8 levels at the same time period, both of which are TGF-induced deleterious effects for ECM homeostasis [8][19][20]. TGF-β1 induced EMT was inhibited by Smad3 inhibition, while other proteins were differentially regulated by other pathways. TGFβ1 is known to orchestrate EMT as a mechanism of regulating ECM homeostasis and cell survival [21][22][23][24]. EMT is a normal process involved in tissue repair [25][26] but deregulated EMT can lead to fibrosis. EMT-associated disease pathogenesis has already been studied in a range of diseases including lung fibrosis and cancer [27]. In the eye, EMT has been associated with subretinal fibrosis leading to age-related macular degeneration (AMD) [23]. The canonical and non-canonical pathways differentially regulate ECM proteins and this maybe one of the mechanisms of the TGF paradox driving increased fibrosis and functional damage in eyes with glaucoma in pseudoexfoliation. Study of these effects in our model particular could give more insights into actual regulation and cross talk of other pathways modulating TGF induced fibrosis in HTM cells. It may also be used to identify putative molecules to reverse EMT, cell death or aggregate formation, which may form important targets for preventing glaucoma in pseudoexfoliation.

To maintain homeostasis, cells rely on protective mechanisms to help them cope with ER stress, pathways referred to collectively as the unfolded protein response (UPR) [28]. The relation between UPR signaling and fibrogenesis are not fully understood. Studies suggest that ER stress facilitates fibrotic remodeling through activation of pro-apoptotic pathways, induction of epithelial—mesenchymal transition, and promotion of inflammatory responses [28][29][30]. In our previous study, we found that the expression of UPR response genes like *XBP1* and *CCT4* were significantly downregulated in PXG [11]. Therefore, we wanted to see if the UPR pathway is also hampered in the in vitro model. We found reduced expression of UPR markers like *XBP1* and *CCT4* at delayed TGF-β1 exposures. This hints at the insufficient UPR activation hampering the process of protein clearance and the misfolded proteins forming insoluble aggregates. Overall, our data indicate that sustained TGF-β1 treatment used in this in vitro HTM cell lime model could be used to mimic PXF human disease. This may enable studying temporal molecular events at different time points and allow us to identify drug targets for preventing cell death or aggregate formation.

References

1. Trantow, C.M.; Mao, M.; Petersen, G.E.; Alward, E.M.; Alward, W.L.; Fingert, J.H.; Anderson, M.G. Lyst mutation in mic e recapitulates iris defects of human exfoliation syndrome. Investig. Ophthalmol. Vis. Sci. 2009, 50, 1205–1214.
2. Yuan, Y.; Schlötzer-Schrehardt, U.; Ritch, R.; Call, M.; Chu, F.B.; Dong, F.; Rice, T.; Zhang, J.; Kao, W.W.-Y. Transient e xpression of Wnt5a elicits ocular features of pseudoexfoliation syndrome in mice. PLoS ONE 2019, 14, e0212569.
3. Kara, S.; Yildirim, N.; Ozer, A.; Colak, O.; Sahin, A. Matrix metalloproteinase-2, tissue inhibitor of matrix metalloproteina se-2, and transforming growth factor beta 1 in the aqueous humor and serum of patients with pseudoexfoliation syndro me. Clin. Ophthalmol. 2014, 8, 305–309.
4. Schlötzer-Schrehardt, U.; Zenkel, M.; Küchle, M.; Sakai, L.Y.; Naumann, G.O. Role of transforming growth factor-beta1 and its latent form binding protein in pseudoexfoliation syndrome. Exp. Eye Res. 2001, 73, 765–780.

5. Chua, J.; Vania, M.; Cheung, C.M.; Ang, M.; Chee, S.P.; Yang, H.; Li, J.; Wong, T.T. Expression profile of inflammatory c ytokines in aqueous from glaucomatous eyes. Mol. Vis. 2012, 18, 431–438.
6. Garweg, J.G.; Zandi, S.; Pfister, I.B.; Skowronska, M.; Gerhardt, C. Comparison of cytokine profiles in the aqueous hu mor of eyes with pseudoexfoliation syndrome and glaucoma. PLoS ONE 2017, 12, e0182571.
7. Gauldie, J.; Kolb, M.; Sime, P.J. A new direction in the pathogenesis of idiopathic pulmonary fibrosis? Respir. Res. 200 2, 3, 1.
8. Nieto, N. Oxidative-stress and IL-6 mediate the fibrogenic effects of Kupffer cells on stellate cells. Hepatology 2006, 44, 1487–1501.
9. Zenkel, M.; Lewczuk, P.; Jünemann, A.; Kruse, F.E.; Naumann, G.O.; Schlötzer-Schrehardt, U. Proinflammatory cytokin es are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. Am. J. Pathol. 2010, 176, 2868–2879.
10. Sahay, P.; Reddy, S.; Prusty, B.K.; Modak, R.; Rao, A. TGFβ1, MMPs and cytokines profiles in ocular surface: Possible tear biomarkers for pseudoexfoliation. PLoS ONE 2021, 16, e0249759.
11. Rao, A.; Chakraborty, M.; Roy, A.; Sahay, P.; Pradhan, A.; Raj, N. Differential miRNA Expression: Signature for Glauco ma in Pseudoexfoliation. Clin. Ophthalmol. 2020, 14, 3025–3038.
12. Tamm, E.R.; Braunger, B.M.; Fuchshofer, R. Intraocular Pressure and the Mechanisms Involved in Resistance of the A queous Humor Flow in the Trabecular Meshwork Outflow Pathways. Prog. Mol. Biol. Transl. Sci. 2015, 134, 301–314.
13. Lee, R.K. The molecular pathophysiology of pseudoexfoliation glaucoma. Curr. Opin. Ophthalmol. 2008, 19, 95–101.
14. Schlötzer-Schrehardt, U.; Naumann, G.O. Trabecular meshwork in pseudoexfoliation syndrome with and without open-angle glaucoma. A morphometric, ultrastructural study. Investig. Ophthalmol. Vis. Sci. 1995, 36, 1750–1764.
15. Zenkel, M.; Schlötzer-Schrehardt, U. The composition of exfoliation material and the cells involved in its production. J. Glaucoma 2014, 23 (Suppl. S1), S12–S14.
16. Aires, I.D.; Ambrósio, A.F.; Santiago, A.R. Modeling Human Glaucoma: Lessons from the in vitro Models. Ophthalmic R es. 2017, 57, 77–86.
17. Evangelho, K.; Mastronardi, C.A.; de-la-Torre, A. Experimental Models of Glaucoma: A Powerful Translational Tool for t he Future Development of New Therapies for Glaucoma in Humans-A Review of the Literature. Medicina 2019, 55, 28 0.
18. Oh, C.K.; Ariue, B.; Alban, R.F.; Shaw, B.; Cho, S.H. PAI-1 promotes extracellular matrix deposition in the airways of a murine asthma model. Biochem. Biophys. Res. Commun. 2002, 294, 1155–1160.
19. Fink, A.L. Protein aggregation: Folding aggregates, inclusion bodies and amyloid. Fold. Des. 1998, 3, R9–R23.
20. Fernando, R.I.; Castillo, M.D.; Litzinger, M.; Hamilton, D.H.; Palena, C. IL-8 signaling plays a critical role in the epithelia l-mesenchymal transition of human carcinoma cells. Cancer Res. 2011, 71, 5296–5306.
21. Palena, C.; Hamilton, D.H.; Fernando, R.I. Influence of IL-8 on the epithelial-mesenchymal transition and the tumor mic roenvironment. Future Oncol. 2012, 8, 713–722.
22. Hao, Y.; Baker, D.; Ten Dijke, P. TGF-β-Mediated Epithelial-Mesenchymal Transition and Cancer Metastasis. Int. J. Mol. Sci. 2019, 20, 2767.
23. Shu, D.Y.; Lovicu, F.J. Myofibroblast transdifferentiation: The dark force in ocular wound healing and fibrosis. Prog. Reti n. Eye Res. 2017, 60, 44–65.
24. Shu, D.Y.; Butcher, E.; Saint-Geniez, M. EMT and EndMT: Emerging Roles in Age-Related Macular Degeneration. Int. J. Mol. Sci. 2020, 21, 4271.
25. Takahashi, E.; Inoue, T.; Fujimoto, T.; Kojima, S.; Tanihara, H. Epithelial mesenchymal transition-like phenomenon in tra becular meshwork cells. Exp. Eye Res. 2014, 118, 72–79.
26. Stone, R.C.; Pastar, I.; Ojeh, N.; Chen, V.; Liu, S.; Garzon, K.I.; Tomic-Canic, M. Epithelial-mesenchymal transition in tis sue repair and fibrosis. Cell Tissue Res. 2016, 365, 495–506.
27. Thiery, J.P.; Acloque, H.; Huang, R.Y.; Nieto, M.A. Epithelial-mesenchymal transitions in development and disease. Cell 2009, 139, 871–890.
28. Georgakopoulos-Soares, I.; Chartoumpekis, D.V.; Kyriazopoulou, V.; Zaravinos, A. EMT Factors and Metabolic Pathwa ys in Cancer. Front. Oncol. 2020, 10, 499.
29. Lenna, S.; Trojanowska, M. The role of endoplasmic reticulum stress and the unfolded protein response in fibrosis. Cur r. Opin. Rheumatol. 2012, 24, 663–668.

30. Zhou, S.; Yang, J.; Wang, M.; Zheng, D.; Liu, Y. Endoplasmic reticulum stress regulates epithelial-mesenchymal transiti on in human lens epithelial cells. Mol. Med. Rep. 2020, 21, 173–180.