IgG-induced signaling pathways in human retinal pigment epithelial cell fibrosis

A novel mechanism linking immune molecules to fibrosis in AMD and DR

Yen-Pu Huang[1], Gang-Hui Lee [1,2*] , Yu-Shan Lin [1,2*], Ming-Jer Tang [1,2*]

[1] Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan[2] Institute of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

*Corresponding author: mjtang1@mail.ncku.edu.tw

Abstract:

Retinal pigment epithelial (RPE) cell fibrosis drives vision loss in diabetic retinopathy (DR) and advanced age-related macular degeneration (AMD). While anti-VEGF therapy suppresses angiogenesis, it fails to prevent fibrotic progression. Here, we identify IgG as a novel pro-fibrotic factor in RPE cells. Using ARPE-19 models, IgG induced collagen remodeling, ECM stiffening, and activation of both Smad-dependent and independent pathways, closely resembling TGF-β signaling. These findings reveal unexpected immune–fibrosis crosstalk in retinal disease and highlight IgG as a potential target for adjunct anti-fibrotic therapy.

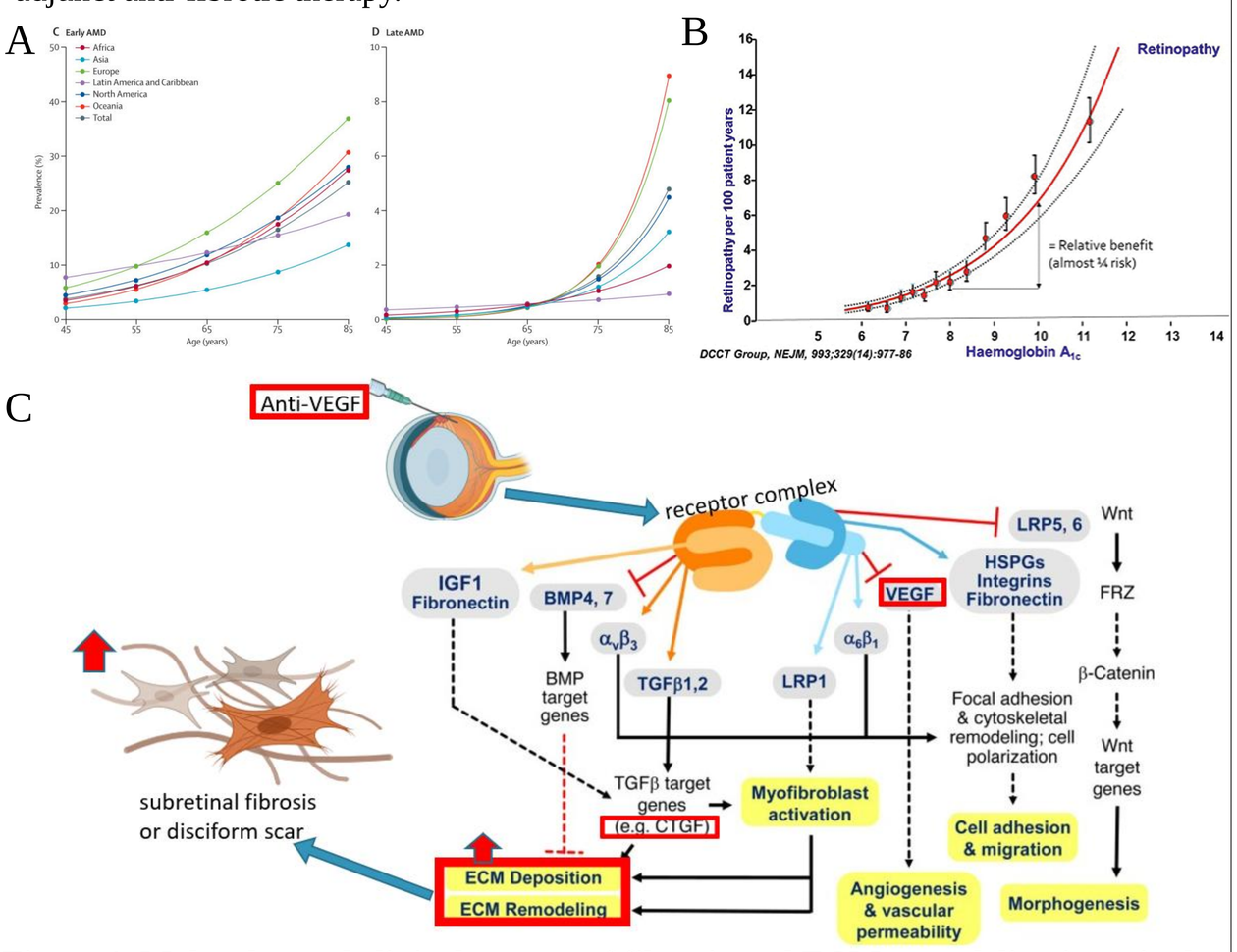
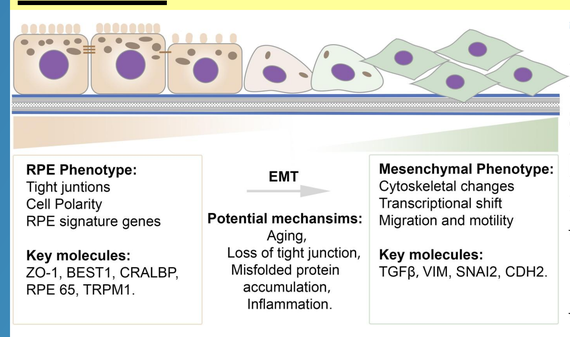


Figure 1. Molecular and clinical context of fibrosis and ECM remodeling in retinal disease.

(A) Global prevalence of early (left) and late (right) AMD by age and region, showing a sharp rise after 70 years [1]. (B) HbA1c–diabetic retinopathy (DR) relationship, with higher HbA1c linked to greater DR risk [2].(C) Anti-VEGF therapy in neovascular AMD limits angiogenesis but cannot fully prevent fibrosis and ECM remodeling [3].

Results:



Time-lapse imaging (48h) shows RPE cells undergoing EMT. IgG- and TGF-β–treated cells progressively adopt a spindle-like, fibroblast morphology, while control cells remain more rounded [4].

(Full time-lapse imaging (48h), scan QR code to watch video ▶)

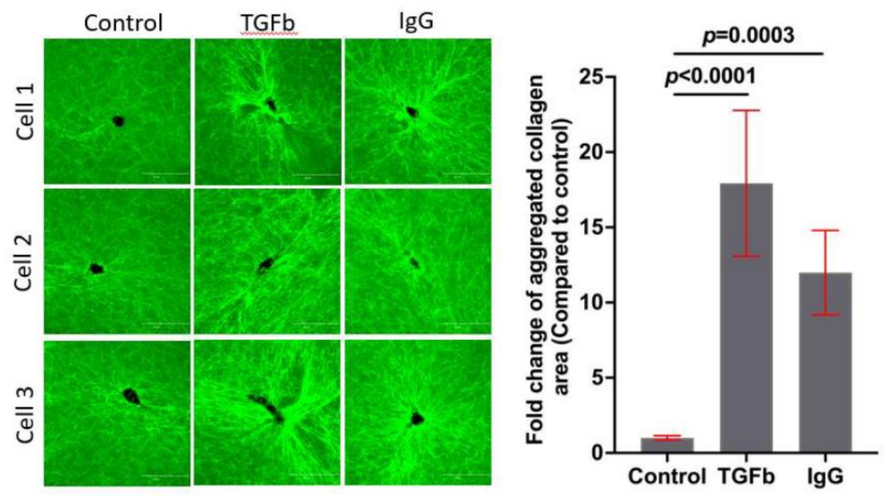


Figure 2. IgG and TGF-β enhance collagen remodeling in live ARPE-19 cells.

ARPE-19 cells on FITC-collagen gels were treated with IgG or TGF-β for 3 days. Confocal imaging and quantification showed significantly increased collagen compaction compared to control.

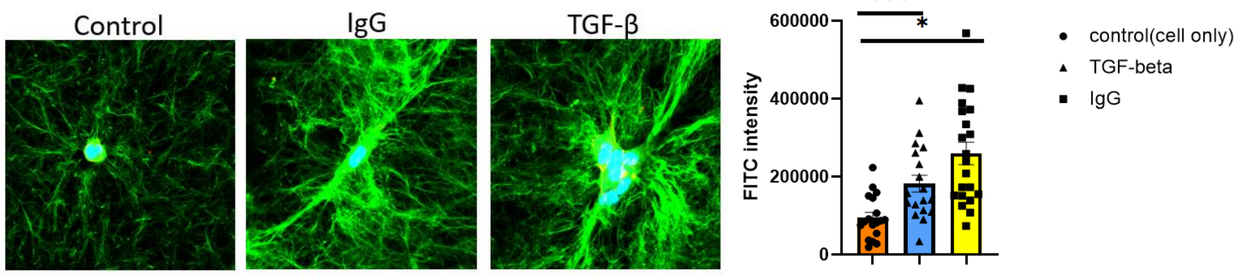


Figure 3. Immunofluorescence analysis of collagen fiber remodeling and quantification of affected area.

Representative immunofluorescence images of ARPE-19 cells cultured on FITC-labeled collagen gels under different treatments: control (cell only), IgG (36.8 μg/ml), and TGF-β (10 μg/ml) for 3 days. Green: FITC-labeled collagen fibers; Blue: cell nuclei (DAPI). IgG and TGF-β treatments resulted in visibly increased collagen compaction compared with control. Quantification of affected area by ImageJ analysis shows significantly higher collagen remodeling in IgG- and TGF-β-treated groups compared to control (**p<0.05**). Data are expressed as mean ± SEM.

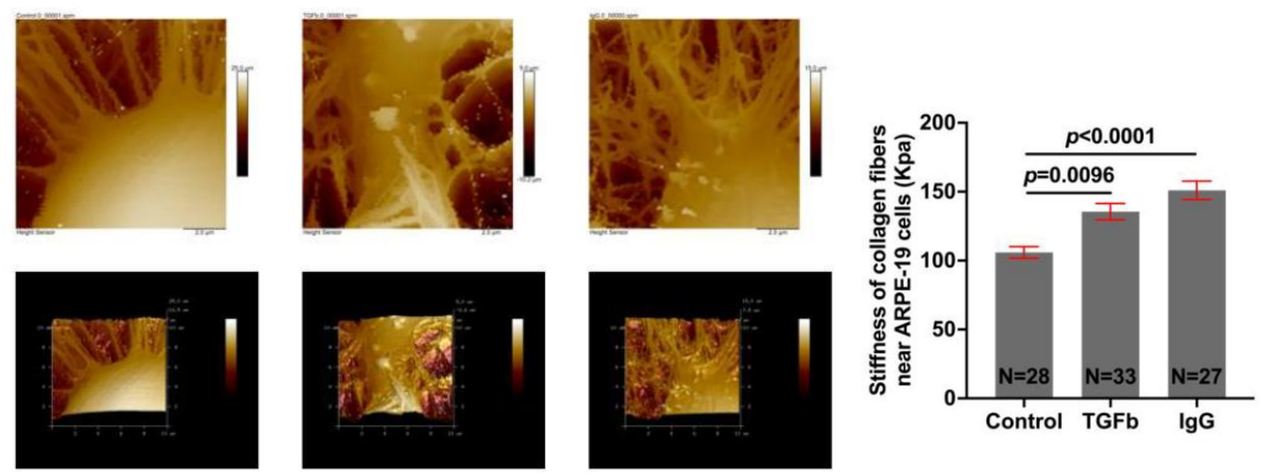
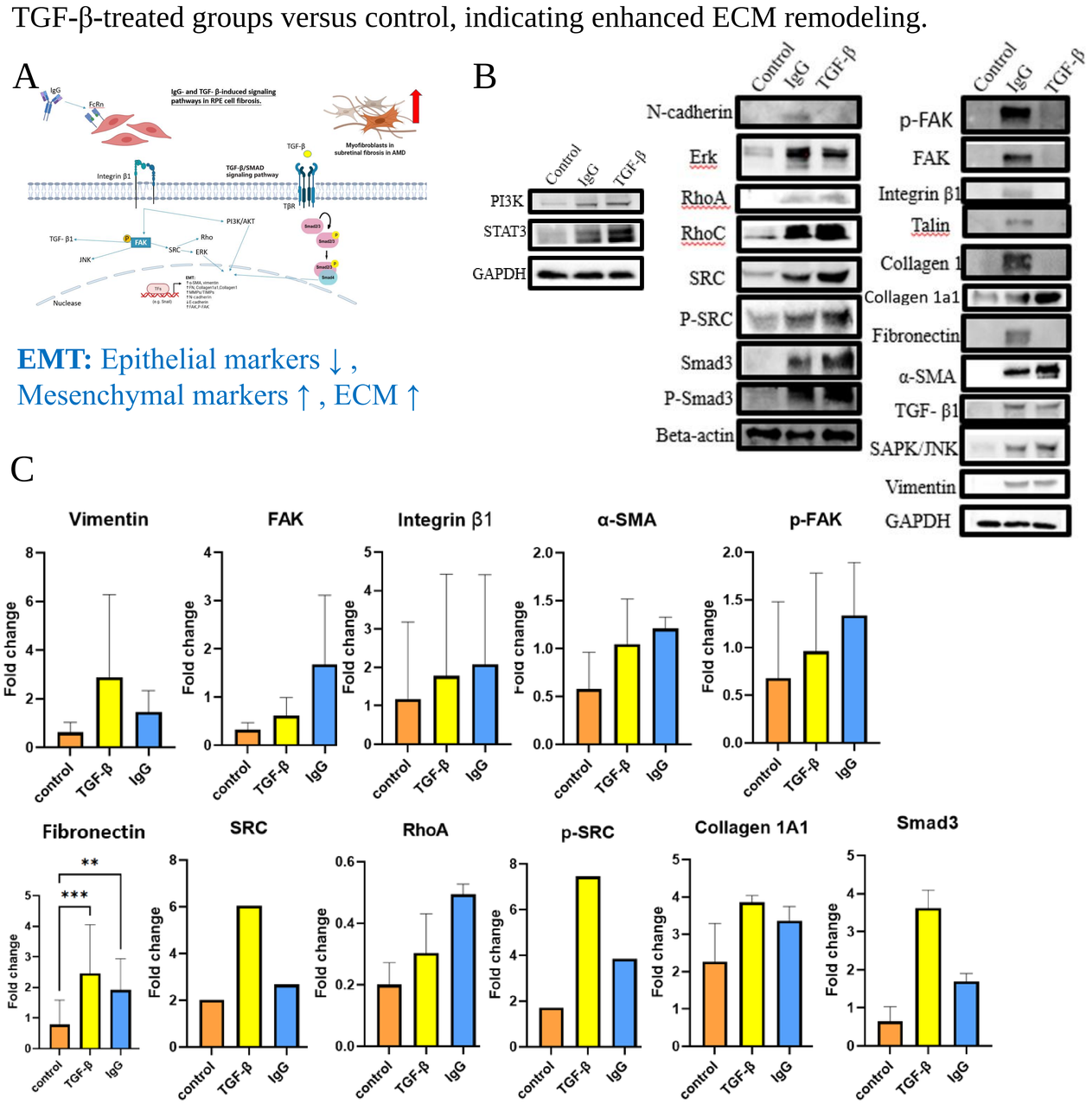


Figure 4. Increased rigidity of collagen fibers after IgG treatment.

Atomic force microscopy (AFM) showed elevated collagen fiber stiffness in IgG- and TGF-β-treated groups versus control, indicating enhanced ECM remodeling.



EMT: Epithelial markers ↓ , Mesenchymal markers ↑ , ECM ↑

Figure 5. Western blot and quantitative analysis of fibrosis-related signaling pathways in ARPE-19 cells

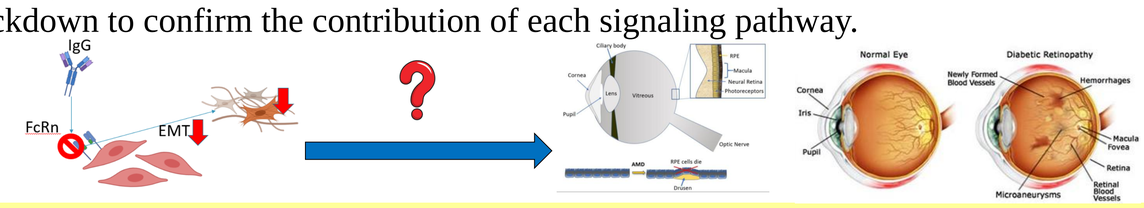
(A) Schematic of IgG- and TGF-β–induced EMT with loss of epithelial markers, gain of mesenchymal markers, and increased ECM deposition.(B) Western blots showing elevated ECM proteins, adhesion molecules, mesenchymal marker, and activation of key pro-fibrotic signaling pathways.(C) Densitometry confirms that IgG (36.8 μg/ml) mimics TGF-β (10 μg/ml) in upregulating ECM/mesenchymal markers and activating fibrosis-related pathways. β-actin and GAPDH served as loading controls.

Conclusion:

IgG, similar to TGF-β, drives collagen fiber remodeling, ECM stiffening, and activation of both Smad-dependent and Smad-independent pro-fibrotic pathways in ARPE-19 cells. This study identifies a previously unrecognized IgG-mediated mechanism in RPE cell fibrosis, uncovering an immune-fibrosis crosstalk in retinal diseases. These findings not only provide new insights into the limitations of anti-VEGF therapy but also highlight **potential targets** for the development of adjunct **anti-fibrotic treatments** for AMD and DR.

Future Work:

1. **Quantitative analysis**: Use image-based fiber analysis (e.g., CurveAlign) and collagen crosslinking assays to further characterize ECM structural changes.
2. **Confirm IgG-specific pathway**: Perform **FcRn knockdown** in ARPE-19 cells to verify whether IgG-induced fibrosis is mediated through the neonatal Fc receptor (FcRn).
3. **Pathway dissection**: Apply specific inhibitors (FAK, RhoA, Smad3) and siRNA knockdown to confirm the contribution of each signaling pathway.



Reference:

[1] Wong, W. L., Su, X., Li, X., Cheung, C. M. G., Klein, R., Cheng, C.-Y., & Wong, T. Y. (2014). Global Prevalence of Age-related Macular Degeneration and Disease Burden Projection for 2020 and 2040: A Systematic Review and Meta-Analysis. The Lancet Global Health, 2(2), e106-e116. https://doi.org/10.1016/S2214-109X(13)70145-1

[2] Rein, D. B., Wittenborn, J. S., Burke-Conte, Z., Gulia, R., Robalik, T., Ehrlich, J. R., Lundeen, E. A., & Flaxman, A. D. (2022). Prevalence of Age-Related Macular Degeneration in the US in 2019. JAMA Ophthalmol, 140(12), 1202-1208. https://doi.org/10.1001/jamaophthalmol.2022.4401

[3] Lipson, K. E., Wong, C., Teng, Y., & Spong, S. (2012). CTGF is a Central Mediator of Tissue Remodeling and Fibrosis and its Inhibition can Reverse the Process of Fibrosis. Fibrogenesis Tissue Repair, 5(Suppl 1), S24. https://doi.org/10.1186/1755-1536-5-s1-s24

[4] Zhou, M., Geathers, J. S., Grillo, S. L., Weber, S. R., Wang, W., Zhao, Y., & Sundstrom, J. M. (2020). Role of Epithelial-Mesenchymal Transition in Retinal Pigment Epithelium Dysfunction. Front Cell Dev Biol, 8, 501. https://doi.org/10.3389/fcell.2020.00501

Acknowledgement

Thanks to Ming-Jer Tang and the senior members of our laboratory for their valuable assistance.Thanks to the College of Medicine, National Cheng Kung University, and the National Science and Technology Council (NSTC) for supporting this retinal fibrosis study.