RESPONSE TO COMMENT ON HU ET AL.

Single-Cell Transcriptomics Reveals Novel Role of Microglia in Fibrovascular Membrane of Proliferative Diabetic Retinopathy. Diabetes 2022;71:762–773

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We are thankful for the opportunity to respond to the comments of Corano Scheri and Fawzi (1). We appreciate their attention and valuable feedback on our article. Here is a point-by-point response to their comments and concerns.

Myeloid cells on the fibrovascular membrane (FVM) express low levels of the canonical microglial markers but do express predominantly macrophage-related genes. Single-cell pseudotime analysis revealed two differentiation pathways of subpopulations of microglia (2), which stemmed from the infiltrating *FCN1*+ monocyte and resident *P2RY12*+ microglia (2). As a dynamically changing cell type, microglia are capable of rapidly responding to local insults by downregulating homeostatic microglial markers and exhibiting heterogeneous disease-associated profiles, for instance, the expression of macrophage markers *TREM2*, *APOE*, and *CTSD* (3). Therefore, the two possible sources of FVM-resident myeloid cells are indistinguishable by transcriptomic profiling.

We apologize that the labeling is not stringent enough to exclude the involvement of monocyte-derived macrophages. In the future, lineage tracing experiment should be implemented to determine the ontogeny of profibrotic retinal myeloid cells in the pathogenesis of FVM.

In our article, we identified the profibrogenic FVM-resident microglia (i.e., myeloid cells) on the basis of the expression of *GPNMB*, *SPP1*, *LIPA*, and *FABP5*. These markers were also expressed in the scar-associated macrophage (SAM) identified in the recent single-cell transcriptomic studies of other fibrotic diseases (lung and liver) (4). The SAM profile has been experimentally proved to promote fibrosis. In our integrative analysis, the transcriptional profile of FVM-resident myeloid cells could be projected to SAMs from liver fibrosis, further indicating similar biological properties of FVM-resident myeloid cells with the profibrotic SAMs (2).

We appreciate their elegant work on the contribution of mesenchymal cells (fibroblasts and pericytes) to the formation of FVM. However, as shown in our data, the proportion of mesenchymal cells was only 8.2%, compared with 73.4% for myeloid cells in FVM samples (2). Unlike SAMs, which were located at the outer edges of fibrotic tissue and functioned in modulating mesenchymal cells (4), FVM-resident myeloid cells directly comprised FVM. In addition, we performed gene signature scoring considering both gene expression and cell proportions, and we found that macrophages had the highest fibrotic scores of all cell types (unpublished data).

Regarding the fibrogenic genes used for scoring, we extracted genes from the extracellular matrix–associated gene set detected in the bulk analysis of FVM (5). It may be more appropriate to evaluate the degree of epiretinal fibrosis than the usage of fibroblast-specific genes (COL1A1, COL1A2, COL5A1, LUM, THBS2, FBLN2, CTGF, etc.). In addition, the presence of fibrogenic myeloid cell was extensively found in human subretinal membrane (6).

We believe that the discovery of the immunopathogenic nature of FVM may highlight the targeted design of therapeutic strategies for FVM in the future.

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References

1. Corano Scheri K, Fawzi AA. Comment on Hu et al. Single-cell transcriptomics reveals novel role of microglia in fibrovascular membrane of proliferative diabetic retinopathy. Diabetes 2022;71:762–773 (Letter). Diabetes 2024;73:e6
2. Hu Z, Mao X, Chen M, et al. Single-cell transcriptomics reveals novel role of microglia in fibrovascular membrane of proliferative diabetic retinopathy. Diabetes 2022;71:762–773

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3. Masuda T, Sankowski R, Staszewski O, et al. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 2019;566:388–392
4. Fabre T, Barron AMS, Christensen SM, et al. Identification of a broadly fibrogenic macrophage subset induced by type 3 inflammation. Sci Immunol 2023;8:eadd8945
5. Yoshida S, Ogura A, Ishikawa K, et al. Gene expression profile of fibrovascular membranes from patients with proliferative diabetic retinopathy. Br J Ophthalmol 2010;94:795–801
6. Little K, Llorián-Salvador M, Tang M, et al. Macrophage to myofibroblast transition contributes to subretinal fibrosis secondary to neovascular age-related macular degeneration. J Neuroinflamm 2020;17:355