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RESEARCH LETTER

Heart and Brain Pericytes Exhibit a Pro-Fibrotic Response After Vascular Injury

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Pericytes are a heterogeneous population of mural cells that surround microvessels in various organs including the heart and brain. Their function, beyond maintaining vascular integrity and contractility, is poorly understood. Recent studies have suggested they contribute to the development of tissue fibrosis.[1] For instance, after spinal cord injury, a subpopulation of pericytes divide and migrate away from blood vessels, where they form the fibrotic scar that constitutes the lesion cavity.[2] Although recent studies suggest pericyte function in homeostasis and after organ injury, their role in mediating fibrosis after vascular ischemia in the heart and brain is not firmly established, owing to differences in injury models, labeling techniques, and transgenic models.[3–5]

We explored the role of pericytes after myocardial infarction (MI) and ischemic stroke by interrogating their gene expression at a single-cell level and their contribution to tissue fibrosis in parallel studies. We used a double transgenic mouse model, *Tbx18*$^{CreER/+}$;*Rosa26*$^{tdT/+}$,[4] to lineage-trace TBX18 (T-box transcription factor 18)-expressing pericytes and define their location, fate, and gene expression profiles at a single-cell resolution during homeostasis and after MI and stroke. Male 2- to 3-month-old mice received 1 mg of tamoxifen intraperitoneally for 4 consecutive days, followed by a 1-week washout period. They then underwent MI (by permanent ligation of the left anterior descending artery), stroke (by photothrombosis), or sham surgery. Seven days later, when initial fibrosis appears in either model, tdTomato-labeled cells were isolated from uninjured and ischemic hearts and brains by fluorescence-activated cell sorting. Before sorting pericytes from the injured organs, the core infarct and peri-infarct regions were prepared separately to distinguish pericytes that may be undergoing a transition into a fibrotic state. The isolated cells were then processed for single-cell RNA sequencing, resulting in a transcriptomic dataset consisting of 37 001 cells from the heart and 15 353 cells from the brain (Figure [A]). Other cell types (ie, endothelial cells, fibroblasts, smooth muscle cells, leukocytes) were excluded from our analysis. Pericytes were identified based on expression of known markers, such as *Rgs5, Mcam, Pdgfrb,* and *Cspg4* for the heart and *Abcc9*, *Pdgfrb*, *Vtn*, *Cspg4*, and *Anpep* in the brain (Figure [B]). The pericyte clusters for both systems were subjected to further downstream analysis (Figure [B]). Our single-cell RNA sequencing analysis revealed a considerable number of biological pathways and upregulated genes related to fibrosis that were enriched in pericytes from both the injured tissues (compared to pericytes from uninjured organs), suggesting similar profibrotic activity of pericytes in a common ischemic injury response. Gene Sets Enrichment Analysis identified a significant (false discovery rate [FDR]<0.05) enrichment of key fibrosis pathways, including inflammation, immune response, and ECM (extracellular matrix) components (Figure [C]).

We next analyzed the gene expression profile of cardiac and brain pericytes separately at a single-cell resolution. Pericytes isolated from sham tissues of the heart and brain formed a distinct cluster from injured pericytes

Key Words: brain ■ fibrosis ■ heart ■ ischemic stroke ■ myocardial infarction ■ pericytes

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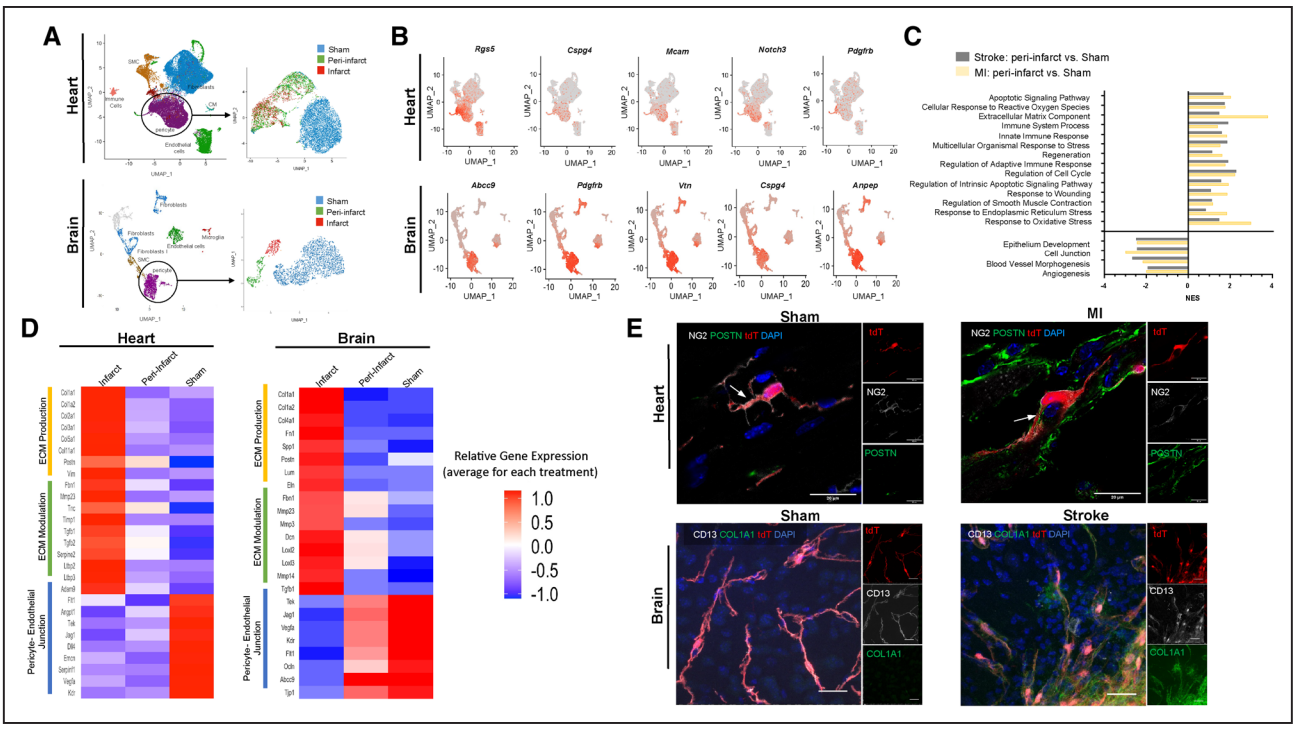

Figure. Characterization of pericytes from brain and heart after ischemic injury.

A, Single-cell RNA sequencing analysis of TBX18 (T-box transcription factor 18)-expressing cells from injured and uninjured hearts and brains (n=3 biologically independent samples). Uniform Manifold Approximation and Projection (UMAP) analysis revealed the presence of other cardiac and brain cell types. Infarct and peri-infarct pericytes cluster distinctly separated from sham pericytes. **B,** Feature plots of known cardiac and brain pericyte markers confirm our identification of pericytes. **C**, Gene Ontology (GO) analysis reveal pathways that are similarly enriched in infarcted heart and brain pericytes when compared to uninjured pericytes. We observed parallel upregulation of pathways associated with immune response, and fibrosis; pathways associated with blood vessel formation were downregulated. **D,** Heatmap of differential genes expression. While profibrotic genes that regulate ECM (extracellular matrix) remodeling were upregulated in cardiac pericytes isolated from the infarct core and to a lesser extent in the peri-infarct region, we observed a reduction in the expression of genes that regulate endothelial cell-pericyte interaction. Expression value on the color scale equates to log-2 fold change of gene expression. **E**, Confocal images show expression of POSTN (periostin; heart) and COL1A1 (collagen 1A1; brain), markers associated with activated fibroblasts, in TBX18-expressing pericytes in the hearts and brains of sham, myocardial infarction (MI), or stroke animals. Images were routinely saved from different animals in each cohort and representative images from each organ system's infarct core were chosen. Scale bar=20 µm. CM indicates cardiomyocytes; DAPI, 4',6'-diamidino-2-phenylindole; NES, Normalized Enrichment Score; NG2, neuron-glial antigen 2; SMC, smooth muscle cells; and tdT, tdTomato.

(Figure [A]). We observed high expression of fibrosis-related genes in the pericytes located within the core infarct regions and, to a lesser extent, in pericytes in the peri-infarct areas (Figure [D]). Conversely, genes associated with the pericyte-endothelium junction and vascular integrity were downregulated in infarct and peri-infarct pericytes compared with pericytes isolated from sham organs (Figure [D]). These changes in gene expression suggest that in response to ischemia, pericytes may directly contribute to ECM remodeling in a fibrotic state.

We next performed immunohistochemistry to confirm the progression of pericytes to a fibrotic phenotype in MI and stroke and define their localization with respect to the ischemic regions. Immunohistochemistry for pericyte and fibrosis markers was performed on frozen sections (Figure [E]). We observed that in the absence of injury, pericytes maintain an intimal connection with the vascular bed and do not express detectable levels of fibrosis markers, such as POSTN (periostin) for the heart and COL1A1 (Collagen 1A1) for the brain (Figure [E]). However, in response to ischemia, we observed accumulation of pericytes expressing POSTN and COL1A1 in the fibrotic regions which was not seen in sham organs (Figure [E]). Our results differ from recent work which investigated the role of TBX18 pericytes in transaortic constriction (heart) and cortical stab wound(brain) models.[4] These have different pathological phenotypes from the models that we used. Transaortic constriction is a chronic form of cardiac injury that yields diffuse interstitial fibrosis across the heart. The cortical stab wound injury does not create an ischemic penumbra, therefore lacking significant reorganization and regeneration upon injury. Here, we performed two clinically relevant pathological models of ischemic injury: MI, which leads to replacement fibrosis and ischemic stroke, which results in a discrete fibrotic scar enveloped by astrocytic scar. Although different injury models could contribute to the observed differences, the clinically relevant models used in the

current study highlight the important role of pericytes in cardiac and brain fibrosis. Our data suggest the fibrotic response of pericytes is highly conserved between the heart and brain.

ARTICLE INFORMATION

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Disclosures

All animal studies were performed according to the guidelines of University of California, Los Angeles (UCLA) Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Studies performed are in accordance with humane treatment of the animals. All sequencing data can be found in the Gene Expression Omnibus (GEO) repository with Gene Series Entry (GSE) accession number GSE178469.

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