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Single-cell Transcriptomics Uncover a Novel Role of Myeloid Cells and T-lymphocytes in the Fibrotic Microenvironment in Peyronie's Disease

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Abstract

Background

Peyronie's disease (PD) is an acquired fibrotic disease affecting the penile tunica albuginea that can lead to curvature and deformities, shortening, and erectile dysfunction. Although immunological mechanisms have been suggested for the pathophysiology of PD, these have not been investigated using single-cell transcriptomics.

Objective

To investigate the immunological signature of plaques from PD patients using immunohistochemistry (IHC) and single-cell RNA sequencing (scRNA-Seq).

Design, setting, and participants

Tunica albuginea biopsy was performed in patients undergoing penile surgery for either PD ($n = 12$) or plication or penile cancer (control, $n = 6$). The inclusion criteria for PD patients were stable chronic disease (≥ 12 mo in duration) and no previous penile surgery or intralesional injection therapy.

Some measurements and statistical analysis

IHC was performed on surgical samples from ten patients with PD and five control subjects. An additional two PD and one control sample were used for scRNA-Seq (droplet-based; 10X Genomics). Cell clusters were visualised using heatmaps and *t*-distributed stochastic neighbour embedding plots (BioTuring v2.7.5).

Results and limitations

IHC revealed the presence of myeloid dendritic cells (DCs; CD68⁺, TLR4⁺, CD206⁺), cytotoxic T lymphocytes (CTLs; CD3⁺, CD8⁺), and B lymphocytes (CD20⁺) in PD plaques, which were absent in controls. scRNA-Seq yielded results for 3312 PD and 5658 control cells. Cell clusters contained fibroblasts (COL1A2⁺), myofibroblasts (COL1A2⁺, ACTA2⁺), smooth muscle cells (ACTA2⁺, DES⁺), endothelial cells (VWF⁺), myeloid cells (CD14⁺), T lymphocytes (CD3D⁺), and neutrophils (ALPL⁺). Myeloid cell subclustering showed infiltration of monocyte-derived cells; control tissue contained classical DCs and resident macrophages. Lymphocyte subclustering revealed mucosal-associated invariant T (MAIT) cells and CTLs in PD. Differential gene expression suggests an increase in inflammatory and immune responses in chronic PD. The study is limited by the small scRNA-seq sample size ($n = 3$) for IHC, mitigated by a larger cohort of historic paraffin-embedded samples ($n = 15$), which showed largely parallel findings. Owing to tissue stiffness and extracellular matrix adhesion, our single-cell yield was lower for PD than for the control sample.

Conclusions

Our data suggest that even in the chronic PD stage (painless and stable curvature) there is a sustained inflammatory reaction. While vascularisation and collagen production are elevated, the inflammation is driven by specialised monocyte-derived CTL and MAIT cells. These findings could uncover new avenues for medical treatment of PD.

Patient summary

We looked at the role of the immune system in patients suffering from Peyronie's disease, a condition causing shortening and curvature of the penis. We found that even in a stable, chronic stage of the disease, there is activation of the immune system. Our results suggest that there is potential for novel treatments for this condition.

Keywords

Peyronie's disease • fibrosis • single-cell RNA sequencing • immune system • dendritic cells •
macrophages • T-lymphocytes

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