**Spatial transcriptomic analysis links immune-rich regions in endometriomas with pain generation and fibrosis**

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**Introduction/Background**

Immune cells, particularly T-cells and macrophages, are prevalent in ectopic lesions and play key roles in driving pain and fibrosis, contributing to the chronic inflammation and tissue remodeling characteristic of endometriosis. Understanding the spatial organization of these immune-rich niches is crucial for elucidating disease pathogenesis and identifying potential therapeutic targets.

**Materials and Methods**

A total of 12 slides from 9 patients (3 with bilateral cysts), containing matched eutopic and ectopic endometrioma sections, were stained for CD45, CD3, pan-cytokeratin, and DAPI. Majority of patients had Stage III endometriosis and were in the follicular phase of their menstrual cycle. Immune-rich regions of interest (ROI) were identified using the Nanostring GeoMx Digital Spatial Profiler, yielding 144 total ROIs. Analysis of these ROIs was performed using the StandR package in R.

**Results**

UMAP projection of ROIs revealed clear separation between ectopic and eutopic samples, with some evidence of patient-specific clustering. Differential expression analysis of the top 50 DEGs showed elevated immune-related genes (e.g., IGHG2, HLA genes, CXCL1, CXCL8) and macrophage markers (e.g., CD68, CD163) in ectopic tissues. Genes involved in lipid and cholesterol metabolism (e.g., APOE, APOC1) were upregulated, with GPNMB elevated in ROIs associated with severe dysmenorrhea, correlating with pain severity and disease stage. Additionally, NGF expression correlated with reported dysmenorrhea. Spatial deconvolution using CIBERSORTx revealed higher macrophage activity in ectopic samples, particularly in those with recurrent cysts and severe dysmenorrhea.

**Conclusion**

Our spatial transcriptomic analysis reveals immune activation and lipid metabolism in ectopic lesions, with GPNMB and NGF correlating with pain severity and disease stage. These findings suggest macrophages and specific biomarkers like GPNMB may be key therapeutic targets for pain and fibrosis management in endometriosis.

**Key words**

Immune, pain, GPNMB