Single-Nuclei RNA-seq Uncovers Distinct Cellular and Molecular Features between Uterine Leiomyoma and Leiomyosarcoma

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INTRODUCTION

Uterine leiomyosarcomas (LMS) are highly aggressive tumors that may arise from preexisting leiomyomas (LM) with distinct genetic and/or cellular traits. The exact identity of the cells that give rise to LMS within LM remains unclear. In this work, we aimed to identify differential molecular and cellular features of LMS and LM, at single-nuclei resolution, that may drive their transformation into a malignant state.

MATERIALS AND METHODS

We profiled ~43,300 nuclei from 4 LM and 7 LMS using the 10X Genomics single-nuclei RNA-seq workflow, with nuclei isolated by droplet microfluidics and sequenced on Illumina platforms. Data were processed with CellRanger v8 and Seurat v5, with clusters annotated using public databases. R-based pipelines enabled analyses of differential cell abundance (DA), gene expression (DGE), and pathway enrichment analyses between LM and LMS.

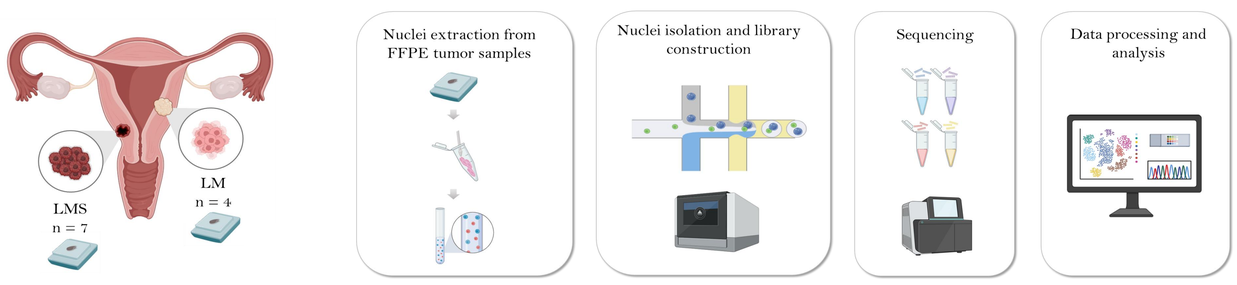

Figure 1. *Schematic representation of the study's workflow.*

RESULTS

We characterized the cellular and molecular landscapes of LM and LMS, covering endothelial, lymphatic endothelial, immune, perivascular, fibroblast, and smooth muscle cell (SMC) populations. A distinct cluster of mitotically active (MA) cells, primarily SMCs and fibroblasts expressing G2S/M phase genes, was enriched in LMS. Given the association of mitotic activity with tumor aggressiveness, this population emerged as a key feature of malignant progression.

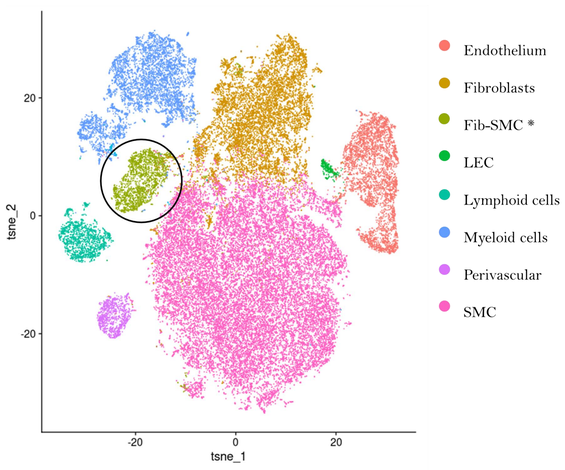

Figure 2. *t-SNE visualization of the main cell types present in LM and LMS tumors. Fib-SMC(circled) represents the intermediate cluster mainly composed of SMC and fibroblasts.*

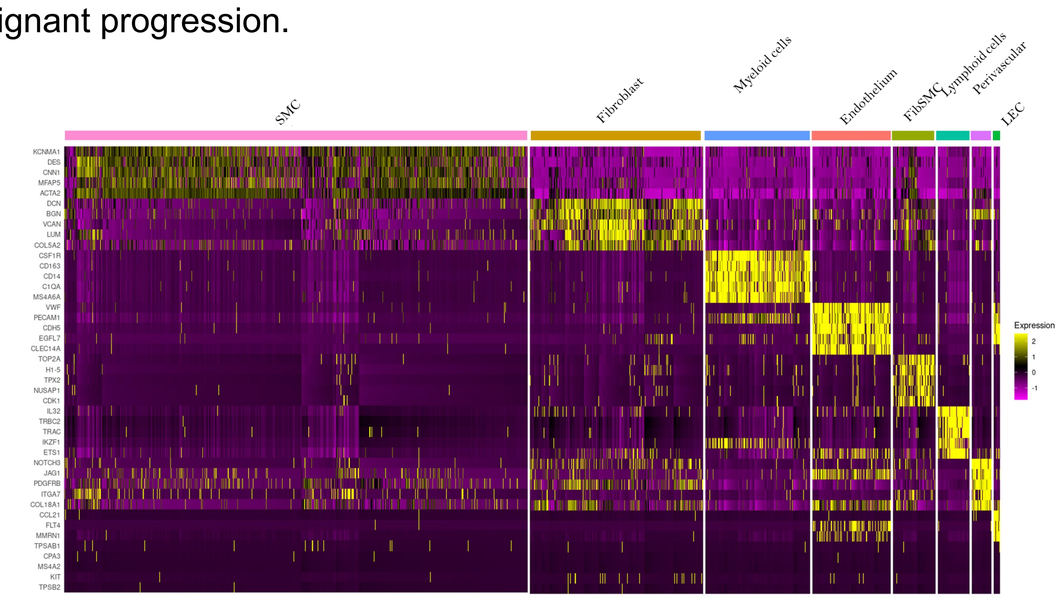

Figure 3. *Heatmap illustrating the expression of marker genes across the main cell types found in these myometrial tumors.*

DA analysis confirmed increased MA cells in LMS, while DGE profiling revealed an overexpression of extracellular matrix (ECM)-associated genes (*NID*, *RCN3*, *COL16A1*) in LMS and cell- cycle (*HMGA2, KLHL13, ZBTB)* as well as proliferation-associated genes (*RERG, SFRP4*) in LM.

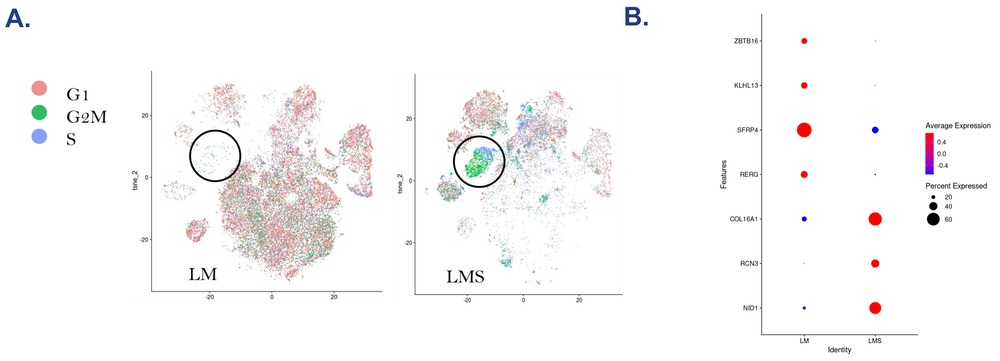

Figure 4. A) *t-SNE distribution of cell cycle activity in LM (up) and LMS (down) cells.* ***B)*** *Dotplot illustrating the DGE of MA cells in LM versus LMS. Color indicates average expression, while dot size represents the percentage of cells expressing specific genes.*

Enrichment analysis based on hallmarks and gene ontology highlighted significant differences in the epithelial-mesenchymal (EMT) transition, along with a disruption in angiogenesis and ECM remodelling, underscoring distinct pathogenic features between LM and LMS.

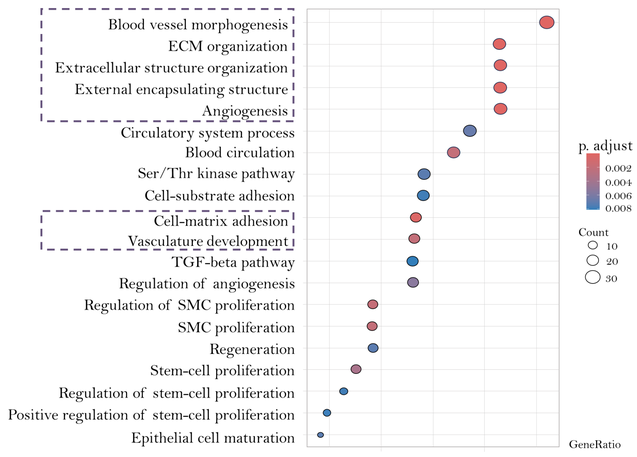

Figure 5. *Significant over-representation of biological processes and pathways (color intensity) shown by each gene set (dot size) from LM and LMS.*

CONCLUSION

Our results underscore the distinct biological pathways in LM and LMS. The enrichment of mitotically active fibroblasts and smooth muscle cells in LMS, coupled with ECM-associated gene upregulation, underscores a proliferative and remodelling phenotype consistent with malignant progression. These insights emphasize the potential for developing therapeutic approaches and improved strategies for clinical management.

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