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Single-cell transcriptomic analysis reveals a unique cluster and differences in gene expression in somatotroph adenomas

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Motivation and Aim: Pituitary adenomas (PAs) are benign tumors of the pituitary gland, accounting for approximately 15 % of all primary brain tumors [1]. They can be either functioning (secreting) or non-functioning (silent). Functioning somatotroph adenoma (FSA) is a type of PAs that primarily secretes growth hormone (GH). The silent form of somatotroph adenomas (SSAs) does not secrete hormones and is more invasive and frequent than its secreting counterparts [2]. In our study, we aim to describe SSAs using single-cell RNA sequencing (scRNA-seq) for the first time. We will compare them with scRNA-seq data of FSAs, also included in our study, to understand the differences in the molecular mechanisms regulating gene expression in each tumor type.

Methods and Algorithms: We obtained pituitaries from four patients (three are FSAs and one SSA), and subjected these tissues to scRNA-seq with a total of 15176 and 4294 cells respectively. The raw sequenced reads were processed with 10X Cell Ranger (v6.1.1), and Default Cell Ranger quality check measurements were used for further comparison methods through the Wilcoxon test. The expression matrices for the filtered cells were submitted to Seurat (v4.9.9 and v5.0.0) for basic analysis, including scaling and normalization. Cell cycle phase predictions were based on reference gene expression processed with Seurat (v5.0.0). Statistical significance was assessed by a two-tailed t-test and Wilcoxon rank-sum test: * ($0.01 < p < 0.05$), ns – not significant – $p > 0.05$.

Results: Based on known tissue-specific marker genes, we identified six main cell clusters in both FSA and SSA samples (Fig. 1a). These cell clusters include the PIT1 lineage (somatotrophs, thyrotrophs, and lactotrophs), the TPIT lineage (corticotrophs), the SF1 lineage (gonadotrophs), stroma cells (endothelial and perivascular cells), immune cells (macrophages and T-cells), and stem cells. The hormone-secreting cells were identified within each lineage based on the enriched gene expression levels of well-known markers. We compared the gene expression profile between FSAs and SSAs. We found that FSAs differentially expressed *SSTR1*, *SSTR5*, and *NPY* genes that code for hormone receptors and secretion regulatory proteins, which correlates with the secreting properties of these tumors rather than the non-secreting SSAs (Fig. 1d).

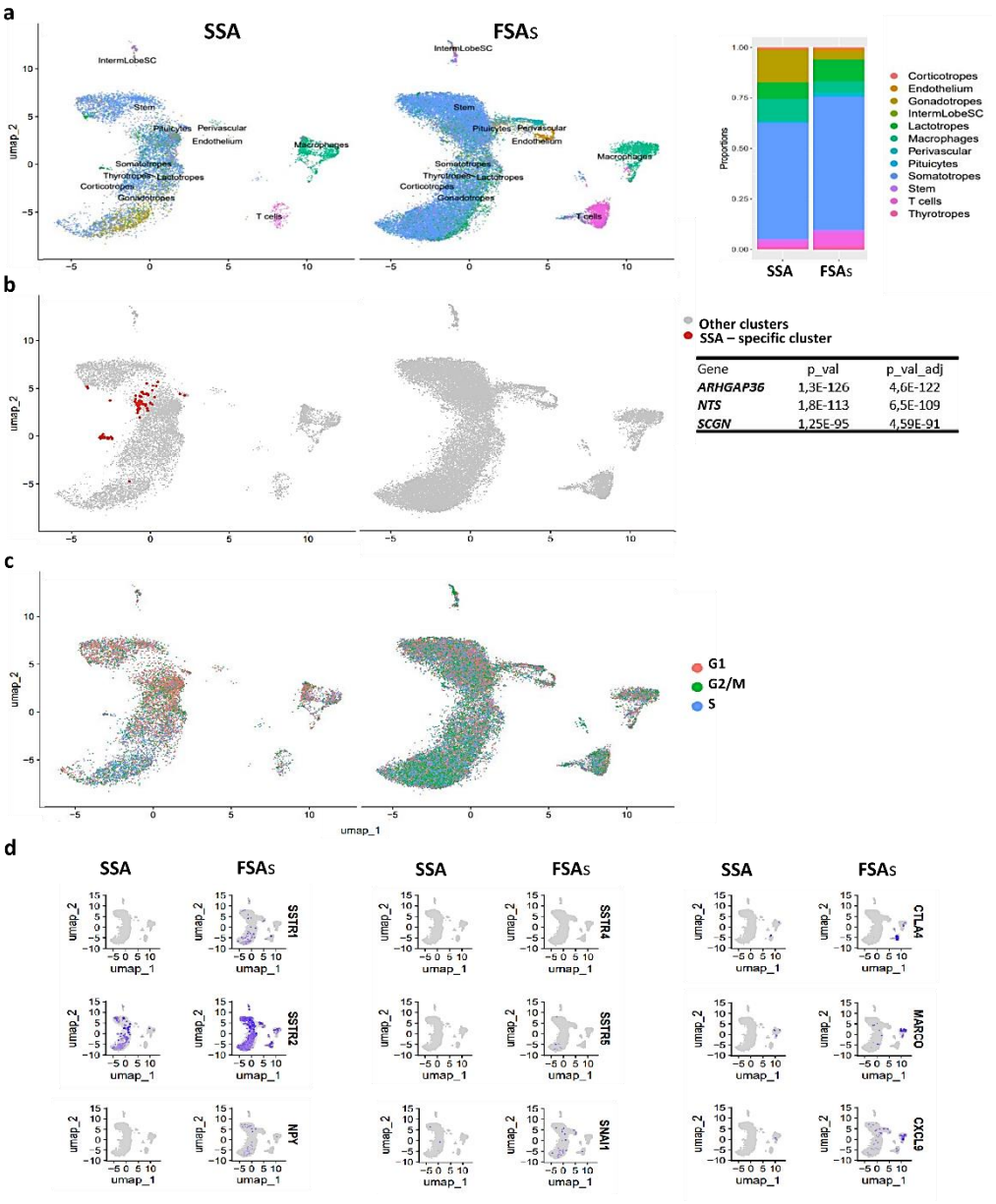

Fig. 1. scRNA-seq data analysis for FSA and SSA samples. *a*: The major cell type compositions found in FSA and SSA samples are represented as UMAP on the left, and a bar plot on the right. *b*: The unique SSA – specific cluster is shown in red, existing only in SSA and not in FSA samples. Examples of differentially expressed (DE) genes associated with the SSA – specific cluster are shown in the table. *c*: Cell cycle estimation for FSA and SSA datasets. *d*: Key specific gene expression according to UMAP for SSA and FSAs cells

FSAs also differentially expressed *SNAI1* – an epithelial to mesenchymal transition (EMT) inducer gene, and immune cell-specific genes (*CTLA4*, *CXCL9*, and *MARCO*) which indicate a difference in the tumor microenvironment and immune profile between FSAs and SSAs (Fig. 1d). Additionally, we detected a novel cluster (SSA-specific cluster) (Fig. 1b), which is found in SSAs but not in FSAs. This cluster shows high activation of cell pathways involved in cell growth, cell differentiation, cell migration, apoptosis, and tumor development like PI3K/AKT/mTOR, JAK/STAT, and TGFB signaling pathways [3–5]. This cluster also highly expresses the *NTS* gene, which is correlated with poor tumor prognosis [6], and genes of ribosomal proteins which might to be involved in cancer initiation [7]. These findings suggest that this cluster plays a significant role in the pathogenesis of SSAs. Furthermore, our scRNA defined a difference in the overall cell cycle status of FSAs and SSAs, where FSAs mostly locate in the G2M phase while SSAs are in the G1 and S phases (Fig. 1c). This reflects a difference in the molecular mechanisms regulating tumor cell growth and proliferation between these two tumor types.

Conclusion: In conclusion, our study of secreting and silent somatotroph adenomas revealed distinct cell clusters in both adenoma types. Furthermore, differences in gene expression related to immune response and tumor microenvironment were observed between functioning and silent adenomas. A unique cluster found only in silent adenomas exhibited high activation of pathways associated with cell growth, differentiation, migration, and tumor development, suggesting a pro-tumor phenotype. Additionally, our data indicate variations in molecular mechanisms regulating cell cycle status in SSAs and FSAs. Overall, these findings provide valuable insights into the distinct characteristics and potential prognostic factors of functioning and silent pituitary adenomas.

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