

OP_A006

SINGLE-CELL SPATIAL PROFILING REVEALS IMMUNE-STEROIDOGENIC CROSSTALK IN ADRENALS OF PATIENTS WITH PRIMARY ALDOSTERONISM

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INTRODUCTION

Primary aldosteronism (PA), most commonly caused by aldosterone-producing adenomas (APAs), represents the leading form of surgically curable secondary hypertension. While genomic studies have elucidated the mutational landscape of APAs, the spatial organisation and functional role of immune populations across APAs, aldosterone-producing micronodules (APMs), and adjacent adrenal cortex remain poorly defined at single-cell resolution.

METHODOLOGY

Single-cell RNA sequencing (scRNA-seq) was integrated with spatial transcriptomics in APAs and paired adjacent adrenal cortex, complemented by immunohistochemical (IHC) validation. Immune populations were spatially mapped using canonical markers (CD14, CD68, CD163, HLA-DR, CD8A, and CD4) across defined adrenocortical regions.

RESULTS

The adrenal microenvironment in PA demonstrates structured immune organisation rather than passive infiltration. CD14⁺ monocyte-lineage cells localize intraparenchymally within APAs ($n = 10$), intercalating between CYP11B2⁺ aldosterone-producing cells and forming a pattern distinct from perivascular immune niches. scRNA-seq further identified a transcriptionally distinct CD14⁺ population within the zona reticularis (zR) that co-expresses steroidogenic markers (CYB5A, SULT2A1, TSPAN12) while lacking canonical monocyte transcripts. IHC supported this observation, demonstrating CD14 expression within adrenocortical zR parenchymal cells ($n = 5$). In parallel, CD4 and HLA-DRA exhibited diffuse cytoplasmic staining within zR parenchymal cells in the absence of classical macrophage marker co-expression (CD68), suggesting non-canonical or context-dependent expression within steroidogenic compartments. CD68⁺ and CD163⁺ macrophages were sparsely distributed across APA, APM and adjacent cortex, consistent with low-density tissue-resident populations, while CD8A⁺ cytotoxic lymphocytes were enriched in APAs and APMs with diffuse parenchymal cytoplasmic staining of CD8A additionally observed within the zR.

CONCLUSION

These findings reveal a previously unrecognized spatially organised immune-steroidogenic interface within the adrenal cortex. The presence of immune-associated transcriptional and protein signatures within zR cells suggests potential functional plasticity of steroidogenic cells, possibly extending to antigen presentation-related pathways, warranting further mechanistic investigation.