00461 Identification of Nephron Progenitor Markers Derived From Human Embryonic Stem Cells by Single-cell RNA Sequencing

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Aims: The steady rise of chronic kidney diseases has caused a major burden for the healthcare system. Yet, most of their molecular pathomechanisms are still understudied due to the lack of functional model systems. Models for kidney diseases and for drug testing mainly employ immortalized kidney cell lines that do not represent their in vivo counterparts, or rely on limited sources of mouse and human primary kidney cells that rapidly de-differentiate in culture. hESC-derived nephron progenitor cells (NPCs) may provide an unlimited cell source for generation of functional kidney models. Most protocols differentiate pluripotent cells to kidney cells that aggregate into organoids, utilizing undefined matrices and xenogenic products with high variability. Here we aim to generate a pure population of NPCs from hESCs in defined systems through the identification of NPC markers.

Methodology: hESCs were differentiated on human recombinant laminin toward mesoderm, metanephric mesenchyme and NPC lineage using relevant Wnt signaling molecule.

Result: By day 30, we observed 82% $WT1^{+}$ cell population via FACS analysis. Quantitive RT-PCR showed significant increases in mRNA expression of kidney lineage markers, particularly those of podocytes (WT1, SYNPO, and NPHS1), and decreases in expression of early metanephric mesenchyme markers (CD133 and CD24). We performed single-cell RNA sequencing on hESCderived cells at different stages to establish signature surface markers, allowing us to purify and enrich for NPC population. From single-cell transcriptome analysis, we have identified a set of genes whose gene expression profiles are highly correlated with those of podocyte lineage genes. Among those, we have selected potential surface markers for NPC enrichment, such as CD83, PLP2, and TM7SF2.

Conclusion: Our highly reproducible protocol allows efficient production of hESC-derived NPCs in a chemically defined, xeno-free culture. These cells promise to provide an unlimited source to construct kidney models for analyzing drug clearance and toxicity, and for studying molecular disease mechanisms.