ABSTRACT

TITLE: CELL TYPE BASED PATHWAY ANALYSIS IN SUBARACHNOID HEMORRHAGE- REVEALED BY SINGLE CELL METHOD

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Key words: Subarachnoid Hemorrhage, neuroinflammation, pathway, single cell sequencing, brain cells, receptor-ligand

INTRODUCTION

Recently, increasing evidence points to a pivotal role of neuroinflammation in the pathogenesis of Subarachnoid hemorrhage (SAH). Microglia, astrocyte, Central nervous system (CNS)-associated macrophage and other kinds of brain cells are important for brain homeostasis and response to external stimuli. However, the pattern of cell interaction and mode of ligand-receptor action after SAH is not clear.

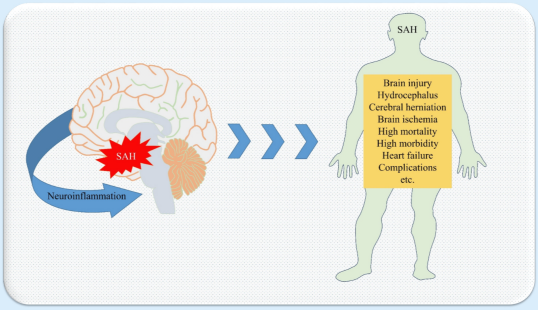

Figure 1: SAH and clinical conditions

METHODS

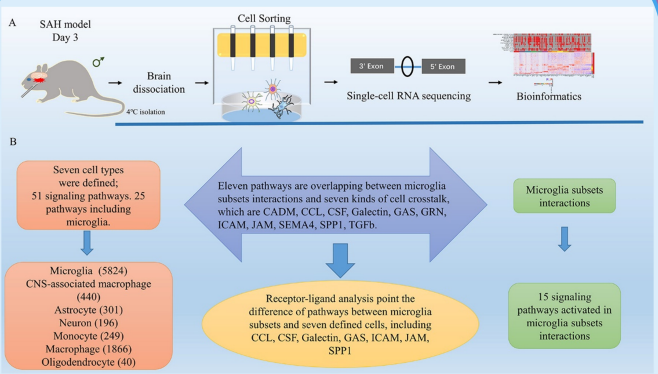

Figure 2: A) The study design; B) Mainly findings. CADM: cell adhesion molecule; CCL: chemokine ligands; CSF: colony-stimulating factor; GAS: growth arrest-specific; GRN: progranulin; ICAM: intercellular adhesion molecule; JAM: junction adhesion molecule; SEMA4: semaphorin 4; SPP1: secreted phosphoprotein 1; TGF-β: transforming growth factor beta.

Endovascular perforation (EVP) murine SAH model was established to reproduce experimental SAH. Post-SAH CD11b+ single-cell suspension was harvested at day 3 and sequenced using 10X single-cell RNA-sequencing platform. Then, the detailed single-cell information of post-SAH cells was analyzed with bioinformatics. The same pathways and related ligand-receptors in microglia and other cells were compared.

ETHICS APPROVAL

The study was approved by the Animal Experimentation Ethics Committee, the Chinese University of Hong Kong (reference number 19-108-GRF).

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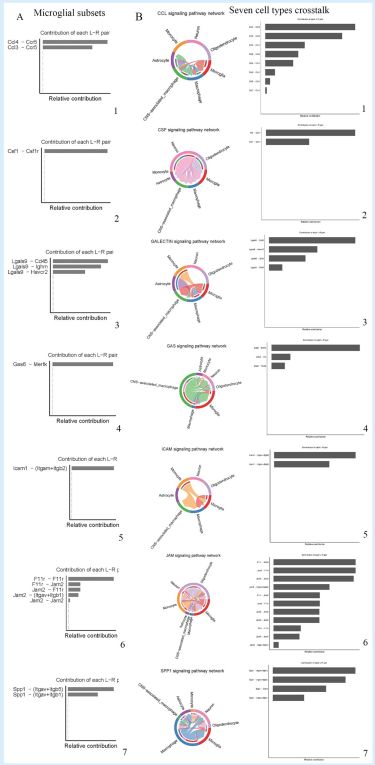
RESULTS

More than 51 pathways were found in the crosstalk of brain cells after SAH, these cells including: microglia (microglia worked in 25 pathways), CNS-associated macrophage, astrocyte, neuron, monocyte and macrophage and Oligodendrocyte. In the microglia subsets' interaction, 15 pathways upregulated, of which four pathways were specific to microglia subsets, including amyloid-β precursor protein (APP), Tumor necrosis factor (TNF), growth differentiation factor (GDF) and oncostatin M (OSM) signaling pathways. We precisely distinguished the ligand-receptor pattern in which microglia differ with other cells in 7 pathways, including chemokine ligands (CCL), colony-stimulating factor (CSF), GALECTIN, growth arrest-specific (GAS), intercellular adhesion molecule (ICAM), junction adhesion molecule (JAM) and secreted phosphoprotein 1 (SPP1) signaling pathways. It can be seen that different ligand-receptor patterns may exist in the same pathway, and these pathways are often very important which may not be determined by a single cell type. Gene ontology (GO) results showed that compared with non-microglia cells, post-SAH microglia with upregulated function like leukocyte, glia cell activation and migration etc., while have downregulated functions of protein-lipid complex remodeling etc.



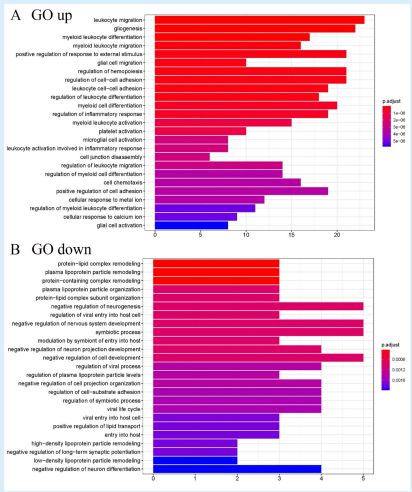


Figure 4: A) Upregulated pathways of microglia vs non-microglia cells in GO (Gene Ontology) results; B) Downregulated pathways of microglia vs non-microglia cells in GO results..

Figure 3: A) Receptor-ligand analysis in post-SAH microglia of seven pathways; B) Receptor-ligand analysis in post-SAH cell of seven pathways. CCL: chemokine ligands; CSF: colony-stimulating factor; GAS: growth arrest-specific; ICAM: intercellular adhesion molecule; JAM: junction adhesion molecule; SPP1: secreted phosphoprotein 1.

CONCLUSIONS

Collectively, we first systematically report the interaction patterns of seven brain cells after SAH. Further the cell type based ligand-receptor analysis of microglia and other cells in the same pathway was comparatively conducted. These results refreshed the current cellular mechanism for neuroinflammation after SAH, and the ligand-receptor results provided a theoretical basis for accurate monitoring and modulating SAH in the future.