Single-cell Transcriptomics Reveals that Ccl6/Ccr2 Pathway Induce Migration and M2 Polarization among Macrophages in Acute Kidney Injury

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Background

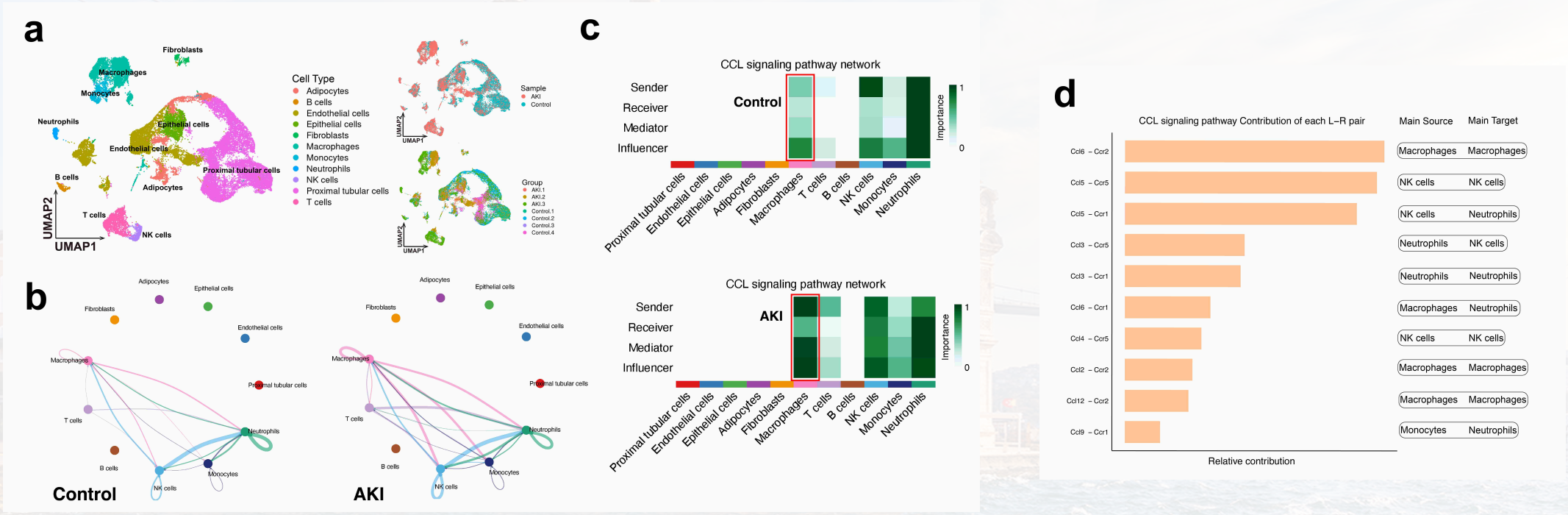
Acute Kidney Injury (AKI) is closely associated with the occurrence of Chronic Kidney Disease (CKD). The M2 polarization of macrophages is involved in the repair after kidney injury while also promoting the occurrence of renal interstitial fibrosis. However, the chemotaxis of macrophages after AKI and the activation mechanism of M2 phenotype macrophages are still unclear.

Methods

We integrated single-cell transcriptomic data from various AKI models 7 days post-injury, corrected for batch effects, and performed dimensionality reduction, clustering, and cell annotation. CellChat was used to analyze macrophage interactions, and Pseudotime Analysis to trace their differentiation. Pseudobulk Analysis identified differentially expressed genes in macrophages. We explored the functions of Ccl6 and Ccr2 through GO enrichment and protein-protein interaction analyses. The AKI model was established by uIRI, with kidneys collected after 7 days.

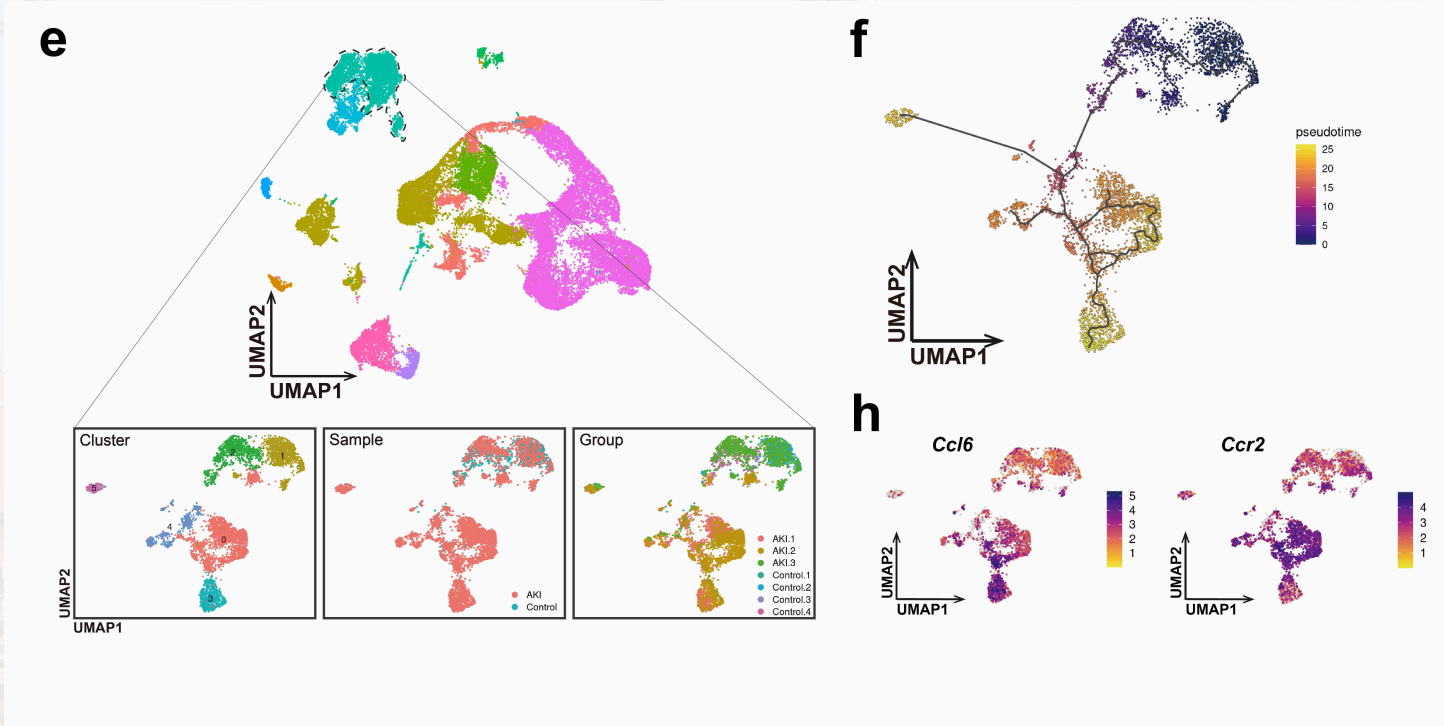
Result 1

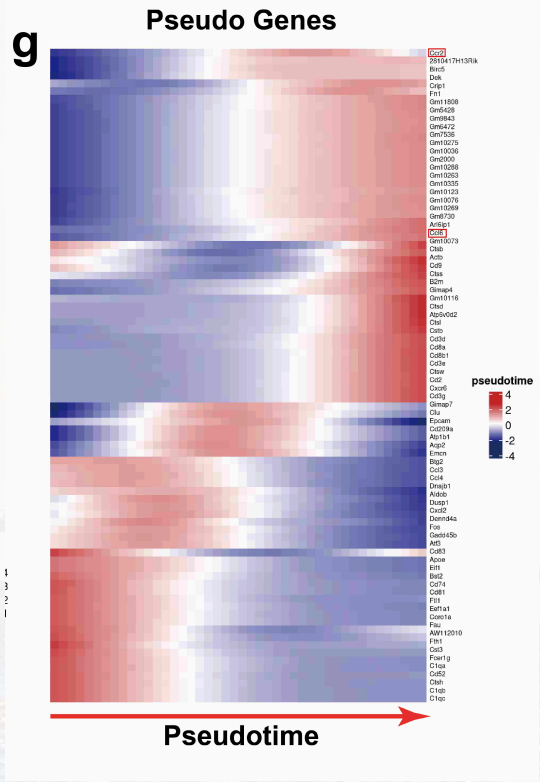
By comparing the ligand-receptor differences in the signaling pathways between the AKI group and the control group, we observed that the signal of ligand-receptor in the CCL signal pathway was significantly strengthened in the macrophages of AKI mice, predominantly mediated by Ccl6 and Ccr2.



Result 2

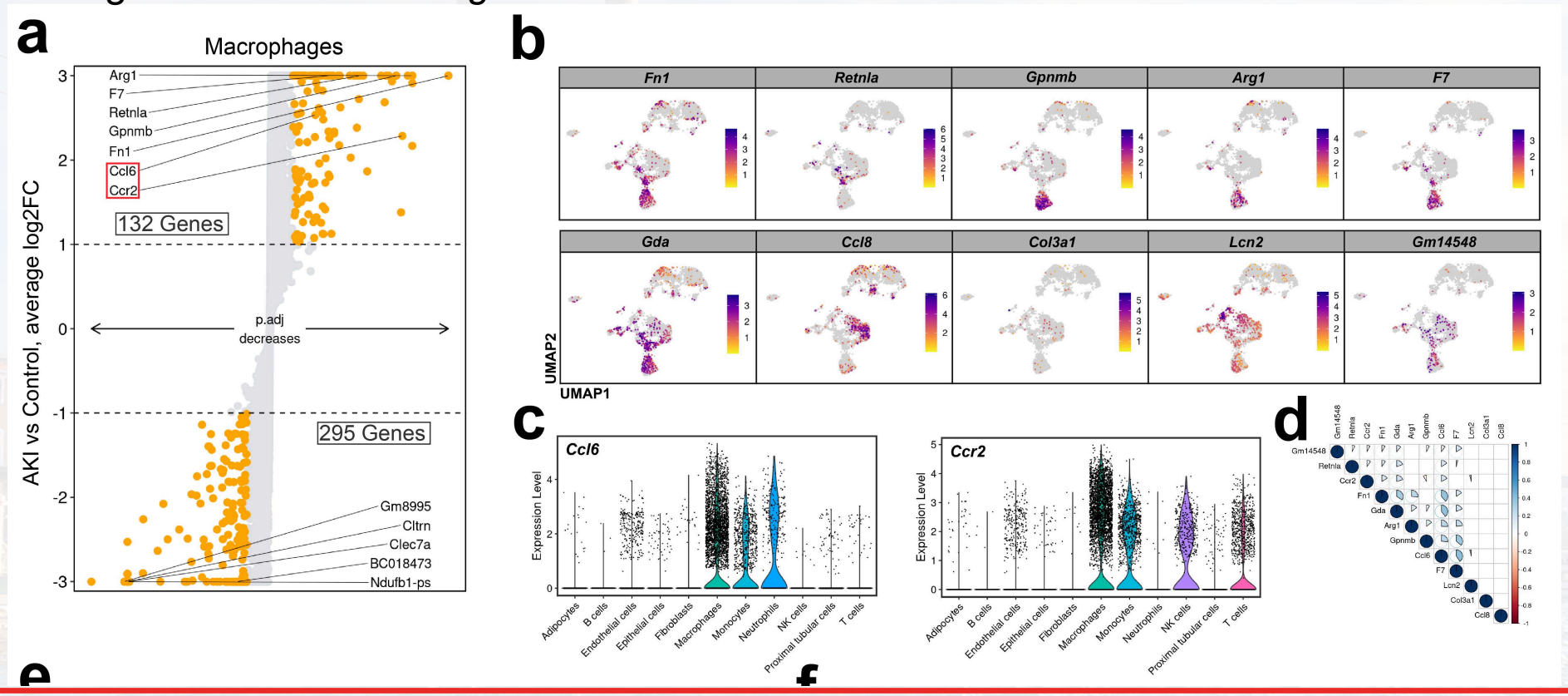
Pseudotime analysis revealed a potential differentiation process from Mac1, 2 to Mac0, 3, 4, with Ccl6and Ccr2 showing significant expression at the terminal stages of the trajectory.





Result 3

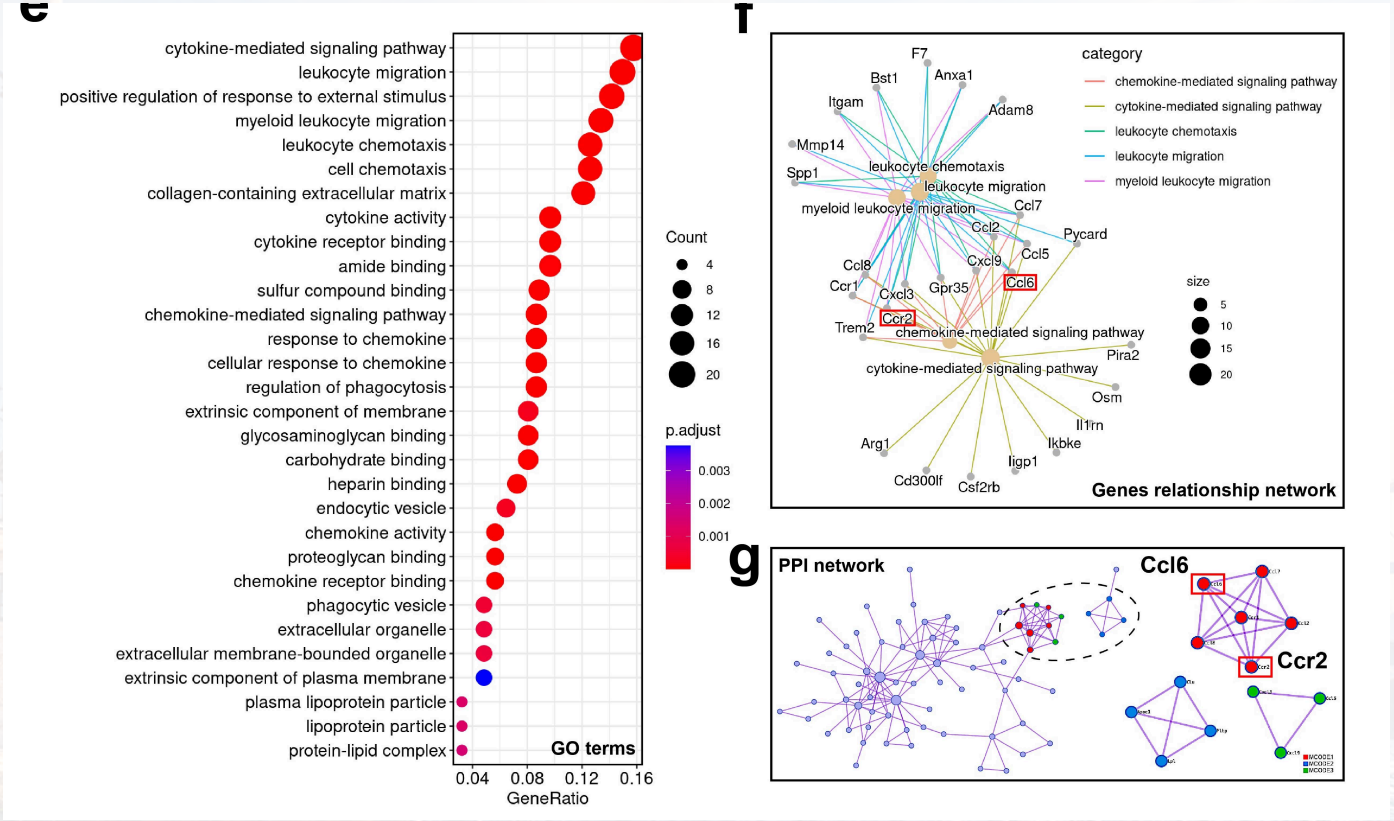
DEGs analysis of macrophages indicated an upregulation of *Ccl6* and *Ccr2* in the AKI group, along with an increased expression of M2 macrophage polarization signature genes *Retnla* and *Arg1.*



Result 4

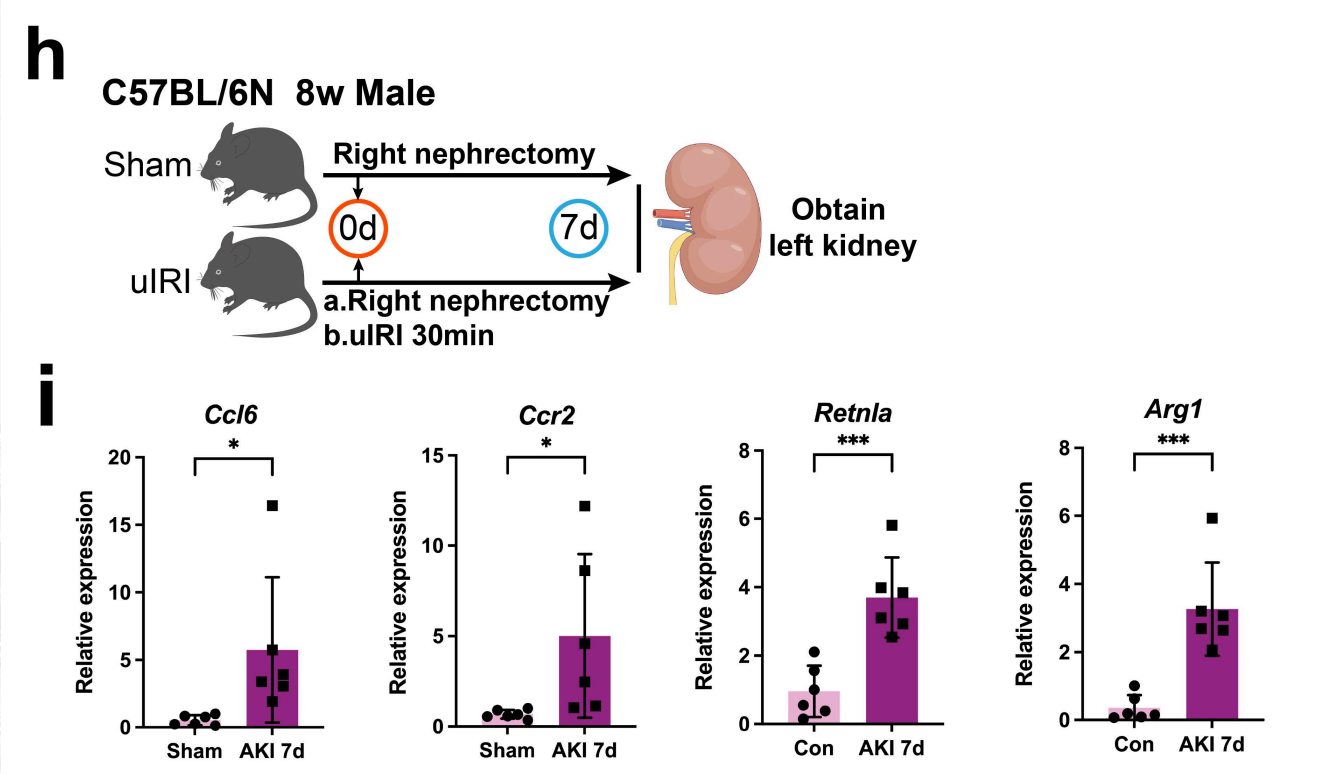
1. Enrichment analysis suggested that Ccl6 and Ccr2 play roles in the cytokine and chemokine-mediated signal pathway.

2. The protein-protein interaction (PPI) network was constructed based on upregulated expressed genes, identifying three core MCODE networks, among which the interaction between Ccl6 and Ccr2 was the most intense.



Result 5

RT-qPCR analysis indicated elevated expression of *Ccl6*, *Ccr2*, *Retnla*, and *Arg1* in the kidneys 7 days post-AKI.



Conclusions

We confirmed that 7 days post-AKI, the macrophages infiltrating the kidney upregulate the expression of the Ccl6. This, in turn, interacts with the circulating Ccr2highmacrophages, inducing a substantial migration of macrophages. Moreover, the Ccl6 and Ccr2 interaction mediates macrophage infiltration and the M2 polarization process, which may lead to renal fibrosis following AKI.

Thanks