Uncovering genomic alterations in DOCA-salt nephropathy rats treated with finerenone

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Background

- The aldosterone antagonist spironolactone has antifibrotic effects, but its clinical use is limited due hyperkalemia, especially in patients with kidney disease
- The novel, nonsteroidal, selective mineralocorticoid receptor (MR) antagonist finerenone has recently been developed with pronounced antifibrotic activity at doses that have only limited effects on potassium homeostasis
- The exact molecular transcriptional targets of spironolactone and finerenone, however, remain unknown
- There are more than 20 different cell types in the kidney; therefore, single cell RNA and single cell epigenome analysis can help to define transcriptional targets

Methods

- We treated uninephrectomized, Sprague–Dawley rats injected with deoxycorticosterone acetate (DOCA) and salt with spironolactone (50 mg/kg/d), finerenone (10 mg/kg/d) or sham
- Outcome parameters included blood pressure, serum and urine electrolytes, albuminuria, renal and cardiac histology
- A single nuclei suspension was prepared from kidneys and hearts for single nuclear RNA and single nuclear open chromatin (assay for transposase-accessible chromatin [ATAC]) profiling using the 10X Genomics Chromium platform as well as bulk RNA sequencing

Results

- The DOCA-salt group developed severe hypertension
- Treatment with finerenone and spironolactone resulted in the same degree of blood pressure reduction
- DOCA-salt-treated rats developed severe myocardial hypertrophy and focal vasculopathy, glomerulosclerosis, and tubulointerstitial fibrosis
- Finerenone significantly attenuated cardiac and renal histological damage
- DOCA-salt-treated rats developed marked albuminuria, which was significantly attenuated by spironolactone and finerenone
- Serum potassium was elevated in the spironolactone group at week 6, but it was unchanged compared with controls in the finerenone group
- Bulk RNA-seq results revealed the reduced enrichment of immune response-related transcripts in the finerenone group compared with the DOCA-salt and spironolactone group
- Single-nuclei gene expression profiling revealed that MR-associated genes were enriched in principal cells (PCs)
- We also identified an injury proximal tubule (PT) cluster; the injury PT cell number ratio in the DOCA-salt and finerenone group was significantly decreased compared with the DOCA+spironolactone and DOCA+vehicle groups

Finerenone improved kidney and heart function in the rat DOCA-salt model

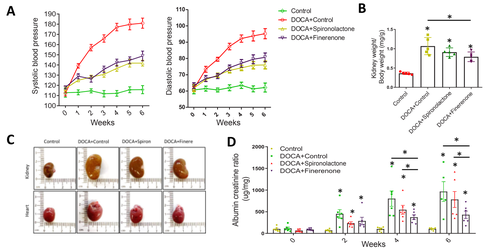


Figure 1. Effects of MR antagonists on (A) Systolic and diastolic blood pressure, (B) kidney weight/body weight ratio in each group, (C) representative kidney and heart image, (D) the urine protein-to-creatinine ratio in each group. Mean ± SEM; *p<0.05 versus control. DOCA, deoxycorticosterone acetate; MR, mineralocorticoid receptor; SEM, standard error of the mean.

Finerenone attenuated renal histological damage in the rat DOCA-salt model

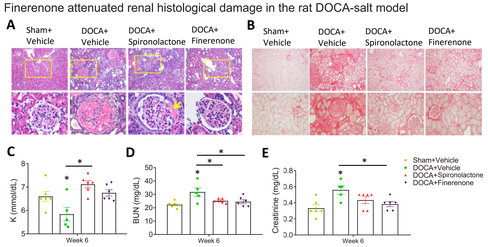
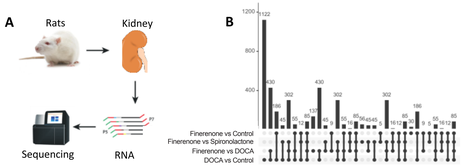


Figure 2. Effect of MR antagonists on (A) Representative renal histopathology, (B) representative renal Sirius red staining, (C) serum potassium level, (D) serum urea nitrogen level, and (E) serum creatinine level. Mean ± SEM; *p<0.05 versus control. BUN, blood urea nitrogen; DOCA, deoxycorticosterone acetate; MR, mineralocorticoid receptor; SEM, standard error of the mean.

Differential expression analysis of whole kidney RNA-Seq data



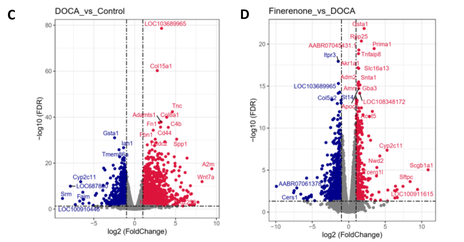


Figure 3. Differential expression analysis of RNA-Seq data. (A) Explanatory of kidney bulk RNA seq, (B) Venn diagram of DEG overlap between each group, (C) volcano plot obtained from DEGs analysis of DOCA-salt and vehicle, (D) volcano plot obtained from DEGs analysis of finerenone and DOCA-salt. DEG, differentially expressed gene, DOCA, deoxycorticosterone acetate.

Single-nuclei RNA expression profiling of control and spironolactone- and finerenone-treated DOCA rat kidneys

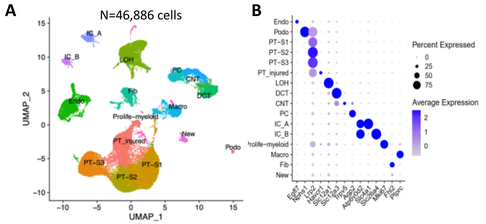


Figure 4. Single-nuclei gene expression profiling identified in an injury PT cluster. (A) Clustering of single-nuclei gene expression profiling, (B) expression of cell type marker genes, (C) relative expression of Kim1 in each cluster. (D) injury PT cell number ratio in different groups. DOCA, deoxycorticosterone acetate; PT, proximal tubule; UMAP, Uniform Manifold Approximation and Projection.

Single-nucleus analysis of expression profiling of control and spironolactone- and finerenone- treated DOCA-salt rat kidneys

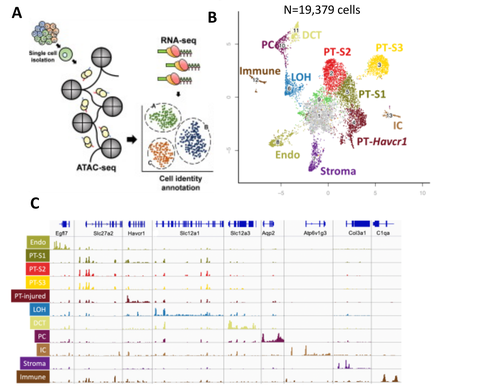


Figure 5. Single-nucleus analysis of accessible chromatin. (A) Schematic of single-nucleus ATAC, (B) UMAP embedding of snATAC-seq data, (C) genome browser view of read density in each snATAC-seq cluster at cell type marker gene transcription start sites. ATAC, assay for transposase-accessible chromatin; snATAC, single nucleus assay for transposase-accessible chromatin; UMAP, Uniform Manifold Approximation and Projection.

Expression of MR and HSD11B2 in the kidney

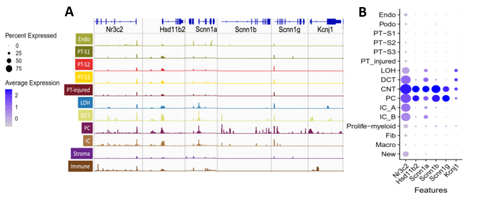


Figure 6. Expression of MRs and HSD11B2 in the kidney. (A) Genome browser view of read density in each snATAC-seq cluster at MR-associated transcription start sites, (B) the expression level of MR-related genes in each cell cluster. MR, mineralocorticoid receptor; snATAC, single nucleus assay for transposase-accessible chromatin.

Single-nuclei gene expression profiling uncovers novel MR targets in PC and CNT cells following MR antagonist treatment

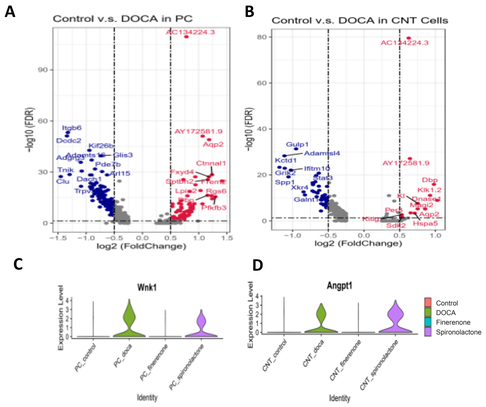


Figure 7. Single-nuclei gene expression profiling. Volcano plot obtained from DEGs analysis of DOCA-salt vs control in PC cluster (A) and CNT cluster (B), (C) Relative expression of Wnk1 in a PC cluster. (D) Relative expression of Angpt1 in a PC cluster. CNT, connecting tubule; DEG, differentially expressed gene, DOCA, deoxycorticosterone acetate; MR, mineralocorticoid receptor; PC, principal cell.

Conclusions

- Treatment with finerenone protected against DOCA-salt-induced cardiac hypertrophy, glomerulosclerosis, and kidney fibrosis without a significant increase in serum potassium
- Combined single-cell RNA and epigenome analysis identified changes in MR targets and kidney injury, defining the mechanism of MR-induced kidney disease