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Aldosterone-regulated miRNAs in mpkCCDc14 cells: the role in aldosterone-mediated apoptosis via Wnt signaling

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Background: Renin-angiotensin-aldosterone is one of the major mechanisms to regulate body water and electrolyte homeostasis. Several studies revealed that mature microRNA (miRNA) acts as an important post-transcriptional regulator. Wnt signaling pathways are known to play an important role in the regulation of ENaC and potassium channel activity and apoptosis. The effects of aldosterone on the Wnt signaling pathways in kidney collecting duct cells are not well understood. The present study aimed to 1) identify the aldosterone-regulated miRNAs and their target genes, and 2) the role of the selected miRNAs in the aldosterone-induced renal apoptosis via Wnt signaling.

Methods: Microarray chip assay (Affymatrix GeneChip miRNA 4.0 array) was performed in the mpkCCDc14 cells (mouse kidney cortical collecting duct cells) in the absence or the presence of 10^{-6} M aldosterone treatment for 3 d, respectively. The candidate miRNAs from the chip assay data were selected by 1) more or less than 30 % of significant fold-changes, or 2) differential expression analysis carried out using the R package 'bridge' (Gottardo R. bridge: Bayesian Robust Inference for Differential Gene Expression. R package version 1.34.0.). To predict putative target genes of identified miRNAs and miRNAs-enriched pathways, DIANA-mirPath program were carried out based on microT-CDS algorithms.

Results: Microarray chip assay demonstrated that 29 miRNAs were significantly up-regulated more than 1.3-fold change and 27 miRNAs were markedly down-regulated less than 0.7-fold change in mpkCCDc14 cells after aldosterone treatment. Using R package 'bridge', posterior probabilities of differential gene expression were calculated and 5 up-regulated and 7 down-regulated miRNAs (more than 1.2 fold change and less than 0.8 fold change compared to control group) were selected with high posterior probabilities (> 0.95) in mpkCCDc14 cells. Twenty four out of 56 miRNAs were further selected by DIANA-mirPath program and 55 KEGG pathways were profiled with 0.01 *P*-value and 0.8 microT threshold. Four out of 12 miRNAs identified by 'bridge' were further selected by DIANA-mirPath program and 29 KEGG pathways were profiled with 0.01 *P*-value and 0.8 microT threshold. In particular, Wnt signaling pathway among the profiled KEGG pathways, which was the highest ranked, was further examined. The quantitative changes of 8 up-regulated and 9 down-regulated mature miRNAs enriched in the Wnt signaling pathways were measured by qPCR in response to aldosterone stimulation in the different concentrations in mpkCCDc14 cells. Further studies are to be performed to evaluate whether

the changes of target genes and proteins by the overexpression of miRNAs could affect the aldosterone-induced apoptosis in the kidney collecting duct.
Conclusion: These results demonstrate that aldosterone stimulation induces significant changes in miRNA expression, which could be involved in a number of signaling pathways including Wnt signaling and hence aldosterone-mediated apoptosis.

Keywords: aldosterone, microRNA, Wnt signaling, collecting duct