Poster Presentation：Electrolyte, Acid-Base, and Hypertension
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The miRNA Expression of Urinary Extracellular Vesicles in Patients with Gitelman Syndrome: The Role of Hsa-let-7d-3p

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Abstract

Background

Gitelman syndrome (GS) is caused by inactivating mutation in the SLC12A3, encoding the thiazide-sensitive sodium chloride cotransporter (NCC), leading to salt wasting and electrolyte imbalance. Our aim is to identify miRNA expressions and explore their role from urinary extracellular vesicles (uEVs) in GS patients.

Methods

Both uEVs from 23 genetically confirmed GS patients and renal biopsied tissues from other 3 GS patients were extracted for small RNA sequencing. Real-time PCR of uEVs (n = 11), immunofluorescence staining of NccS707X/S707X knock-in mice (GS animal model), and dual luciferase reporter assays were performed for validation.

Results

Small RNA sequencing identified 338 miRNAs from uEVs and 652 miRNAs from renal biopsied tissues. Amongst differentially expressed miRNAs, 20 were up-regulated and 23 down-regulated in uEVs, whilst renal biopsied tissues showed 30 up-regulated and 23 down-regulated miRNAs. Four miRNAs (hsa-let-7d-3p, hsa-miR-362-5p, hsa-miR-30c-5p and hsa-miR-30b-5p) overlapped from uEVs and renal tissues. In particular, the terms of the distinct up-regulated hsa-let-7d-3p target genes were related to ion transport and membrane depolarization, especially in NEDD4L. Real-time PCR of uEVs from another 11 GS patients confirmed significantly elevated hsa-let-7d-3p compared to healthy controls. The decrease in the Nedd4l expression in collecting duct was also confirmed in NccS707X/S707X knock-in mice. Dual luciferase assays further demonstrated that hsa-let-7d-3p negatively regulated the expression of NEDD4L.

Conclusions

The expression of miRNA could be isolated from uEVs and their differentially expressed miRNAs, especially has-let-7d-3p, may play a critical role in renal tubular transporters, new insights into the regulation of tubular transporters in GS.

Keywords：extracellular vesicle, Gitelman syndrome, hsa-let-7d-3p, miRNA

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Potassium Deficiency Activating GCN2 eIF2 Kinase and Integrated Stress Response Mediating the Loss of AQP2

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Abstract

Background: Potassium (K+) deficiency could cause a reduction in urinary concentration ability, resulting in nephrogenic diabetes insipidus (NDI), but the detailed mechanism remains unclear. Recently, transcriptomic and proteomic data from acquired NDI models reveal that inflammatory signaling is associated with AQP2 loss. We aim to explore the early signaling after K+ deficiency in collecting ducts.

Method: We performed RNA-Seq and LC-MS/MS on microdissected rat cortical collecting ducts (CCDs) to identify early signaling pathway after establishment of K+ deprivation mediating the loss of AQP2. The signaling was validated by the mpkCCD cells treated with or without K+ medium.

Results: Immunoblotting of bulk kidney showed a decrease in AQP2 protein abundance at 12 hours of K+ deprivation diet, and urine osmolality was significantly decreased at 24 hours, confirming the animal model of K+ deprivation-induced NDI. RNA-Seq data of microdissected CCDs at 6 hours after K+ deprivation showed Aqp2, Aqp3, and Atp1a1 were significantly downregulated. It also revealed that chemokine transcripts (Ccl20 and Ccl28) were significantly increased. Gene Ontology Biological Process terms among statistically over-represented in the list of 88 “Increased Transcripts” are related to cell chemotaxis, cellular response to lipopolysaccharide, consistent with an inflammatory response. LC-MS/MS data of microdissected CCDs at 12 hours after K+ deprivation revealed upregulated EIF2S1 (eIF2α), that was confirmed by immunoblotting of rat kidney after K+ deprivation. In vitro mpkCCD cells, Ccl20 and Cxcl16 increased significantly at 3 hours when the Aqp2 markedly decreased at 12 hours after K+ deprivation compared to normal K+ medium (NK controls). Of note, p-GCN2 and p-eIF2α started to increase at 1 hour after K+ deprivation compared to NK controls, and N-acetyl cysteine decreased the p-GCN2 and p-eIF2α and also rescued the loss of AQP2.

Conclusion: Our small samples RNA-Seq and LC-MS/MS from rats microdissected CCDs showed early cellular signaling changes in activation of GCN2 eIF2 Kinase and integrated stress response, inflammatory signaling causing loss of aquaporin-2 after K+ deficiency.

Keywords：Aquaporin-2, cortical collecting duct, K+deficiency, proteomics, transcriptome

Poster Presentation：Electrolyte, Acid-Base, and Hypertension
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Inhibition of FAK signaling in inner medullary cells affects urine concentration in the collecting duct

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Abstract

Background: Renal epithelial cells (REC) are polarized, with the apical side oriented towards the tubular lumen and the basal domain of the plasma membrane attached to the extracellular matrix. REC polarity gives rise to tubular organization in the nephron and develops specific functions in distinct segments of the renal tubule. The FAK signaling is as fundamental of maintaining cell polarity in REC. REC polarity in principal cells reflects the apical AQP2 and basal-lateral AQP4 polarity in the PM. The role of FAK signaling in the apical polarization of AQP2 and the basal-lateral polarization of AQP4 in epithelial cells of the inner medullary collecting duct (IMCD) remains unclear.

Methods: In present study, we found out that the mIMCD-3 cells at lower passage numbers bear principal cell trait and expressed AQP2 and AQP4. To gain insight into FAK signaling to AQP2 and AQP4 polarization, the FAK inhibitor VS-4718 was applied to investigate role of FAK in AQP2-AQP4 polarity in the mIMCD-3 cells.

Results: As the mIMCD-3 cells exposed to FAK inhibitor, AQP2 and AQP4 both are absent of their subcellular localization at PM in mIMCD-3 cells. In contrast, FAK is activated by osmotic pressure forces, and the presence of AQP2 and AQP4 at the plasma membrane has been concurrently observed.

Conclusion: Our data suggests that FAK signaling in principal cells constitutively regulates AQP4 at both the basolateral and apical regions of the plasma membrane. FAK signaling may regulate water reabsorption and urine concentration in the CD.

Keywords：AQP2, AQP4, FAK inhibitor

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RRM2B deficiency impairs aquaporin-2 expression via the ATM-NFAT5 signaling axis in renal collecting duct

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Abstract

Background:

Ribonucleotide-diphosphate reductase subunit M2 (RRM2B) is essential for mitochondrial DNA repair and oxidative stress response, while aquaporins (AQPs) regulate water homeostasis across membranes in various physiological and pathological contexts. Although both are crucial for cellular function, the link between RRM2B deficiency and AQP expression remains uncharacterized. Understanding this connection may reveal novel mechanisms by which mitochondrial dysfunction influences water transport under stress conditions.

Methods:

Immunohistochemical analysis of phosphorylated Ataxia telangiectasia mutated (ATM) kinase, a known binding partner of RRM2B, was performed to assess ATM signaling activity in RRM2B-deficient mice. To evaluate the downstream effects, the expression of AQP2, a target of Nuclear Factor of Activated T Cells 5 (NFAT5) regulation, was examined, given the role of ATM in NFAT5 transcriptional activation. Additionally, to investigate the RRM2B/ATM/NFAT5 signaling axis in renal principal cells, NFAT5 and AQP2 expression levels were analyzed in mouse inner medullary collecting duct (mIMCD3) cells treated with the ATM inhibitor KU-55933.

Results:

ATM and NFAT5 are both present in the nucleus of principal cells without RRM2b deficiency or ATM inhibition. The NFAT5 and AQP2 expression levels decrease in RRM2b-deficient mice and principal cells exposed to KU-55933.

Conclusion:

The RRM2B/ATM-NFAT5 signaling axis manages AQP2 expression.

Keywords：Ribonucleotide-diphosphate reductase subunit M2; aquaporins; Nuclear Factor of Activated T Cells 5; inner medullary collecting duct

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Single-Cell Transcriptomic Characterization of Adrenal Glands with Aldosterone-Producing Adenomas

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Abstract

Aldosterone-producing adenomas (APAs) are adrenocortical tumours in the adrenal gland that autonomously secrete aldosterone, a hormone that regulates blood pressure. They are a major cause for primary aldosteronism and when left untreated leads to hypertension and related co-morbidities. Most APAs harbour known somatic aldosterone-driving mutations that are responsible for the dysregulated aldosterone production and in some cases, co-production of hybrid steroids (i.e. 18-hydroxycortisol and 18-oxocortisol). However, the development of the APA from the adrenal cortex are still not fully understood. This study aims to explore the transcriptomic landscape of adrenal glands harbouring APAs using single-cell RNA sequencing. 10x Genomics Chromium Single Cell Gene Expression Flex assay was performed on formalin-fixed paraffin-embedded (FFPE) adrenal sections containing APAs harbouring common somatic aldosterone-driving mutations in KCNJ5, CACNA1D, ATP1A1, and ATP2B3, or wild-type for these genes (n=6). Data analysis with Seurat R package (v5) identified diverse distinct cell populations within the adrenal sections — adrenocortical cells, medullary cells, immune cells, endothelial cells, capsule, and stromal cells. The adrenocortical cells were further subclustered as APA cells, aldosterone-producing micronodule cells (APM), zona glomerulosa (ZG) cells, zona fasciculata (ZF) cells, and zona reticularis (ZR) cells using known canonical markers. Interestingly, one adrenocortical cell cluster expressed markers for both ZG (ANO4, DACH1, VCAN) and ZF (CYP11B1, CYP17A1) suggesting the existence of ZG-ZF intermediary cells capable of producing hybrid steroids as the enzymes that are normally limited to ZG cells and ZF cells respectively, are now present in the same cell. Differential gene expression analysis revealed that this hybrid cluster highly expressed heat shock proteins—HSPA1A, HSPA1B

(members of Hsp70 family), and HSPB1 (Hsp20). Heat shock proteins are molecular chaperones that facilitate proper protein folding and cellular repair under stress conditions. Pathway analysis conducted using Reactome (v93) indicated these genes were involved in stress response pathways, including the mitochondrial unfolded protein response, cellular response to heat stress, and regulation of HSF1-mediated heat shock response. Spatial transcriptomics using Visium CytAssist showed sparse expression of HSPA1A and HSPA1B in distinct cells in normal adrenal tissue and APA; whereas HSPB1 was ubiquitously expressed but at lower levels in APA cells. In conclusion, this study highlights a significant enrichment of stress-response pathways within an intermediary ZG-ZF adrenocortical cell population. Understanding the stress-related pathway in normal adrenal tissue adjacent to APAs may offer new insights to adrenal steroidogenesis, APA pathology, and potential therapeutic targets.

Keywords：Hypertension, Primary aldosteronism, Aldosterone-Producing Adenoma, Hyperaldosteronism, Somatic Mutations, Single-Cell RNA sequencing, Spatial Transcriptomics