TSS-Seq analysis of low pH-induced gene expression in intercalated cells of the renal collecting duct

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Background

Recent studies have suggested that metabolic acidosis accelerates the progression of chronic kidney disease. Dietary acid intake accumulates acids and decreases the pH in urine and the interstitium of the kidney. Little is known about the molecular mechanisms of acidosis-induced kidney injury. In the present study, we performed comprehensive analyses to characterize the global effect of low pH on intercalated cells of the renal collecting duct and molecular biology experiments to support the findings obtained from the analyses.

Methods

- We employed Transcription Start Site-Sequencing (TSS-Seq) to provide low pH-induced gene transcripts in a rat intercalated cell line (IN-IC cells) (1).
- Cells were incubated either at pH 7.4 or 7.0 for 24h, then total RNA was extracted. Two biological replicates were performed. cDNA library for CAGE (Cap Analysis Gene Expression) was created (2).
- Deep sequencing was performed by Illumina HiSeq2500 sequencer. TopHut 2 software was used to map TSS-Seq reads. RECLU was used to identify differentially expressed transcripts and motif discovery analysis.
- CAGE library preparation, sequencing, matting, and gene expression analysis were performed by DNAFORM (Kanagawa, Japan).
- Gene ontology (GO) analysis was carried out using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (3).
- To evaluate ubiquitin-proteasome pathway, cells were incubated either at pH 7.4 or 7.0 for 12h, then further incubated in the presence of MG132, a proteasome inhibitor, for 8h. Western blotting was examined using anti-ubiquitin antibody. Quantitative PCR was examined to measure the expressions of specific mRNAs after cells were incubated either in pH 7.4 or 7.0.

Results

Expression analysis by RECLU

- 697 transcripts (TSSs) were significantly up- or down-regulated ($p < 0.05$).
- Among them, 478 and 11 transcripts were selected by a log fold change ≥ 1.0 or ≤ 1.0, respectively.
- 305 up- and 10 down-regulated transcripts were corresponded to gene ID

	Up	Down
Number of TSSs significantly changed ($p < 0.05$)	651	128
Log_2 fold change ≥ 1.0 or ≤ 1.0	424	38
Genes given NCBI Ref. Seq. IDs	193	34

Significantly Up- or down-regulated at pH 7.0 relative to in pH 7.4.

GO analysis by DAVID

- Genes that were up-regulated ≥ 1.0 fold change (Log2), were included for the analysis.

Functional Annotation Clustering

193 Genes whose transcription is up-regulated ≥ 1.0 in Log2 in pH 7.0 Analyzed by DAVID (High stringency, Enrichment score > 1.5).

GO term	Genes
Phosphate metabolic process	Ptprj, Trim28, Ptpra, Cdk9, Mark3, Spag9, Plk3, Sbk1, Ppm1j, Map3k3, Ulk1, Gsk3a, Ulk2, Map3k1, Aak1, Ppp2ca, Grk6, Prkaca, Jak2, Brd4, Fgf2
Negative regulation of transcription	Nacc1, Gclc, Rcor2, Yaf2, Glis2, Trim28, Nab1, Gatad2a, Mdm2, Ncor1, Fgf2, Foxp4, Akirin2
Positive regulation of apoptosis	Xpa, Nacc1, Cebpb, Hipk1, Map3k1, Mtch1, Tgm2, Sort1, Jak2, Faf1
Ribonucleotide binding	Sept3, Gclc, Ube2z, Kif27, Gna11, Arfrp1, Pde3b, Itpka, Arl5a, Map3k3, Sbk1, Aak1, Map3k1, Tgm2, Rhobtb1, Prkaca, Cdk9, Mark3, Kif1c, kif1b, Plk3, Atp2a2, Gsk3a, Ulk2, Pi4k2a, Smarca5, Grk6, Dgkz, Jak2, Ar18a
Acid-amino acid ligase activity	Ube2e3, Gclc,Ube2z, Map3k1, Mdm2, Ube2q1, Ube2r2
Regulation of protein kinase activity	Spag9, Map3k1, Dgkz, Prkaca, Jak2, Fgf2, Dlg1, Dvl1
Protein catabolic process	Xpa, Arih1, Ube2e3, Ube2z, Map11c3a, Usp9x, Map3k1, Mdm2, Usp25, Ube2r2
Regulation of programmed cell death	Xpa, Nacc1, Gclc, Cebpb, Hipk1, Map3k1, Mtch1, Tgm2, Sort1, Jak2, Faf1, Fgf2, Traf4, Angptl4
Positive regulation of cell migration	Spag9, Irs2, Plcg1, Jak2, Fgf2
Regulation of nervous system development	Twhah, Serpinef1, Ulk1, Ulk2, Timp2, Fgf2, Numbl

Known roles of low-pH induced genes in the kidney

- **Cdk9**: a key molecule that promotes renal fibrosis in mice with unilateral obstruction (Qu et al. *Kidney Int.* 2015).
- **Ulk1**: induces autophagy in acute kidney injury (Livingstone et al. *Semin Nephrol.* 2014).
- **Fgf2**: promotes epithelial-mesenchymal transition (EMT) and renal interstitial fibrosis (Strutz et al. *Kidney Int.* 2002.; Livinigstone et al. *Autophagy.* 2016).
- **Jak2**: belongs to Jak2/Stat pathway that is activated in various renal disease models (González-Guerrero et al. *Toxicol Appl Pharmacol.* 2013; Matsui et al. *J Surg Res.* 2012).
- **Tgm2**: induces renal fibrosis of aging kidney (Sasai et al. *Biosci Biotechnol Biochem.* 2012; Lin et al. *Kidney Int.* 2016).
- **Gclc**: upregulated by Nrf2 and inhibits TGFβ-1 in renal tugular epithelial cells (Ryoo et al. *Arch Pharm Res.* 2015; Christopher et al. *Mol Aspects Med.* 2008).
- **Glis2**: whose loss induces growth of cysts caused by nephronophthisis (Lu et al. *Kidney Int.* 2016).
- **Map3k3**: activates NFAT5, a transcription factor, which induces the expression of osmoprotective genes (Padda et al. *Am J Physiol Renal Physiol.* 2006).

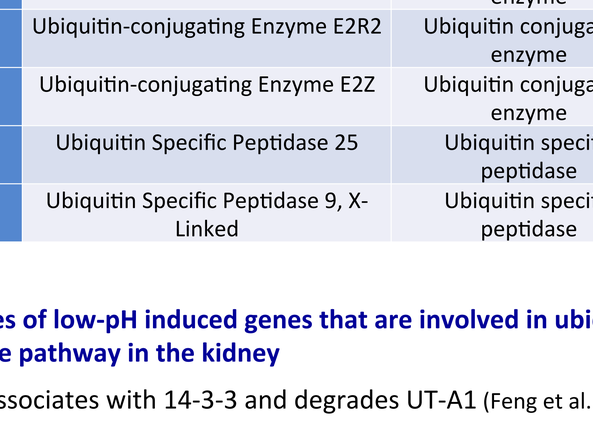
Genes involved in ubiquitin proteasome pathway

Gene	Description	Function
Angptl4	Angiopoietin-Like 4	Ubiquitin-protein ligase
Ankrd13a	Ankyrin Repeat Domain 13a	Ubiquitin binding protein
Arih1	Ariadne RBR E3 Ubiquitin Protein Ligase 1	E3 ubiquitin-protein ligase
Faf1	Fas(TNFRSF6) Associated Factor 1	Ubiquitin protein ligase binding
Josd1	Josephin Domain Containing 1	Deubiquitinating enzyme
Map3k1	Mitgen-Activating Protein Kinase Kinase Kinase 1	E3 ubiquitin protein ligase
Mdm2	MDM2 Proto-Oncogene	Nuclear-localized E3 ubiquitin ligase
Otud4	OTU Deubiquitinase 4	Deubiquitinating enzyme
Psme4	Proteasome Activator Subunit 4	Associated component of proteasome
Siah1	Siah E3 Ubiquitin Protein Ligase 1	Ubiquitin ligase
Trim28	Tripartite Motif Containing 28	Ubiquitin ligase
Ube2e3	Ubiquitin-conjugating Enzyme E2E3	Ubiquitin conjugating enzyme
Ube2h	Ubiquitin-conjugating Enzyme E2H	Ubiquitin conjugating enzyme
Ube2q1	Ubiquitin-conjugating Enzyme E2E3	Ubiquitin conjugating enzyme
Ube2r2	Ubiquitin-conjugating Enzyme E2R2	Ubiquitin conjugating enzyme
Ube2z	Ubiquitin-conjugating Enzyme E2Z	Ubiquitin conjugating enzyme
Usp25	Ubiquitin Specific Peptidase 25	Ubiquitin specific peptidase
Usp9x	Ubiquitin Specific Peptidase 9, X-Linked	Ubiquitin specific peptidase

Known roles of low-pH induced genes that are involved in ubiquitin proteasome pathway in the kidney

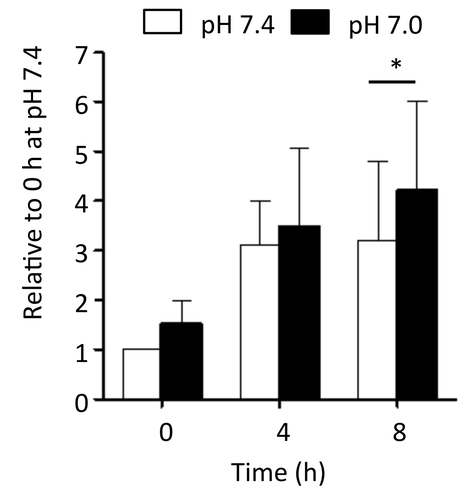
- **Mdm2**: associates with 14-3-3 and degrades UT-A1 (Feng et al. *Am J Physiol Renal Physiol.* 2015).
- **Siah1**:degrades homeo-domain interacting protein kinase 2 (HIPK2), which is a key molecule to induce fibrosis and EMT that appears in human immunodeficiency virus (HIV)-associated kidney diseases (Jin et al. *Nat Med.* 2012).
- **Ube2e3**: interacts with E3 ligase Nedd4-2 and regulates ENaC expression in principal cells of the collecting duct (Debonneville et al. *Mol Cell Biol.* 2004).

Low pH activates ubiquitin-proteasome pathway



- MG132 increased and low pH enhanced the expressions of ubiquitinated proteins in time dependent manner.

Quantitative analysis of ubiquitinated proteins in Western blot.



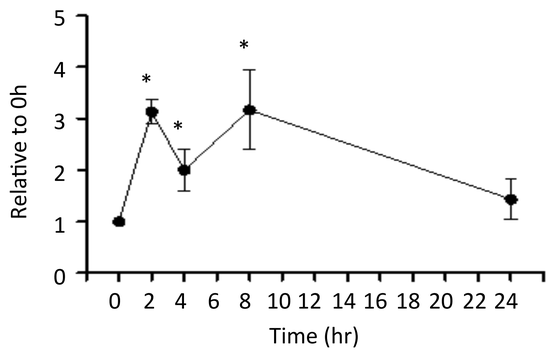
$* p < 0.05$

The amount of band densities that locate on more than 75 kDa was measured. n = 4.

Motif Discovery Analysis of up-regulated genes

Motif no.	Consensus	Foreground	Background	P-value	Known motifs (P-value)
GLAM2_009	GCCBCCGCSCCCGCCCCCGCCCBC	36	2,996	7.33E-03	EGR1(4.54859e-09), SP1(5.1807e-06), EGR2(7.6456e-06), SP2(9.37891e-06), ZNF263(2.67598e-05), E2F3(7.12131e-05)
AMD_009	CMAKSCAGGCCTGARNYY	4	1,299	4.60E-03	Zfx(6.973e-05)
GLAM2_009	GVRGSNGSGGCSGGGGGMGGGGGSGGCGBGG	80	3,341	6.59E-05	SP2(5.97832e-07), SP1(1.60588e-06), EGR1(2.09958e-06), ZNF263(1.87841e-05), E2F3(2.23805e-05), KLF5(9.67166e-05)
AMD_001	GGSGGCGGSGGSGGSGGCGGSGGSG	187	2,761	1.48E-31	EGR1(8.38472e-07), SP2(6.57943e-05)
GLAM2_010	GVRGSNGSGGCSGGGGGMGGGGGSGGCGBGG	80	1,137	-1.31E-03	SP2(5.97832e-07), SP1(1.60588e-06), EGR1(2.09958e-06), ZNF263(1.87841e-05), E2F3(2.23805e-05), KLF5(9.67166e-06)

Effect of low pH on the expression of EGR1 mRNA



Summary

- Low pH stimulated various gene expressions that are involved in renal fibrosis in IN-IC cell line.
- Low pH induced global ubiquitination of protein.
- EGR1 possibly regulates many gene expressions in response to low-pH.

Conclusion

- Metabolic acidosis could induce various pathways that facilitates renal fibrosis upon kidney disease.

References

1. Izumi Y, Hori K, Nakayama Y et al. Aldosterone requires vasopressin V1a receptors on intercalated cells to mediate acid-base homeostasis. *J Am Soc Nephrol.* 2011;22(4):673-80.
2. Kodzius R, Kojima M, Nishiyori H et al. CAGE: cap analysis of gene expression. *Nat Methods*. 2006; 3(3):211-22.
3. Dennis G Jr, Sherman BT, Hosack DA et al. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4: P3, 2003.

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Acid-base, uric acid & nephrolithiasis
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