Poster SP270

Dynamic Regulation of Circulating Long Noncoding RNAs in End-stage Renal Disease

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Background

Long non-coding RNAs (lncRNAs) are a heterogeneous group of non-coding transcripts longer than 200 nucleotides. While the roles of lncRNAs in human diseases including cancer and neurodegenerative disorders are beginning to emerge, it remains unclear how lncRNA regulation contributes to the pathogenesis linked to end-stage renal disease (ESRD).

The present study aimed to test the hypothesis that cell-free, circulating lncRNA expression pattern can reflect the disease state and the underlying pathophysiology of ESRD.

Materials and Methods

This study is carried out in the National Taiwan University Hospital and its Jin-Shan branch hospital. Adult patients with various stages 1-5 of chronic kidney disease (CKD) are enrolled at out-patient clinic. Patients with ESRD under maintenance hemodialysis are also included. This study was approved by the Institutional Review Board of the National Taiwan University Hospital, Taipei, Taiwan (201409019RINB). All participants signed a written informed consent before inclusion in the study.

Cell free, circulating lncRNA and mRNA expression profiling was conducted on the total RNA isolated from plasma samples of ESRD patients (n=4) and age-/gender-matched healthy subjects (n=4) using next-gen RNA sequencing (Ion AmpliSeq Human Transcriptome, Thermo Fisher) on an Ion Proton sequencer.

Microarray data of the renal biopsy samples from an independent cohort of ESRD (n=48) and healthy control subjects (n=8) samples were obtained from the NCBI GEO repository (Affymetrix HG-U133_Plus_2 arrays, GEO accession number: GSE66494). Re-annotation R packages with lncRNA Chip Definition Files (CDFs) were downloaded from GATExplorer website (http://bioinfow.dep.usal.es/xgate/principal.php).

Results

- 56,687,576 sequencing reads were obtained from 4 control and 4 ESRD plasma RNA samples, where 92.3% of the reads were mapped to the human genome.
- Among the 1,279 lncRNAs detected in human plasma, 85 were significantly dysregulated (20 up-, 65 down-regulated) with ESRD.
- Unsupervised hierarchical clustering **(Figure 1)** demonstrates that plasma lncRNA expression pattern distinguishes ESRD from control subjects.
- Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the dysregulated lncRNAs based on their neighboring mRNAs (cis-mRNA) revealed significant (corrected $P<0.01$) enrichment of genes in peroxisomes, the essential cellular component for detoxification process in the kidney.
- 20 out of these 85 cis-mRNAs are significantly dysregulated in ESRD, with 12 concordantly and 8 discordantly regulated with their neighboring lncRNAs, respectively. **(Table 1)**
- Among the 8 (lncRNA : mRNA) gene pairs that show discordant regulation, 5 are natural anti-sense transcript (NAT) lncRNAs and their target genes (SEPSECS, STXBP5, LYPLAL1, PSMD6, MBNL1, SOCS2), all of which are highly enriched in kidney tissue.

Figure 1. Differentially expression of plasma lncRNA in control and ESRD patients. (A) Heat map and hierarchical clustering of the differentially expressed plasma lncRNA. (B) Volcano plot of the differentially expressed plasma lncRNA. X-axis indicates fold change (ESRD/Ctrl), whereas Y-axis indicates *P* value

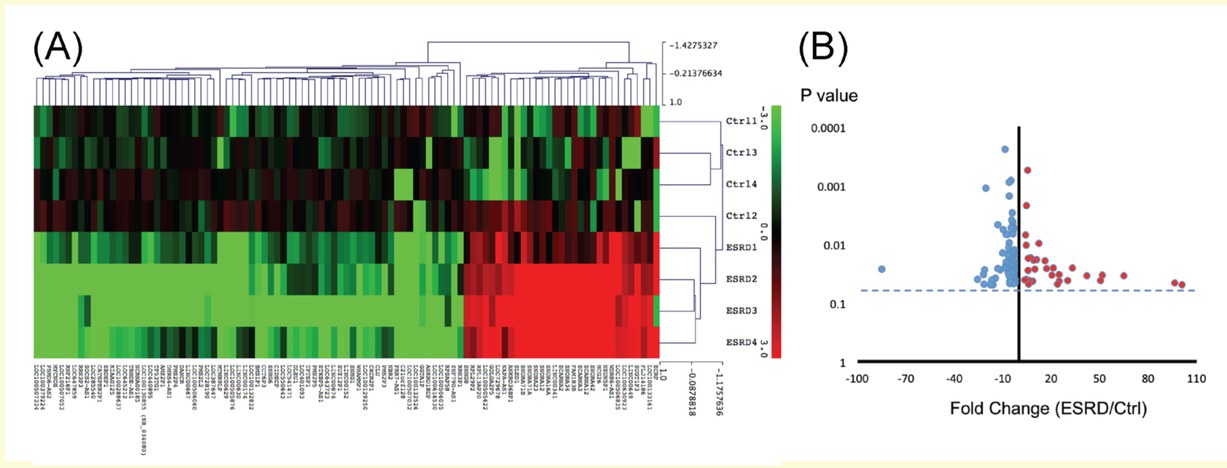

Table 1. Expression pattern and inter-relationship of ESRD-linked lncRNAs and their cis-mRNAs

lncRNA	Natural Anti-Sense Transcript?	Fold Change (ESRD vs Ctrl)	*P* value	cis-mRNA	cis-mRNA Fold Change (ESRD vs Ctrl)	*P* value	Relationship with lncRNA
AMZ2P1	No	-3.19	0.030	PRKRIP1	5.11	0.050	Discordant
LOC401093	No	-3.20	0.005	RPL13AP20	4.62	0.002	Discordant
LOC729678	Yes	12.30	0.010	SEPSECS	-7.42	0.007	Discordant
NSUN5P1	Yes	21.26	0.026	STXBP5	-4.05	0.013	Discordant
SCARNA12	Yes	30.19	0.042	LYPLAL1	-13.03	0.011	Discordant
SNORA16A	Yes	17.06	0.026	PSMD6	-3.33	0.029	Discordant
SNORA42	Yes	64.95	0.035	MBNL1	-2.55	0.050	Discordant
WDR86-AS1	Yes	51.58	0.034	SOCS2	-2.55	0.050	Discordant
BMS1P1	No	-8.45	0.024	CROT	-4.47	0.008	Concordant
CCT6P3	No	-8.07	0.007	CRLF3	-8.09	0.007	Concordant
CIDECP	No	-4.01	0.007	BRCA1	-4.02	0.007	Concordant
LINC00630	No	-7.20	0.026	TPST1	-24.11	0.001	Concordant
LOC100129250	No	-4.81	0.005	DANCR	-2.99	0.028	Concordant
LOC100505876	No	-21.45	0.027	LMLN	-8.17	0.001	Concordant
LOC100507032	No	-16.93	0.040	PDIA3	-6.14	0.004	Concordant
LOC100507053	No	-8.52	0.026	LIMS3	-2.95	0.008	Concordant
LOC541471	No	-3.87	0.035	PMS2L2	-3.88	0.034	Concordant
PMS2L2	No	-3.87	0.034	CLU	-2.71	0.016	Concordant
RNF216P1	No	-6.35	0.015	CIDEC	-4.02	0.007	Concordant
SNHG8	No	3.57	0.041	RNASE2	11.92	0.019	Concordant

- Microarray dataset obtained from an independent renal biopsy samples of 48 patients with chronic kidney disease (CKD) and 8 healthy control subjects (GSE66494) **(Table 2)** revealed that ~20% (16 out of 85) of the dysregulated plasma lncRNA with ESRD are similarly dysregulated in the kidney tissues from CKD patients.

Table 2. Dysregulated plasma lncRNAs in ESRD that are concordantly regulated in CKD kidney tissues

lncRNA	Fold Change in Plasma (ESRD vs Ctrl)	*P* value	Fold Change in Kidney Tissue (CKD vs Ctrl)	*P* value
GOLGA2P5	4.46	0.040	2.35	<0.001
SNHG8	3.57	0.041	5.59	<0.001
ECRP	23.82	0.048	3.47	<0.001
CLU	-2.71	0.016	-2.48	<0.001
LINC00152	-4.90	0.035	-2.34	<0.001
ANKRD18DP	-17.44	0.050	-3.27	<0.001
MYCNOS	-11.79	0.027	-2.76	0.001
KIAA0125	-6.05	0.001	-3.49	0.001
C21orf128	-13.23	0.026	-2.60	0.001
RNF216P1	-6.35	0.015	-1.55	0.001
LINC00667	-2.82	0.040	-0.55	0.002
DLEU1	-3.65	0.020	-2.18	0.005
TP53TG1	-6.19	0.010	-1.61	0.008
MTMR9LP	-16.73	0.042	-1.45	0.012
LINC00674	-2.74	0.013	-1.49	0.018
AMZ2P1	-3.19	0.030	-1.43	0.032

Conclusion

- **Circulating lncRNAs are dynamically regulated in ESRD.**
- **The changes in circulating lncRNAs not only reflect the disease status and the pathophysiology of renal failure, but also serve as a liquid biopsy that mirrors the renal noncoding transcriptome change with advanced kidney diseases.**

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