Supplemental Material

The Role of RBM25/LUC7L3 in Abnormal Cardiac Sodium Channel Splicing Regulation in Human Heart Failure

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Comparison of Microarray Gene Expression in Human Heart Failure Tissue

Clinical Specimens

The microarray study samples were composed of end-stage cardiomyopathy hearts (n=10) and nonfailing control hearts (n=6).[1] End-stage cardiomyopathy heart samples were obtained at the time of left ventricular assist device (LVAD) placement or cardiac transplantation. Subjects with end-stage cardiomyopathy exhibited severely reduced ejection fraction, left ventricular dilation, elevated pulmonary arterial and wedge pressures, and a reduced cardiac index. The control subjects were younger (median age 42 years with an interquartile range of 24–50 years) and predominantly male.

Data Analysis Methods

The GeneSifter gene expression microarray data analysis system was used to identify and compare significant differentially expressed genes from human (GEO accession: GSE1869)[1] heart failure tissue-derived gene expression data. The data were uploaded to GeneSifter by Batch Upload with the option to use Affymetrix probe IDs. No data points were missing from any

of the files. The z score was used as a measure of the significance of observed genes compared to a normal distribution. A z score was considered significant if it was > 2 or < -2, implying the genes were significantly over-represented or under-represented. Statistically significant gene changes were identified using an unpaired Student's t test, *p* value < 0.05 and a 5% Benjamini and Hochberg false discovery rate (FDR) correction. These settings are similar to those used by Kittleson et al.[1] A range of fold change cutoffs was used. For functional analysis, gene ontology (GO) reports were generated according to the method of Doniger et al.[2] Genes associated with RNA splicing were found under the biological process GO term "GO:0008380 RNA splicing". The upregulated splicing factors are listed in Table 1. No significant downregulation of splicing factors was observed. Hierarchical clustering of these genes across samples was done using the average correlation approach through Bioconductor (Fred Hutchinson Cancer Research Center). The heatmap (Figure 1) represents the relationship of genes and samples to one another with green representing downregulated and red representing upregulated genes relative to the mean expression value of each gene.

References

1. Kittleson MM, Minhas KM, Irizarry RA, Ye SQ, Edness G, Breton E, Conte JV, Tomaselli G, Garcia JGN and Hare JM. Gene expression analysis of ischemic and nonischemic cardiomyopathy: shared and distinct genes in the development of heart failure. Physiol Genomics. 2005; 21:299-307.

2. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC and Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol. 2003; 4:R7.

Results

Table 1. RNA splicing genes differentially regulated in human heart failure

Gene Name	Gene ID	Fold Change
Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)	SFPQ	2.05
Ubiquitin specific peptidase 39	Usp39	2.02
Quaking homolog, KH domain RNA binding (mouse)	QKI	1.91
Heterogeneous nuclear ribonucleoprotein H3 (2H9)	HNRPH3	1.86
Cisplatin resistance-associated overexpressed protein	LUC7L3	1.77
YTH domain containing 1	YTHDC1	1.77
Splicing factor, arginine/serine-rich 7, 35kDa	SFRS7	1.66
HLA-B associated transcript 1	BAT1	1.65
TAR DNA binding protein	TARDBP	1.61
Influenza virus NS1A binding protein	IVNS1ABP	1.61
DEAD (Asp-Glu-Ala-Asp) box polypeptide 1	Ddx1	1.60
Transformer-2 alpha	TRA2A	1.57
Peptidylprolyl isomerase G (cyclophilin G)	PPIG	1.55
RNA binding motif protein 39	RBM39	1.49
Splicing factor, arginine/serine-rich 11	SFRS11	1.49
RNA binding motif protein 25	RBM25	1.49
Heterogeneous nuclear ribonucleoprotein A1	HNRNPA1	1.49

Table 2. Primers for target genes

Gene	Primers
SCN5A	5'-TTACGCACCTTCCGAGTCCTCC-3'
	5'-GATGAGGGCAAAGACGCTGAGG- 3'
HSCN5AE28C/R	5'-TCTCTTCTCCCCTCCTGCTGGTCA-3'
HSCN5AE28D/R	5'-GGAAGAGCGTCGGGGAGAAGAAGTA-3'
RMB25	5'-TGTCTTTTCCACCTCATTTGAATCG-3'
	5'- ATTGGTACAGGAATCATTGGGGT-3'
LUC7L3	5'-GGACCAAGATCAGAACGTGTATTTG-3'
	5'-CAGTTGTTGGATGAGTTAATGGGC-3'
β-actin	5'-GGATCGGCGGCTCCAT-3'
	5'-CATACTCCTGCTTGCTGATCCA-3'

Table 3. Clinical information on heart failure tissue samples

Patient ID	Age	M/F*	RACE	CAD*	Beta Blocker	ACEI*	Antiarrhythmic	LVEF* (%)
HF-1	59	M	Black	Y	Y	Y	N	<20
HF-2	72	F	Black	N	Y	Y	N	<20
HF-3	42	F	Black	N	Y	N	N	<20
HF-5	75	M	White	Y	Y	N	N	<20
HF-6	65	M	White	Y	N	N	Y	<20
HF-7	70	M	White	Y	Y	N	Y	<20
HF-8	58	M	White	Y	Y	Y	N	<20
HF-9	58	F	Black	Y	Y	N	Y	<20
HF-10	34	M	Black	N	Y	Y	N	<20
HF-11	58	M	Black	N	Y	N	N	<20

***** M/F: male/female; CAD: coronary artery disease; ACEI: Angiotensin I-converting enzyme inhibitor; LVEF: left ventricular ejection fraction

Figure 1. Heatmap representing a hierarchical clustering of genes differentially regulated in human heart failure.