□ 외국 연수 연구발표(지상발표) □

Differential Renal Response to Volume Depletion, Ischemia, Nephrotoxin in Rats; Microarray Analysis

Jo, Sang-Kyung, MD, Yuen, Peter, PhD., Star, Robert, MD.

Renal Diagnostics and Therapeutics Unit, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Introduction

Ischemia/reperfusion injury or nephrotoxic insults are the most common cause of acute renal failure (ARF), a clinical condition still associated with a very high mortality and morbidity despite significant advances in supportive care, like hemodialysis or hemofiltration. The pathophysiology of ARF includes persistent vasoconstriction, injury to tubular cell, endothelial cell and inflammation. But the molecular mechanisms underlying these phenomema remain incompletely understood and hold important promise for possible therapeutic interventions.

Microarray technique combined with bioinformatic tool can provide the advantage of analyzing thousands of genes simultaneously, with the potential to uncover the new pathogenetic mechanisms in a diverse disease status.

In the present study, we employed oligonucleotide microarray and bioinformatics to determine the early changes in mRNA expression in rat models of ischemic and nephrotoxic ARF and also to find the novel genes that can serve as a biomarker or therapeutic target.

Methods

1. Animals

A total of 23, 6-8 wk old Sprague-Dawley rats were assigned to 7 different experimental groups; normal, volume depletion, sham, ischemia/reperfusion 2 hr, 8 hr, mercuric chloride 2 hr, 8 hr, respectively.

2. RNA isolation and microarray

Total RNA was isolated with TRI_{ZOL} reagent and subjected to further purification using an RNeasy minikit. We used total RNA prepared from 3-5 animals for each group and total 27 arrays were performed including 5 biological duplicates. Microarray analysis was performed according to the CodeLink Gene Expression Bioarray user guide. Briefly, after the synthesis of double strand cDNA, in vitro transcription reaction was done in the presense of biotin-UTP to produce biotin labeled cRNA. A total of 10 μg of fragmented cRNA product was used for hybridization reaction to CodeLink Rat Unigene Set (Amersham), containing 30-mer oligonucleotide corresponding to 9911 genes. The scanned data was processed by CodeLink Expression Analysis Software and each array was normalized by median centering.

3. Real time PCR

To validate the microarray data, Taqman^{R} quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR) was performed for 10 selected rat genes. 18S ribosomal RNA was used as an internal control to normalize all

the data. Abundance of each gene was determined relative to the abundance of 18S.

Results

1. Clustering of samples

The gene expression profiles of 27 samples were compared by the hierarchical clustering according to their patterns of expressions. In general, normal, sham operated and volume depleted animals clustered together on the basis of corresponding similarities in gene expression and all animals clustered into correct groups confiming that array can detect biological information in early time point after insults (Fig. 1). We performed the prinicipal component analysis (PCA) using 720 genes which passed that filtering criteria (ANOVA, $p<0.001$) and found that ischemia and nephrotoxic insults have a different injury trajectory (Fig. 2).

2. Differentially expressed genes

The most extensive regulation of gene expression occurred 8 hr after reperfusion. Almost 500

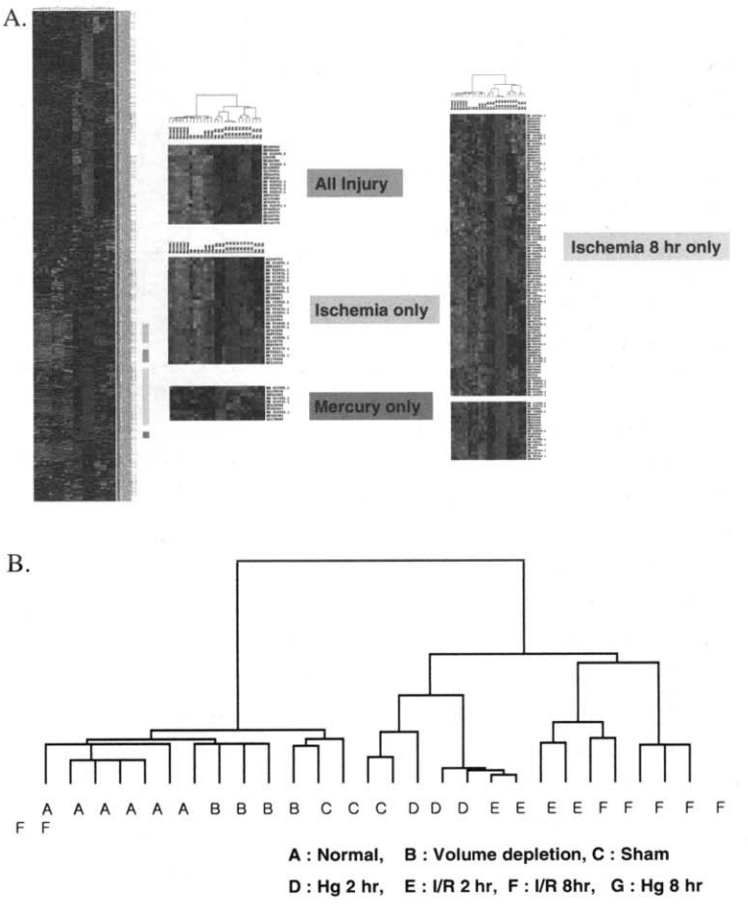

Fig. 1. *Hierarchical clustering of interesting genes.* ***(A)*** *Cluster analysis were performed on log transformed values of the fold ratio and presented with Treeview.* ***(B)*** *Dendrogram showing that all animals clustered into the correct groups.*

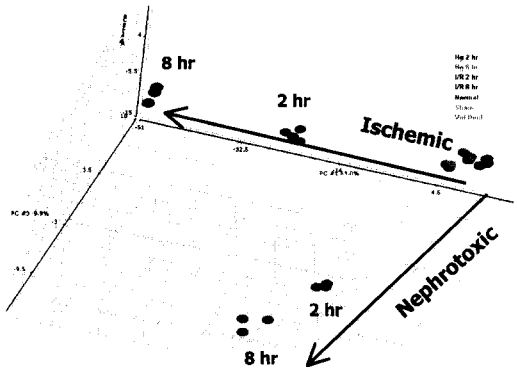

Fig. 2. *Principal component analysis. 720 genes selected by ANOVA were used for PCA. Ischemic and nephrotoxic insults have different injury trajectories.*

Individual Groups			Combinations		
Group	Hit	Exclusive Hit	Group	Hit	Exclusive
Sham	0	0	All conditions		26
VD	13	Not calc	Type		
IR 2 hr	224	51	I/R	165	107
IR 8 hr	737	496	Mercury	43	6
Hg 2 hr	74	11	Timing		
Hg 8 hr	160	32	Early injury	40	3
			Late injury	117	59

Fig. 3. *Differentially expressed genes in different types of insults at different time points. Hit : ANOVA and T-test <0.01 and 2-fold changes (up or down), Exclusive hit : hit not found in other groups.*

genes were found to be over or downregulated 2 fold or more. Of those lists, there were chaperone protein heat shock protein 70 (HSP70), stress gene heme oxygenase-1 (HO-1), transcription factors like Early Growth Response-1 (EGR-1), activating transcription factor-3 (ATF-3) and various inflammation related cytokine, chemokine, like interleukin-6 (IL-6), monocyte chemoattractant protein (MCP-1), growth regulated oncogene (Gro-1) which are already known. In contrast, there were only 160 genes differentially regulated in mercuric chloride groups and most of them are related to stress reponse or extracellar matrix related genes (Fig. 3, 4).

Because we were interested in finding of novel genes that can serve a new biological marker or therapeutic targets in various types of injury, we

Previously Identified

	I/R	Hg	2 hr	8 hr
HSP 70	✓	✓	✓	✓
HOX-1	✓	✓	✓	✓
MCP-1	✓			✓
IL-6	✓		✓	✓
Clusterin	✓	✓		✓
Endothelin	✓		✓	✓
ATF-3	✓	✓	✓	✓
Kim-1	✓			✓
EGR-1	✓	✓	✓	✓
EGR-2	✓	✓	✓	✓
CYR61	✓		✓	

All Genes

	All	I/R	Hg	2 hr	8 hr
TF	6	6			
Stress	3				1
Metabolic	2	3			7
Inflammation		10			
Cell signaling	2	6			
Cytoskeleton		2			
ECM			1		1
Growth factor		1		1	
Others	5	7	2		8
EST	8	13	3	2	40

Fig. 4. *Classification of hits.*

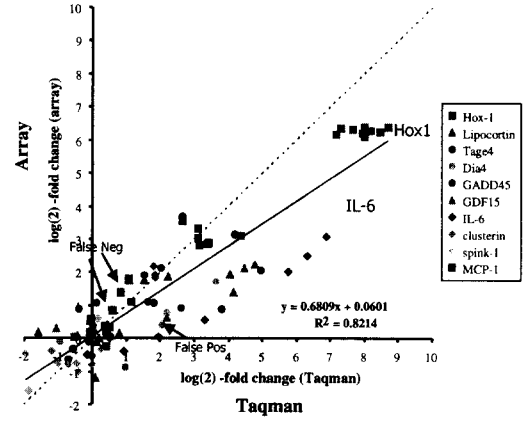

Fig. 5. *Validation by Taqman RT-PCR. Same RNA samples were used and plotted as log2 fold compared to normal. Array value showed directional accuracy, but some genes were more compressed in array (eg : IL-6).*

analysed 26 genes those are differentially regulated in all injury groups. In addition to previously reported genes, like HSP-70, HO-1, we found several genes that has not been described previously. Of those, EGR-1 has been lately identified as a master switch in regulation of diverse group of gene families mediating the injury in lung ischemia and canbe a therapeutic target in kidney injury.

3. Validation

To confirm the microarray data, we chose 10 up or downregulated genes and perform a real time quantitative PCR using Taqman® probe and

found a directional accuracy in 10 genes we measured (Fig. 5).

Conclusion

In this study, we report first that ischemia and nephrotoxic ARF can be clealy distinguished by patterns of gene expression early after renal injury and also several common genes upregulated throughout both types of injury may serve as early biomarker or therapeutic targets.