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Analysis of *FOS*, *BTG2*, and *NR4A* in the function of renal medullary hypertension

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ABSTRACT. The aim of this study was to identify differentially expressed genes (DEGs) in renal medullary hypertension and reveal their pathogenic mechanisms. We downloaded the gene expression profile of GSE28360 from the Gene Expression Omnibus database. The profile included 14 samples (5 normal and 9 hypertension). The DEGs in normal and disease samples were distinguished with a false-discovery rate threshold of <0.05 and a fold-change value of >2 or <-2. We put the selected genes into the online program String 8.3 to obtain the protein-protein interaction network and selected the hub proteins. These hub proteins were then placed in the PANTHER database to determine hub protein-related pathways and explain their functions. Finally, we cleared up the single-nucleotide polymorphisms (SNPs) of the hub genes via combing with the National Center for Biotechnology SNP database. A total of 13 genes were identified as DEGs between normal and disease samples. Five selected hub proteins, B-cell translocation gene 2 (BTG2), FBJ murine osteosarcoma viral oncogene homolog (FOS), nuclear receptor subfamily 4, group A, member 1 (NR4A1), NR4A member 2 (NR4A2), and NR4A member 3 (NR4A3), were mainly related to angiogenesis and B-cell activation. After SNP analysis, 103, 103, 595, 150, and 493 SNPs were found to correspond

to *BTG2*, *FOS*, *NR4A1*, *NR4A2*, and *NR4A3*, respectively. Our results suggest that pathways of angiogenesis and B-cell activation may involve in the progression of renal medulla hypertension.

Key words: Differentially expressed gene; Hub protein; Renal dysfunction; Renal medullary hypertension

INTRODUCTION

Renal dysfunction has been a recognized feature of essential hypertension since the 19th century. An enormous amount of accumulated data from humans and experimental animal models supports the view that renal dysfunction underlies the development of all forms of hypertension (Norman Jr et al., 1978; Hall et al., 1984; Hall et al., 1986; Mattson et al., 1991). Subsequent studies have also shown that the renal medulla plays an important role in sodium and water homeostasis and the long-term control of arterial blood pressure.

Despite this overwhelming body of evidence, the view that renal dysfunction underlies the development of all forms of hypertension is not universally accepted (Cowley et al., 1995). The mechanism of the renal medulla in the initiation and development of hypertension has been suggested by two observations. One observation made in the 1960s by Dr. Eric Muirhead described medullary interstitial cells producing an antihypertensive principle (Cowley Jr, 1994). The other observation was made in the mid-1980s by Allen et al., who reported that renal medullary blood flow was reduced in several forms of experimental hypertension (Cowley Jr, 1992; Cowley Jr and Roman, 1996). Our study revealed the relationship between hypertension and the renal medulla using a bioinformatics method.

Systems biology approaches such as network-based methods have recently been successfully applied to elucidate the mechanism of diseases (Ideker and Sharan, 2008; Zhao et al., 2008). With the rapid development of systems biology, bioinformatics analysis for renal medullary hypertension has the potential to improve understanding of the complexity of molecular pathways underlying hypertension and help uncover the processes of disease initiation.

In this study, we aim to investigate the molecular mechanism of renal medulla hypertension. Initially, we identified differentially expressed genes (DEGs) and used them as the basis for a protein-protein network. We then placed 5 hub proteins into PANTHER online (Protein Analysis through Evolutionary Relationships) for further analysis and revealed 11 bio-pathways in which they participated. Finally, we researched the single-nucleotide polymorphisms (SNPs) of these 5 hub proteins in the National Center for Biotechnology (NCBI) SNP database (dbSNP) to understand the molecular mechanism of renal medullary hypertension.

MATERIAL AND METHODS

Processing and analysis of Affymetrix microarray data

We downloaded GSE28360, which included 14 specimens (Marques et al., 2011) (5 normal and 9 renal medulla hypertension expression samples) from the Gene Expression Omnibus database based on GPL6244 ([HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array). The data were preprocessed with the Affy package R language (Fujita et al., 2006). The differ-

ential expressional value between the hypertensive renal medulla and the normal renal medulla specimens was calculated with the limma package (Smyth, 2005), and multiple testing correction was performed with Bayesian analysis (Benjamini and Hochberg, 1995). Genes with a false discovery rate of <0.05 and fold change values of >2 or <-2 were selected as DEGs.

Screening of hub proteins

The selected DEGs were analyzed with String 8.3 software (Szklarczyk et al., 2011b), which identifies interactional gene pairs by calculating the combined score between the DEGs for gene sequence characteristics and physicochemical properties. Based on this information, we constructed a protein-protein network and screened the hub proteins with the highest number of interactions (Szklarczyk et al., 2011a).

Exploring bio-pathways

The hub proteins were placed in the PANTHER database (PANTHER classification system, http://www.pantherdb.org/) (Mi et al., 2010) to explore the bio-pathway in which the hub proteins participate ($P < 0.05$). PANTHER classifies genes by their functions using published scientific experimental evidence and evolutionary relationships to predict function even in the absence of direct experimental evidence. With the increase in the amount of protein data available, detailed biological interactions in bio-pathways have been displayed via interactive methods. The system searches primarily signaling pathways, molecular functions, and biological processes.

Searching SNPs of hub proteins

We identified SNPs of hub proteins using gene SNP information in the NCBI dbSNP (Sherry et al., 2001).

RESULTS

Screening DEGs of the renal medulla in hypertension and normal samples

After pre-processing the data - that is, standardizing expression profiling data to a high degree - we screened 13 DEGs (false discovery rate < 0.05, |logFC| > 2). One of them was a downregulatory gene, and others were upregulatory genes (Table 1).

Constructing the protein-protein network

String 8.3 was used to calculate the co-expression value of DEGs, and 9 co-expression pairs including 5 genes were obtained because their combined scores were >0.5 (Table 2 and Figure 1). As shown in Figure 2, the hub genes *FOS* (FBJ murine osteosarcoma viral oncogene homolog), *BTG2* (B-cell translocation gene 2), *NR4A1* (Nuclear receptor subfamily 4, group A, member 1), *NR4A2* (NR4A member 2), and *NR4A*3 (NR4A member 3) had co-expression relationships, and their connective nodes were >3. Therefore, we selected these genes for further study.

Table 1. DEGs of renal medulla between the hypertension and normal samples.

Gene symbol	FDR Value	LogFC
LOC162632	0.014082	-1.11
FOS	0.027986	1.04
DOC2B	0.013091	1.05
BTG2	0.023562	1.07
PDK4	0.030155	1.07
CFB	0.026141	1.1
KGFLP2	0.00818	1.1
SPCS2	0.018977	1.1
HBEGF	0.00629	1.17
CFC1	0.036731	1.25
NR4A1	0.002643	1.53
NR4A3	0.005428	1.58
NR4A2	0.01389	1.63

Table 2. Combined score of hub proteins.

node1	node2	Combined score
NR4A2	FOS	0.86
BTG2	NR4A1	0.801
NR4A3	NR4A1	0.67
NR4A2	BTG2	0.681
NR4A3	BTG2	0.553
NR4A2	NR4A1	0.972
NR4A2	NR4A3	0.745
FOS	NR4A1	0.896
FOS	BTG2	0.758

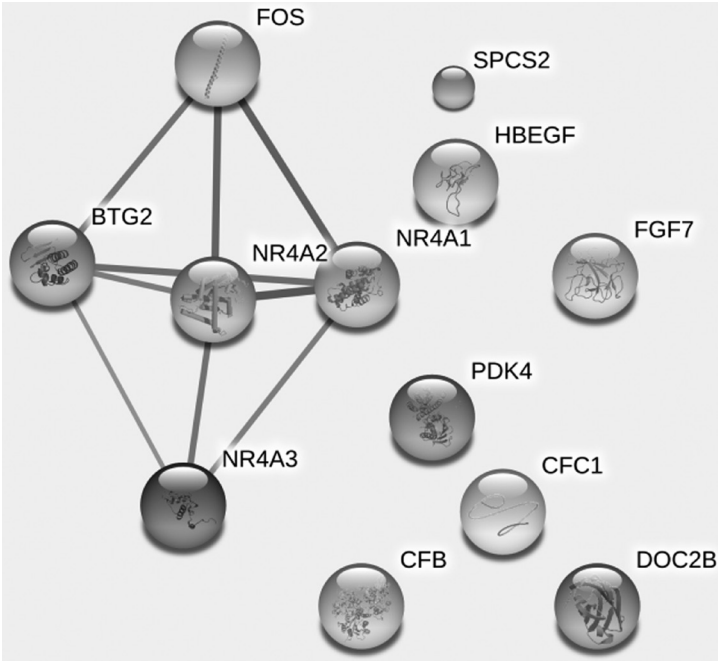

Figure 1. Protein-protein network of DEGs.

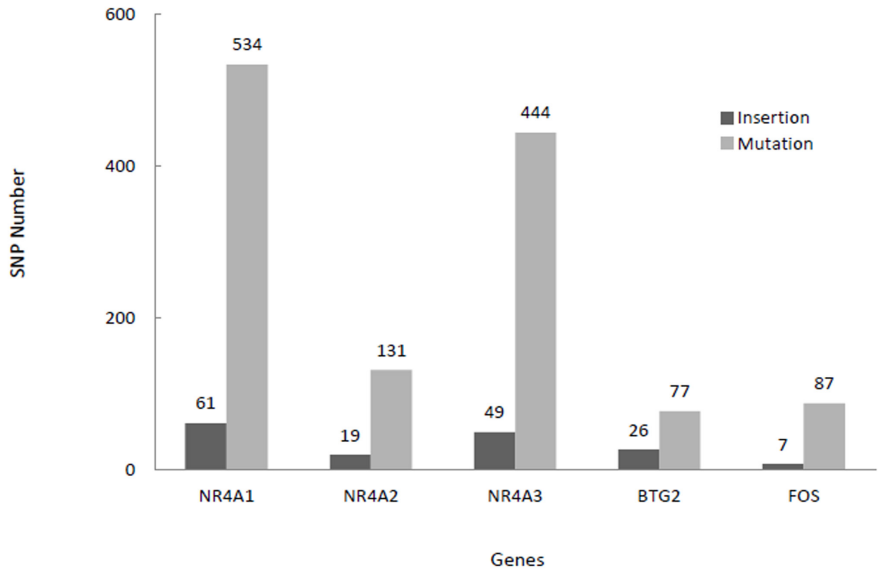

Figure 2. SNP charts of hub proteins. Dark gray and light gray columns represent insertion and mutation, respectively.

Bio-pathway analysis

Five hub proteins were placed into PANTHER online for further analysis. A total of 11 bio-pathways in which they participate were identified. These pathways are related to angiogenesis and B-cell activation (Table 3). *FOS* and the *NR4A* family were related to angiogenesis, whereas *BTG2* was related to B-cell activation.

Table 3. Bio-pathways of 5 hub proteins.

ID	Pathway	Components	P	Genes
P00005	Angiogenesis	77	1.76E-07	FOS, NR4A
P00010	B cell activation	36	2.70E-06	BTG2
P05918	p38 MAPK pathway	36	1.94E-03	FOS
P00029	Huntington disease	60	3.18E-02	FOS

Searching hub protein SNPs

The SNPs of 5 hub proteins were found in the NCBI dbSNP. *FOS*, *BTG2*, and *NR4A1*, *NR4A2*, and *NR4A3* had 103, 103, 595, 150, and 493 SNPs, respectively, which were divided into 2 subtypes of insertion and mutation (Figure 2). Mutation SNPs in each gene accounted for the major proportion. The mutations C\T, A\G, A\G, A\G, and C\T in *FOS*, *BTG2*, and *NR4A1*, *NR4A2*, *NR4A3* were most frequent.

DISCUSSION

The evidence summarized above establishes that the renal medulla plays an important role

in sodium and water homeostasis and long-term control of arterial blood pressure. The analysis was based on samples of hypertensive and normotensive renal medulla data. Through the analysis of functional genes, we found that the *NR4A* family, *FOS*, and *BTG2* were overexpressed in the renal medullary tissue samples of hypertensive patients.

Some researchers have concluded that *FOS* is a cellular proto-oncogene belonging to the immediate early gene family of transcription factors (Monje et al., 2005). *FOS* interacts with *C-jun* to upregulate the transcription of a diverse range of genes involved in biological processes from proliferation and differentiation to defense against pathogen invasion and cell damage (Bossis et al., 2005). FOS is a potent mitogen in endothelial cells that, and it also marginally enhances the proliferation of and increases alkaline phosphatase in human dental pulp cells (Matsushita et al., 2000). However, these effects are not observed in human skin fibroblasts. We found that *FOS* was closely to angiogenesis, which is a new discovery of *FOS* function. *FOS* was overexpressed in hypertensive renal medulla tissue samples, suggesting that it could stimulate the renal medulla pipeline to generate pressure.

Recently, members of the *NR4A* family have been implicated in the control of skeletal muscle metabolism (Pearen et al., 2006, 2008). This gene family includes *NR4A1*, *NR4A2*, and *NR4A3* (Maheux et al., 2005; Maxwell and Muscat, 2006). Early functional studies have described a critical role of *NR4A* receptors in regulating differentiation, proliferation, and apoptosis. Notably, these genes are involved in the transcriptional control of metabolism and vascular remodeling (Zhao and Bruemmer, 2010). Many studies have proved that 3 MR4A genes are expressed in developing neointima and advanced atherosclerotic lesions. Emerging evidence also indicates that their function in vascular biology centers on inflammation, proliferation, apoptosis, thrombosis, and angiogenesis. We have now used a bioinformatics method to prove that the *NR4A* family functions in angiogenesis, and the overexpression of NR4A genes controls cell viability, proliferation, and inflammation.

BTG2, also known as BTG family member 2 or nerve growth factor-inducible anti-proliferative protein PC3 or nerve growth factor-inducible protein TIS21, is a protein that, in humans, is encoded by *BTG2* (Rouault et al., 1996). *BTG2* was previously identified as an antiproliferative gene, and its precise biological functions remain to be elucidated. *BTG2* expression is induced *in vivo* during neurogenesis and is associated with neurogenic asymmetric division in neural progenitor cells (Iacopetti et al., 1999). The discovery that *BTG2* is related to B-cell activation using a bioinformatics method in this study proves the role of such techniques in revealing disease mechanisms. However, this conclusion needs further verification.

Over-expression of the *NR4A* family, *FOS*, and *BTG2* cause functional abnormalities and lead to the development of hypertensive disorders. Our results suggest that these genes may be targets and diagnostic markers for renal medullary hypertension or vascular disease. During drug design, relevant SNPs can be referenced in the NCBI dbSNP database. C\T, A\G, A\G, A\G, and C\T were the important mutations in *FOS*, *BTG2*, and *NR4A1*, *NR4A2*, and *NR4A3* and can be considered a renal medullary hypertension disease guide.

Although at present the understanding, and treatment of renal medullary hypertension have significantly improved, further understanding of the disease and standard treatments are desirable future pursuits.

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