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Screening of genes related with intervertebral disc disease by dynamic differential interaction network analysis

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Abstract. – AIM: Gene expression profiles for intervertebral disc (IVD) cells treated with different osmolarities were compared to identify key genes associated with intervertebral disc diseases.

MATERIALS AND METHODS: Microarray data was downloaded from Gene Expression Omnibus (GEO) database and pre-processed using package of R. Gene co-expression was determined with Pearson correlation coefficient. Interaction networks were established with the protein-protein interaction (PPI) information obtained from Human Protein Reference Database (HPRD database) for the two conditions: isosmoticity and hyperosmosis, and then a comparative analysis was done to identify disease-related genes. The functional annotation was performed for these genes using network ontology analysis (NOA), which also confirmed the effectiveness of this method.

RESULTS: A total of 45 feature genes were obtained through comparing 7 samples treated under isosmotic conditions and 9 high osmotic conditions. Biological processes and molecular functions were then revealed by NOA.

CONCLUSIONS: A range of disease-related genes were obtained, which might serve as the potential biomarkers or drug targets. More works are needed to further elucidate their roles in the development of intervertebral disc diseases like intervertebral disc herniation.

Key Words:
Intervertebral disc, Differential co-expressed genes, Co-expressed network, Disease-related genes.

Introduction

Intervertebral disc (IVD) is located between vertebrae of the human spine, and comprised of cartilage, annulus fibrosus and nucleus pulposus. And the nucleus pulposus contains mucopolysaccharide protein complexes, chondroitin sulfate and water (60%-90% of total weight)[1]. Alterations occur in IVD in response to various external stimuli, especially altered osmotic pressure, which leads to hydration in IVD. And long-term exposure to high osmotic pressure results in intervertebral disc herniation (IDH).

Changes in a series of genes and proteins rise up in response to hyperosmotic stimuli[2-6]. Previous study has indicated that vitamin D receptor is associated with IDH[7]. The polymorphisms of this gene is linked with a variety of disease, such as osteoporosis, osteoarthritis, cancer, and cardiovascular disease[8]. Sox9 gene therapy was also proposed to treat intervertebral disc disease[9]. However, generally, there was few genes related with IDH have been revealed. Therefore, microarray technology was adopted to observe gene expression in intervertebral disc cells under isosmotic conditions and high osmotic conditions were compared in present study to identify feature genes implicated in the IDH, which may be developed into biomarkers or drug targets.

As we know, interactions in multiple genes or pathways are necessary to fulfill biological functions and diseases rise from alterations in a range of genes or pathways, which impose challenges to identification of disease-related genes[10-11]. Several methods have been developed[12-13], but these methods focus on differential expression analysis and ignore the dynamic interactions between proteins. Comparative analysis about interaction networks reflecting disease state and normal state takes advantages in disclosing the mechanisms. Therefore, such method was set up in present study and several IDH-related genes were identified from a previously collected microarray data set.

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Materials and Methods

Microarray Data and Protein-Protein Interaction

Microarray data set GSE1648[14] including IVD samples under different osmotic pressures was downloaded from DEO database (http://www.ncbi.nlm.nih.gov/geo/). Human Genome U133A array (Affymetrix, Santa Clara, CA, USA) was used to obtain gene expression profile. A total of 11 samples were contained in the data set, 4 isosmoticity and 7 hyperosmosis. Package Robust Multiarray Average (RMA)[15] of *R* was chosen for data normalization.

Information about protein-protein interactions (PPI) in Homo sapiens were downloaded from Human Protein Reference Database (HPRD) database (http://www.hprd.org)[16-17]. Self-interactions and duplicate one were removed and finally 37080 interactions among 9465 proteins were retained.

Differential Analysis of Gene Co-Expression Networks

As we know, co-expressed genes are often subject to common regulation and participate in the same or similar biological processes and pathways[18]. Thus, information on gene co-expression was extracted with Pearson correlation coefficient from the gene expression data of the samples under isotonic and hyper-osmotic conditions, respectively.

Then PPIs was screened with the co-expression information. Considering that Protein-Protein Interactions (PPIs) with low correlation coefficient don't exist in corresponding samples, those with high correlation coefficient were retained. Therefore, PPINs (PPI Networks) were established for samples under different osmolarites.

The PPIs shared by both networks were eliminated and the altered PPIs were thus uncovered, which indicated the different states of the IVD.

Screening of Genes Related With IDH

Differential PPIs in disease sample and controls were collectively referred to as disease-related interactions, indicating the dynamic changes in regulatory network. Genes in these interactions might play important roles in the development of disease and thus were called potential disease-related genes.

Validation and Functional Annotation For the Potential Disease-Related Genes

Validation of the potential disease-related genes was performed on the basis of the Cancer-Genes database[19]. Then these genes were divided into two groups: up-regulated and down-regulated. NOA (Network Ontology Analysis)[20] was adopted for functional annotation.

Results

In the organism, the interactions between multiple proteins or pathways are the basis of biological process. In present study, differential PPIs between disease samples and normal samples were identified. Genes involved in these interactions are potential biomarkers or drug targets, and further studies may pave the way for their applications in diagnosis and treatment of IDH.

Differential PPIs and Potential Disease-Related Genes

PPIN for samples from isotonic conditions is shown in Figure 1 and that for samples from high osmotic conditions is shown in Figure 2. Differential protein-protein interactions were obtained through comparison. Genes included in these interactions and shared in both groups were regarded as potential IDH-related genes.

From Table I, it was clear that the number of gene co-expression decreased obviously in IDV samples under high osmotic conditions compared those under isotonic conditions. Therefore, we speculated that alterations occurred in protein levels and structures due to hyperosmosis, which in-

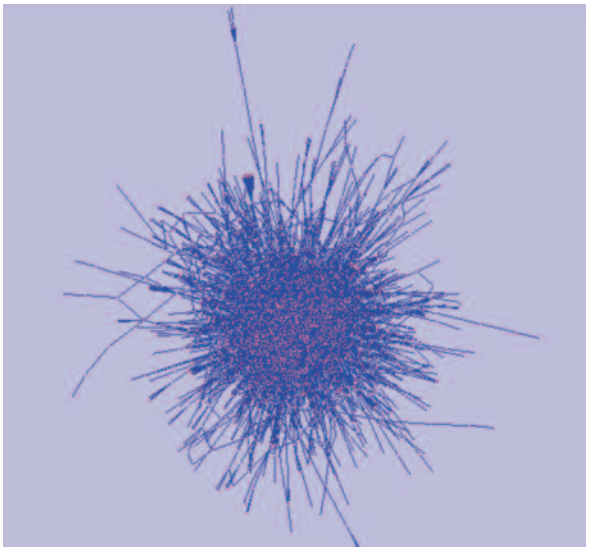

Figure 1. The PPIN for samples treated with isotonic conditions.

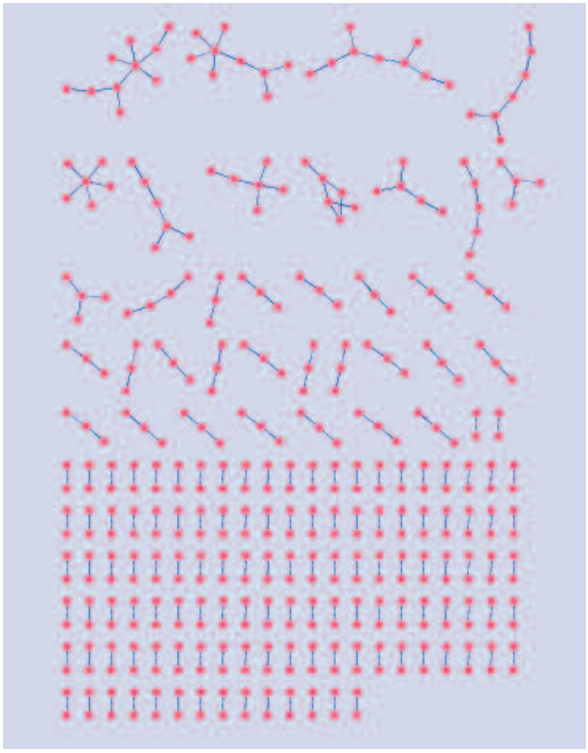

Figure 2. The PPIN for samples treated with high osmotic conditions.

terrupted the biological processed and finally lead to the incidence of IDH.

Screening of IDH-Related Genes

As the differential interactions were acquired, a total of 303 genes shared in both states were screened out. Expression levels for these genes were extracted from the 11 microarray data and normalized. Differential expression analysis was conducted with SAM method and 45 differentially expressed genes (DEGs) were finally revealed, 21 up-regulated and 24 down-regulated (Supplementary Table I).

Functional Annotation for ADH-related Genes

The significantly over-represented molecular functions and biological processes were for the up- and down-regulated genes are shown in Tables II, III.

Discussion

Alterations in a range of genes or pathways occur in the initiation and development of complicated disease. Identifying these perturbations with a systematic view is helpful in diagnosis and treatment of corresponding disease[21]. In present study, we proposed a new method based up on differential network analysis, differing from traditional differential expression analysis[22-23]. Finally, a range of genes related with IDH were obtained.

S-phase kinase-associated protein 1 (SKP1) is a component of SCF complexes[24], which are implicated in the regulated ubiquitination of protein substrates and, thus, the degradation by proteosome. Accordingly, proteasome subunit beta 5 (PSMB5) and proteasome subunit alpha 4 (PSMA4) were also up-regulated. Previous studies have reported its roles in cell fate determination[25]. It up-regulation in samples treated with high osmotic pressure suggested that protein profile

Table I. The basic information of the PPINs and disease-related genes.

	Isosmoticity		Hyperosmosis
Interactions	5628		451
Nodes	3661		570
Differential interactions		5273	
Overlapped genes		303	
Candidate genes		45	

Supplementary Table I. ADH-related genes.

Up-Regulated Genes	Down-Regulated Genes
SGK1	SKP1
HLA-G	APEX1
TUBB	ID3
GNAS	ANXA2
CD44	ACTG1
PPIA	SDC2
CD59	COL4A2
BSG	HNRNPA1
SPTAN1	COL1A1
GAPDH	LIMS1
DLG1	PSMB5
ILK	EGFR
YWHAZ	HDAC2
BAT3	ZMYND11
YWHAQ	POLR2B
SFPQ	AIMP2
ITGB1	KHDRBS1
PTGES3	PSMA4
POLR2A	FYN
LDLR	TMED10
SHC1	TXN
	SNAP23
	EEF1A1
	PFN2

Table II. Functional annotation for up-regulated ADH-related genes biological process.

GO: term	Corrected *p*-value	Term
GO:0007160	0.0705	Cell-matrix adhesion
GO:0031589	0.1256	Cell-substrate adhesion
GO:0001658	0.3859	Branching involved in ureteric bud morphogenesis
Molecular function		
GO:0005515	0.0022	protein binding
GO:0003968	0.1731	RNA-directed RNA polymerase activity
GO:001512	0.3460	lactate transmembrane transporter activity
GO:0004365	0.3460	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity
GO:0008943	0.3460	glyceraldehyde-3-phosphate dehydrogenase activity

Table III. Functional annotation for down-regulated ADH-related genes biological process.

GO: term	Corrected *p*-value	Term
GO:0009987	0.0068	cellular process
GO:0048519	0.0676	negative regulation of biological process
GO:0051437	0.0913	positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle
GO:0031324	0.0960	negative regulation of cellular metabolic process
GO:0006903	0.0981	vesicle targeting
GO:0051439	0.1044	regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle
GO:0010605	0.1116	negative regulation of macromolecule metabolic process
GO:0051443	0.1138	positive regulation of ubiquitin-protein ligase activity
GO:0051351	0.1289	positive regulation of ligase activity
GO:0051438	0.1690	regulation of ubiquitin-protein ligase activity
Moleculalr function		
GO:0005515	1.6E-5	protein binding
GO:0005546	0.0384	phosphatidylinositol-4,5-isphosphate binding
GO:0004298	0.0668	threonine-type endopeptidase activity
GO:0070003	0.0668	threonine-type peptidase activity
GO:0008092	0.1351	cytoskeletal protein binding
GO:0016564	0.2075	transcription repressor activity
GO:0004710	0.4441	MAP/ERK kinase kinase activity
GO:0005006	0.4441	epidermal growth factor receptor activity
GO:0042802	0.4520	identical protein binding

changed greatly in response to this stimulation. APEX nuclease 1 (APEX1) is involved in DNA repair and it was also up-regulated, indicating increased frequency of DNA damage imposed by hyperosmosis. Syndecan 2 (SDC2) is a member of the syndecan proteoglycan family that mediate cell binding, cell signaling, and cytoskeletal organization. The up-regulation implied significant changes in several aspects. Changes in extracellular matrix organization were expectable and confirmed by the up-regulation of annexin A2 (ANXA2), collagen IV alpha 2 (COL4A2) and collagen I alpha 1 (COL1A1). Several proteins associated with protein transport were up-regulated, such as synaptosomal-associated protein (SNAP23) that implicated in vesicular transport[26], and transmembrane emp24-like trafficking protein 10 (TMED10) that involved in vesicular protein trafficking[27]. This might be responsible for changes in extracellular matrix.

A number of down-regulated genes were also obtained. CD44 is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration[28]. CD59 is a cell surface glycoprotein that regulates complement-mediated cell lysis, and it is involved in lymphocyte signal transduction. Down-regulation in these genes might be associated with decreased defense response, suggesting that cell under high osmotic pressure pay less "attention" in this function. Cells might exhibit a lower degree in cell adhesion as integrin beta 1 (ITGB1, a subunit of integrin)[29] was down-regulated. Integrin-linked kinase (ILK) takes a part in regulation of integrin-mediated signal transduction[30] and it was down-regulated, indicating a less degree of cell communication. Another typical down-regulated protein was discs large homolog 1 (DLG1), which plays a role in junction formation[31], signal transduction, cell proliferation and lymphocyte activation[32]. The declining activities in cell adhesion, cell communication and defense reactions are signs of degradation, which is closely related with IDH[33-34].

The most significantly over-represented molecular functions for both up- and down-regulated

genes are same as protein binding, in accordance with our speculation that alterations in interaction network are responsible for disease development. In addition, quite a few of proteins are found to be located in extracellular matrix and plasma membrane, not only suggesting their potential key roles, but also showing their promising applications for diagnosis and treatment.

Conclusions

Our study well deciphered the dynamic changes in PPIN and many genes associated with IDH were uncovered. Further researches are necessary to advance their clinical applications. Moreover, interactions between transcription factors and genes, miRNAs and genes, and so on are good points to understanding the regulatory mechanisms.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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