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Screening of differentially expressed genes in pathological scar tissues using expression microarray

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ABSTRACT. Pathological scar tissues and normal skin tissues were differentiated by screening for differentially expressed genes in pathologic scar tissues via gene expression microarray. The differentially expressed gene data was analyzed by gene ontology and pathway analyses. There were 5001 up- or down-regulated genes in 2-fold differentially expressed genes, 956 up- or down-regulated genes in 5-fold differentially expressed genes, and 114 up- or down-regulated genes in 20-fold differentially expressed genes. Therefore, significant differences were observed in the gene expression in pathological scar tissues and normal foreskin tissues. The development of pathological scar tissues has been correlated to changes in multiple genes and pathways, which are believed to form a dynamic network connection.

Key words: Pathological scar tissue; Differentially expressed gene; Gene chip

INTRODUCTION

Scar tissues are the end result of skin wound healing; however, uncontrolled synthesis and metabolism of skin collagen during the process of healing results in a continuous hyperthyroidism state, which leads to hyperplasia of the collagen fiber. This results in the formation of a pathological scar, which is characterized by a big protrusion out of the skin, an irregular shape, and the formation of red, hard benign masses. The formation of pathological scar is closely related to abnormal gene expression in the pathological scar tissue. In this study, Genechip U133 Plus 2.0 gene expression microarray was used to compare the differential expression of genes in pathological scar and normal skin tissues; this was achieved by screening for differentially expressed genes in both types of tissues, and conducting a preliminary identification of the function of these genes.

MATERIAL AND METHODS

Clinical data

The total number of patients was 8 and was divided into two groups: the pathological scar group (3 cases) and the normal skin group (5 cases). Pathological scar tissue samples were obtained from patients with pathological scars treated at the Department of Dermatology in the People's Liberation Army (PLA) General Hospital. The scar tissue samples were cut and sent to the Department of Pathology at the PLA General Hospital, for a formal diagnosis of pathological scars. The normal skin tissues were obtained from patients undergoing circumcision at the Department of Urology at PLA General Hospital. Informed consent was obtained from all patients who provided samples (or who were operated on). The case data has been summarized in Table 1.

Table 1. Patient characteristics.

No.	Gender	Age	Donor site	Diagnosis	Disease history (years)
1	Male	35	Chest	Folliculitis	1
2	Male	41	Chest	Furuncle	2
3	Male	29	Chest	After surgery	1.6

Screening for differentially expressed genes

The ratio of signal value was used to analyze the chip in order to screen for differentially expressed genes. The Genechip was scanned by laser scanning confocal microscope (OLS4100, Olympus Imaging Co., Ltd, China). The ratio of signal value was analyzed online at http://rana.lbl.gov/EisenSoftware.htm. The differential expression was analyzed online at http://cybert.microarray.ics.uci.edu. Those whose ratio was above 50 times were considered as up-regulation, while those below 50 times were considered as down-regulation. The *t*-test was used for analysis and a value of $P < 0.05$ was considered to be significantly.

Analysis of differentially expressed genes with Gene Ontology (GO) and pathway analyses

The GO analysis was conducted using the DAVID Bioinformatics Resources 6.7 software platform, provided by the United States National Institute of Allergy and Infectious Diseas-

es; on the other hand, pathway analyses were performed online at http://david.abcc.ncifcrf.gov/.

RESULTS

RNA integrity

The obtained RNA samples gave clear bands when subjected to formaldehyde denaturing gel electrophoresis. The band brightness of 28S:18S rRNA was greater than or close to 1:1. The quality of the bands was in line with the basic requirement of the Affymetrix expression microarray system (Figure 1A and B).

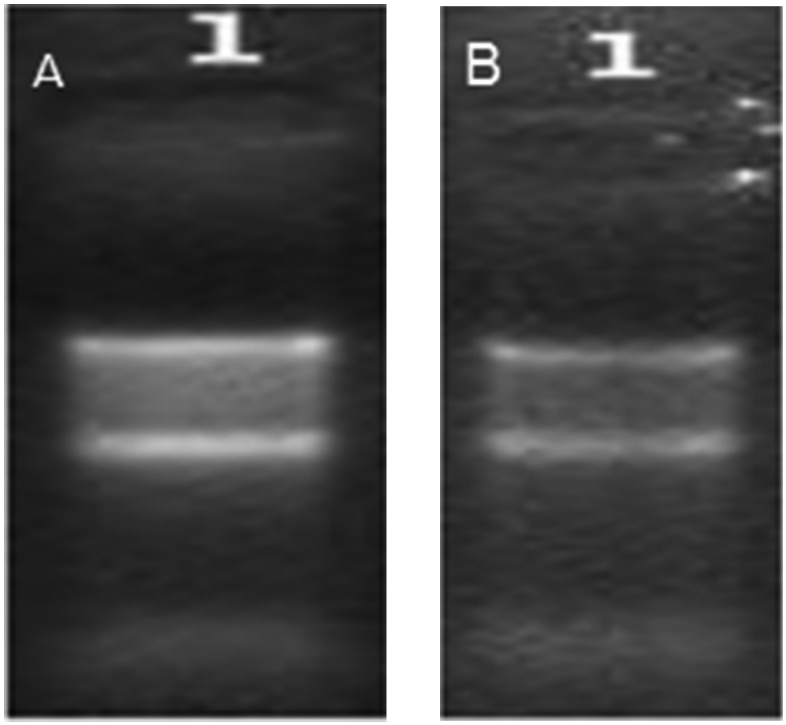

Figure 1. Electrophoresis strip of RNA extracted from normal foreskin tissue (**A**), and pathological scar tissue (**B**) from the chest of the patient.

Results of screening for differential expression of genes

The ratio of signal value was used to screen the whole gene expression profile obtained using the Genechip U133 plus 2.0 microarray. The pathological scar tissue showed differential expression of genes compared to normal human foreskin tissue. There were 5001 up- or down-regulated genes in 2-fold differentially expressed genes (2942 up and 2059 down) and 956 up- or down-regulated genes in 5-fold differentially expressed genes (699 up and 357 down). There were 144 up- or down-regulated genes in 20-fold differentially expressed genes (89 up and 55 down) and 38 up- or down-regulated genes in 50-fold differentially expressed genes (19 up and 19 down).(Tables 2 and 3).

Table 2. List of differentially expressed genes in the pathological scar tissue up-regulated by more than 50 times compared to the normal foreskin tissue.

Probe Set ID	Ratio	Gene Title	Gene symbol
213790_at	394.753	ADAM metallopeptidase domain 12	*ADAM12*
202952_s_at	340.571	ADAM metallopeptidase domain 12	*ADAM12*
204320_at	326.908	Collagen, type XI, alpha 1	*COL11A1*
226777_at	270.562	-	-
37892_at	219.961	Collagen, type XI, alpha 1	*COL11A1*
229479_at	117.305	-	-
229802_at	94.4577	-	-
236044_at	93.6993	Phosphatidic acid phosphatase type 2 domain containing 1A	*PPAPDC1A*
229152_at	92.2468	Chromosome 4 open reading frame 7	*C4orf7*
224396_s_at	84.1051	Asporin	*ASPN*
204052_s_at	83.8197	Secreted frizzled-related protein 4	*SFRP4*
204776_at	83.508	Thrombospondin 4	*THBS4*
213909_at	78.8505	Leucine rich repeat containing 15	*LRRC15*
226237_at	68.9421	-	-
217428_s_at	66.1755	Collagen, type X, alpha 1	*COL10A1*
209840_s_at	58.5571	Leucine rich repeat neuronal 3	*LRRN3*
231766_s_at	57.0013	Collagen, type XII, alpha 1	*COL12A1*
206796_at	56.5772	WNT1 inducible signaling pathway protein 1	*WISP1*
243802_at	54.5355	Dynein, axonemal, heavy chain 12	*DNAH12*
205713_s_at	47.9473	Cartilage oligomeric matrix protein	*COMP*

Table 3. List of differentially expressed genes in the pathological scar tissue down-regulated by more than 20 times compared to normal foreskin tissues.

Probe Set ID	Ratio	Gene Title	Gene Symbol
207935_s_at	0.0012	Keratin 13	*KRT13*
1553946_at	0.0024	Dermicidin	*DCD*
213240_s_at	0.0038	Keratin 4	*KRT4*
206378_at	0.0039	Secretoglobin, family 2A, member 2	*SCGB2A2*
229477_at	0.0068	Thyroid hormone responsive (SPOT14 homolog, rat)	*THRSP*
41469_at	0.0076	Peptidase inhibitor 3, skin-derived	*PI3*
229476_s_at	0.0085	Thyroid hormone responsive (SPOT14 homolog, rat)	*THRSP*
203691_at	0.0087	Peptidase inhibitor 3, skin-derived	*PI3*
205549_at	0.0089	Purkinje cell protein 4	*PCP4*
1558846_at	0.0097	Pancreatic lipase-related protein 3	*PNLIPRP3*
234513_at	0.0139	Elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	*ELOVL3*
219554_at	0.0149	Rh family, C glycoprotein	*RHCG*
207430_s_at	0.0178	Microseminoprotein, beta-	*MSMB*
205029_s_at	0.0186	Fatty acid binding protein 7, brain	*FABP7*
212531_at	0.0189	Lipocalin 2	*LCN2*
205783_at	0.0193	Kallikrein-related peptidase 13	*KLK13*
210297_s_at	0.0193	Microseminoprotein, beta-	*MSMB*
207802_at	0.0199	Cysteine-rich secretory protein 3	*CRISP3*
205030_at	0.02	Fatty acid binding protein 7, brain	*FABP7*
214240_at	0.0213	Galanin prepropeptide	*GAL*

Results of GO analysis

GO analysis for molecular function

The up-regulated differentially expressed genes were associated with the following specific molecular functions: interactions with protein and metal ions, composition of extracellular matrix, peptidase activity, integration of binding protein, transcription factor activity (Table 4 lists the top 10 results by P value).

On the other hand, down-regulated differentially expressed genes were mainly related to the following molecular functions: enzyme activity, interactions between and movement of ions, and ion channel activity (Table 5 lists the top 10 results based on P value).

Table 4. Results of GO analysis of up-regulated differentially expressed genes affecting molecular function.

GO molecular function	Count	P value
GO:0005515 protein binding	110	5.55E-92
GO:0005509 calcium ion binding	50	1.13E-68
GO:0005201 extracellular matrix structural constituent	18	7.90E-37
GO:0008233 peptidase activity	22	2.13E-27
GO:0004222 metalloendopeptidase activity	14	9.11E-26
GO:0005178 integrin binding	12	2.17E-25
GO:0008270 zinc ion binding	30	3.64E-23
GO:0003700 transcription factor activity	22	9.57E-23
GO:0046872 metal ion binding	35	4.97E-21
GO:0003823 antigen binding	13	1.33E-20

Table 5. Results of GO analysis of down-regulated differentially expressed genes affecting molecular function.

GO molecular function	Count	P value
GO:0005515 protein binding	53	2.48E-34
GO:0004252 serine-type endopeptidase activity	14	6.56E-25
GO:0016491 oxidoreductase activity	19	7.18E-24
GO:0004867 serine-type endopeptidase inhibitor activity	12	1.23E-23
GO:0008233 peptidase activity	17	1.00E-21
GO:0005509 calcium ion binding	18	9.79E-20
GO:0020037 heme binding	10	7.82E-18
GO:0046872 metal ion binding	27	6.60E-17
GO:0005198 structural molecule activity	14	2.19E-16
GO:0042803 protein homodimerization activity	11	2.22E-16

GO analysis for biological process

The up-regulated differentially expressed genes were mainly associated with the following biological processes: cell adhesion, formation of collagen fibers, decomposition of collagen, cell differentiation, cell signal transduction, cell hypoxia response, cell matrix adhesion, integrin-mediated signal pathway, and nerve bone development (Table 6 lists the top 10 results by P value).

The down-regulated differentially expressed genes were related to other biological processes, such as cellular redox reaction, cell differentiation, lipid metabolism and synthesis, and inflammatory reaction (Table 7 lists the top 10 results by P value).

Table 6. Results of GO analysis of up-regulated differentially expressed genes affecting the biological process.

GO biological process	Count	P value
GO:0007155 cell adhesion	52	2.61E-76
GO:0007275 development	38	5.35E-32
GO:0006955 immune response	26	3.72E-29
GO:0030199 collagen fibril organization	11	3.45E-27
GO:0006508 proteolysis	23	4.27E-23
GO:0006355 regulation of transcription, DNA-dependent	28	9.79E-21
GO:0001501 skeletal development	13	7.26E-18
GO:0055114 oxidation reduction	16	1.13E-17
GO:0007399 nervous system development	14	4.67E-13
GO:0030154 cell differentiation	16	1.70E-12

Table 7. Results of GO analysis of down-regulated differentially expressed genes affecting the biological process.

GO biological process	Count	P value
GO:0055114 oxidation reduction	26	1.10E-36
GO:0006508 proteolysis	21	5.09E-23
GO:0008544 epidermis development	11	1.07E-18
GO:0030216 keratinocyte differentiation	8	7.63E-18
GO:0031424 keratinization	8	1.18E-17
GO:0030855 epithelial cell differentiation	6	4.29E-12
GO:0006629 lipid metabolism	10	9.89E-10
GO:0019370 leukotriene biosynthesis	4	1.41E-09
GO:0016338 calcium-independent cell-cell adhesion	4	2.66E-09
GO:0001666 response to hypoxia	5	4.36E-09

GO analysis for cell components

The up-regulated differentially expressed genes were associated with the extracellular domain, extracellular matrix, dynamic cell membrane protein, extracellular space, endoplasmic reticulum, Golgi body, basement membrane, collagen, type V collagen, platelet alpha granule, and other such cellular components (Table 7 lists the top 10 results by P value).

On the other hand, the down-regulated differentially expressed genes were mainly related to the extracellular matrix, cell membrane, endoplasmic reticulum, plasma membrane, cornified envelope, and intermediate fiber cellular components (Table 8 lists the top 10 results by P value).

Results of pathway analysis

The DAVID software platform was used to analyze the 956 genes that were differentially expressed by >5 times (599, up-regulation; 357, down-regulation); 61 pathways were analyzed in the KEGG map, out of which 23 were associated with the up-regulated differentially expressed genes and 38 were related to the down-regulated differentially expressed genes (Table 8 and 9 list the top 10 results by P value).

Table 8. Results of GO analysis of down-regulated differentially expressed genes affecting the cellular composition.

GO cellular component	Count	P value
GO:0005576 extracellular region	72	4.45E-95
GO:0016020 membrane	62	6.37E-45
GO:0005737 cytoplasm	59	6.28E-42
GO:0016021 integral to membrane	53	3.03E-41
GO:0005783 endoplasmic reticulum	27	6.07E-34
GO:0005886 plasma membrane	38	1.62E-30
GO:0005789 endoplasmic reticulum membrane	21	7.43E-29
GO:0005615 extracellular space	20	6.54E-27
GO:0005887 integral to plasma membrane	23	7.19E-25
GO:0001533 cornified envelope	9	1.60E-23

Table 9. Biological pathways affected by the down-regulated differentially expressed genes.

Pathway	P value	Genes
Drug metabolism - cytochrome P450	9.12E-07	*MGST1*; *MGST1*; *MGST1*; *MAOA*; *FMO5*; *UGT2A1*; *MAOA*; *CYP3A5*; *ALDH3A1*; *CYP3A5*
Arachidonic acid metabolism	5.92E-06	*ALOX15B*; *ALOX15B*; *CYP2J2*; *GPX3*; *ALOX12*; *ALOX12B*; *GPX3*
Urea cycle and metabolism of amino groups	7.33E-06	*ARG2*; *ARG1*; *MAOA*; *MAOA*; *ALDH3A1*
Androgen and estrogen metabolism	5.03E-05	*SULT1E1*; *SULT1E1*; *HSD17B2*; *HSD11B1*; *UGT2A1*
Tyrosine metabolism	5.98E-05	*PNPLA3*; *MAOA*; *MAOA*; *ALDH3A1*; *TYR*
Phenylalanine metabolism	1.28E-04	*PNPLA3*; *MAOA*; *MAOA*; *ALDH3A1*
Retinol metabolism	2.13E-04	*DGAT2*; *DGAT2*; *UGT2A1*; *CYP3A5*; *CYP3A5*; *DHRS9*
Biosynthesis of steroids	2.66E-04	*TM7SF2*; *DHCR7*; *SC4MOL*
Metabolism of xenobiotics by cytochrome P450	2.83E-04	*MGST1*; *MGST1*; *MGST1*; *UGT2A1*; *CYP3A5*; *ALDH3A1*; *CYP3A5*
Histidine metabolism	3.62E-04	*HAL*; *MAOA*; *MAOA*; *ALDH3A1*
Tight junction	3.83E-04	*MYH11*; *MYH11*; *MYH11*; *CLDN17*; *CLDN8*; *CLDN11*; *CLDN4*
Sphingolipid metabolism	7.17E-04	*SGPP2*; *ACER1*; *DEGS2*

DISCUSSION

A majority of the research conducted into scar formation-related genes thus far has focused on the identification of the role played by individual genes. However, previous research has also shown that the formation of scars during skin wound healing is a result of a complicated, dynamic network of gene expression and metabolic regulation (Chen et al., 2012; Ray et al., 2013). Therefore, research into individual genes has some limitations (Wang, 2007) compared to fast and accurate gene analysis methods, which are used to screen for the genes that are associated with the formation of pathological scars. An effective method to explain the pathogenesis of hypertrophic scars is to explore the relationship between genes and pathways. Gene chip technology can be used to screen a large number of genes in parallel (Ayari and Bricca, 2013; Yoshida and Ishibashi, 2013). Gene expression profiling enables researchers to obtain a vast amount of information on related gene expression through tissue analysis (using a small amount of sample). Analysis of the expression spectrum of gene chips has been widely applied to study the differential expression of genes in normal and carcinoma tissues; in fact, this has facilitated the discovery of many important and relevant characteristics of carcinoma tissues. (Simpson et al., 2008; Shi et al., 2009). In this study, the GeneChip® Human Genome U133 Plus 2.0 expression microarray was used to analyze and screen for differentially expressed genes in pathological scar and normal foreskin tissues. Gene chips can be used to analyze a large number of gene expressions simultaneously, producing extensive experimental data; however, the up- and down-regulated genes cannot be identified in a majority of the cases. Gene expression data is linked to the molecular function or known signaling pathways, which facilitates the interpretation of the microarray data, and may help identify mechanisms underlying changes in gene expression (Zhao and Shao, 2009).

In this study, we utilized the GO and Pathway gene function analysis methods in order to analyze the differential expression of genes. The GO database contains 17,348 annotated genes; these are divided into three categories based on the role played by these genes in molecular function, biological processes, and cellular components. Analysis of the obtained microarray data can help researchers identify the common GO function branch that can accommodate a genetic change, thereby identifying the gene changes that could affect the bio-

logical function. Pathway analysis is a widely used microarray data analysis method used to identify changes in gene function. Pathway analysis data has been used to study the changes in biological pathways affected by differential gene expression; this method uses individual gene expression data, and therefore differs from the GO classification. In this method, the list of differentially expressed genes is inputted, the pathway analysis software is imported, the pathways that are affected by the differential expression of these genes is identified, and the pathway that is significantly affected by the changes in gene expression is calculated. In this study, the pathway analysis was conducted using information extracted from the KEGG database. Each of the biological pathways are specially illustrated in the KEGG database, and all KEGG biological pathways can be expanded to and crossed with other pathways (Zhao and Shao, 2009).

Based on the results of the GO analysis, the up-regulated differentially expressed genes were mainly associated with the binding of protein and metal ions (molecular function); these were also correlated to the composition of extracellular matrix, peptidase activity, integration of protein binding, and transcription factor activity. The down-regulated differentially expressed genes were mainly associated with enzyme activity, ion binding and movement, and ion pathway activity. The up-regulated differentially expressed genes were also correlated with several biological processes, such as cell adhesion, collagen fiber formation, decomposition of collagen, cell differentiation, cell signal transduction, cell hypoxia response, cell matrix adhesion, integrin mediated signaling pathway, and nerve skeletal development. The downregulated differentially expressed genes, on the other hand, were mainly related to the cellular redox reaction, cell differentiation, lipid metabolism and synthesis, and inflammatory reaction. The results of pathway analysis revealed that the up-regulated differentially expressed genes were involved in the following pathways: extracellular matrix receptor response, focal adhesion connection, biosynthesis of O-GalNAc chitosan, transforming growth factor-β signaling pathway, the interaction between cytokines and their receptors, toll-like receptor signaling pathway, actin cytoskeleton system, cell adhesion molecules, and tumor related signaling pathways. A part of the above-mentioned signaling pathway has been shown to be closely related with the development of pathological scars. In conclusion, we verified the reliability of chip testing; based on the results of data analysis, the unconfirmed signal pathway must be investigated further to elucidate the mechanism of pathological scar formation.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Ayari H and Bricca G (2013). Identification of two genes potentially associated in iron-heme homeostasis in human carotid plaque using microarray analysis. *J. Biosci.* 38: 311-315.

Chen MR, An G, Liu SL and Wei FC (2012). Gene expression of transforming growth factor beta receptor ii in the epidermis of pathological scar. *Zhonghua Shao Shang Za Zhi* 28: 278-281.

Ray S, Ju X, Sun H, Finnerty CC, et al. (2013). The IL-6 trans-signaling-STAT3 pathway mediates ECM and cellular

proliferation in fibroblasts from hypertrophic scar. *J. Invest. Dermatol.* 133: 1212-1220.
Shi WD, Meng ZQ, Chen Z, Lin JH, et al. (2009). Identification of liver metastasis-related genes in a novel human pancreatic carcinoma cell model by microarray analysis. *Cancer Lett.* 283: 84-91.
Simpson CD, Anyiwe K and Schimmer AD (2008). Anoikis resistance and tumor metastasis. *Cancer Lett.* 272: 177-185.
Wang Q (2007). The expression and significance of NEDD4-1 gene in pathological scar. *Zhongguoweichuangwaikezazhi* 7: 385-387.
Yoshida S and Ishibashi T (2013). Development of novel molecular targeting therapy for diabetic retinopathy based on genome-wide gene expression profiling. *Hukuoka acta medica* 104: 240-247.
Zhao M and Shao GR (2009). The expression and significance of NEDD4-1 gene in pathological scar. *Xiandaishengwuyixuejinzhan* 9: 382-384.