Analysis of Differentially Expressed Genes between Affected and Control Limbs in Cases with Primary Lymphedema

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Abstract **Objective** To Study some possibly related genes in the occurrence or development of primary lymphedema in limb and discuss their functions. **Methods** Young female patients with primary lymphedema in unilateral leg were chosen at the clinic of 9th Shanghai Peoples Hospital. A piece of skin tissue with cutis and hypodermis was taken from symmetric sites of the affected and the normal leg of the patients respectively. Then RNA was isolated and made as cDNA probe. After hybridization and washing, it was scanned by ScanArray4000 of General Scanning Corporation and analysed by GenePix Pro 3.0. Finally, the function of the positive genes was aquired in Medline data base and bioinformatical analysis were proceeded. **Results** Among the 4096 target genes, there were 27(0.66%) genes expressed differently between the affected and the normal limbs in all cases. Those genes possibly played important roles in the mechanism of primary lymphedema in limbs. **Conclusion** DNA microarray technology was an effective technique in screening the differentially expressed genes between the affected and the normal limbs of primary lymphedema. Further analysis of the obtained genes would help to understand the molecular mechanism of primary lymphedema in limb.

Key words Primary lymphedema; Gene express; Molecular mechanism; Microarray

Microarray of expressed genes was a new technique in that thousands of probes with specific genes or their cDNA fragments were fixed to form a genechip and it was detected of mRNA or its reversal transcript cDNA from different individuals (normal and affected), different tissue (normal and invaded), etc. As a result, the expressed specification of the genes was analysed and judged comprehensively. In the development process of primary lymphedema in limbs, some studies had demonstrated the relationship of VEGF –C and its receptor with Milroys Disease [1,2], FOXC2 with LD syndrom[3,4]. It was recognized that lymphedema was a kind of disease with different phenotypes and influenced by all sorts of inherent and environmental factors, which meant that one genotype might result in many phenotypes, one phenotype might results from different genotypes[5]. The study should be carried out to find the relationship of many sorts of genetic and environmental factors in order to make the interaction of the related factors clear in the paths of genes action and signals transduction. Therefore, microarray of genes expression was used in the study in order to find the gene expressional difference of cutis and hypodermis between the affected and normal limb, which might be conducive to learn the molecular mechnism of lymphedema in limbs.

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MATERIALS AND METHODS

Clinical Sampling

Four patients of young women who suffered with lymphedema in one leg and their lymphoscintigraphy showed the blockade of lymph pathway, was chosen in the 9th Peoples Hospital of Shanghai. With the approval of hospital ethic committee and written permission of the patients, two pieces of skin tissues about 4.0 cm ×3.0 cm ×2.0 cm with cutis and hypodermis were taken from symmetrical sites of the affected and normal leg, which were labeled as test(T) and control(C) group respectively after being cleaned and frozen immediately.

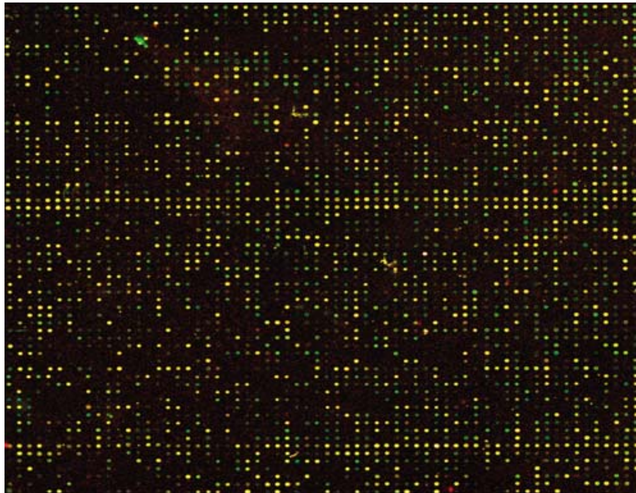

Fig.1 Superposition figure labeled by bicolor fluorescence

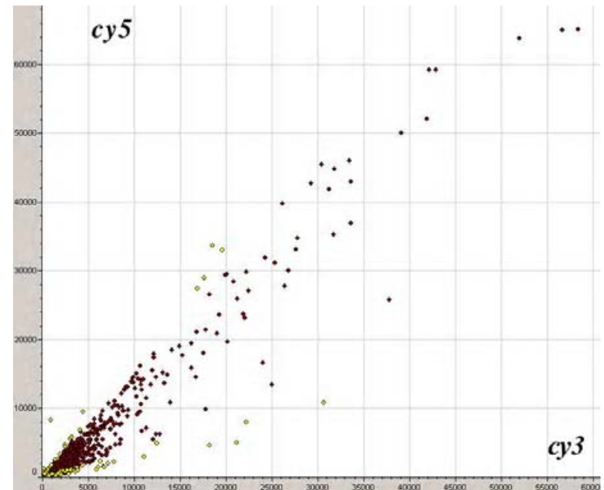

Fig.2 Scatter diagram of Hybrid signal intensity

Choosing Microarray

H40s microarray of expression spectrum (manufactured by Biostar Co) was chosen to examine the samples. Procedures included:

1. Extraction of mRNA and preparation of probe;
2. Preparation of microarray;
3. Hybridization and washing;
4. Detection and analysis.

The microarray were scanned by ScanArray4000 from the General Scanning Company, in addition, the intensity and ratio of Cy3 and Cy5 signals were analyzed by software, GenePix Pro 3.0. The ratio value bigger than 2 was regarded as higher gene expression, and the ratio value smaller than 0.5 as lower gene expression.

RESULTS

Results of RNA Quality Determination

More than 50μg total RNA with high purity was obtained after sample extraction which was qualified for microarray.

Results of Hybridization on the Microarray

Fig. 1 represents the superposition figure labeled by bicolor fluorescence, which is the result of hybridization of the sample from the affected and normal leg with the microarray. Firstly, the green points show stronger Cy3 signal, which mean the gene expression is stronger in the control leg, that is to say, the gene expression is lower in the affected leg. Secondly, the red points demonstrat stronger Cy5 signal, which mean the gene expression is stronger or higher in the affected leg. Thirdly, the yellow points show similar intensity of the two kinds of signals, which mean that the gene expression is similar in two legs. Fig. 2 is scatter diagram of hybrid signal intensity, on which X–axis and Y–axis represented the intensive value of Cy3 and Cy5 fluorescent signals respectively, and every point represented a signal of hybridization on the microarray. The red points indicate that the ratio of Y to X was between 0.5 and 2.0, which means no significant difference of gene expression. On the other hand, the yellow points indicate that the ratio of Y to X was out of the field from 0.5 to 2.0, which means significant difference of expression. Further, the yellow points near to the Y–axis demonstrate stronger Cy5 signal which means higher expression and the ones near to the X–ray show stronger Cy3 signal which means lower expression.

The common part of genes was 27 which expressed differently among the four pairs of samples (see table. 1).

DISCUSSIONS

Lymphedema in limb is a pathologic state of lymph

Tab.1. Common part of genes expressed differently among the samples

Accession	Average Ratio	Definition
1	0.268	Homo sapiens peroxiredoxin 3 (PRDX3), nuclear gene encoding mitochondrial protein, mRNA
2	0.281	Homo sapiens microsomal glutathione S-transferase 1 (MGST1), mRNA
3	0.311	603059843F1 Homo sapiens cDNA, 5´ end
4	0.316	Homo sapiens mRNA; cDNA DKFZp564E1962 (from clone DKFZp564E1962); partial cds
5	0.343	Homo sapiens solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5 (SLC25A5), nuclear gene encoding mitochondrial protein, mRNA
6	0.373	Homo sapiens annexin A1 (ANXA1), mRNA
7	0.378	Homo sapiens cDNA FLJ31365 fis, clone NB9N41000135, highly similar to RAS-RELATED PROTEIN RAB-1A
8	0.390	/
9	0.392	Homo sapiens ATPase, H+ transporting, lysosomal (vacuolar proton pump) membrane sector associated protein M8-9 (APT6M8-9), mRNA
10	0.397	Homo sapiens RAP1A, member of RAS oncogene family (RAP1A), mRNA
11	0.404	Homo sapiens adaptor-related protein complex 3, sigma 1 subunit (AP3S1), mRNA
12	0.411	Homo sapiens GABA(A) receptor-associated protein-like 2 (GABARAPL2), mRNA
13	0.425	Homo sapiens mRNA for KIAA1058 protein, partial cds
14	0.427	Homo sapiens eukaryotic translation initiation factor 3, subunit 6 (48kD) (EIF3S6), mRNA
15	0.429	Homo sapiens vitamin A responsive; cytoskeleton related (JWA), mRNA
16	0.433	MXRA1 Homo sapiens cDNA
17	0.443	Homo sapiens hypothetical protein FLJ20051 (FLJ20051), mRNA
18	0.448	Homo sapiens ribosomal protein L18 (RPL18), mRNA
19	0.452	Homo sapiens signal recognition particle 9kD (SRP9), mRNA
20	0.459	Homo sapiens heterogeneous nuclear ribonucleoprotein F (HNRPF), mRNA
21	2.380	Homo sapiens mRNA, chromosome 1 specific transcript KIAA0495
22	2.411	Homo sapiens cDNA: FLJ22062 fis, clone HEP10276, highly similar to AF151890 Homo sapiens CGI-132 protein mRNA
23	2.412	Homo sapiens zinc finger protein 264 (ZNF264), mRNA
24	2.495	Human mRNA for KIAA0188 gene, partial cds
25	2.558	zn87h12.r1 Homo sapiens cDNA, 5´ end
26	4.335	Homo sapiens dual specificity phosphatase 1 (DUSP1), mRNA
27	7.199	Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA

pathway blockade, which causes lymph fluid accumulation in superficial soft tissue and secondary proliferation of fibrous connective tissue, secondary fatty sclerosis, thicker fasciae, resulting in the limb swelling. The reason for lymphedema could be of congenital hypoplasia of lymph vessels or acquired diseases. However, it was observed by Wrone[6] that the cause of lymphedema was not single, more than 1.7 percent of patients with melanomatosis suffered lymphedema after biopsy of lymph node. It was possible that some primary factors led to lymphedema because the biopsy was such a small injury. In addition, it also inferred that the important role of genetic susceptibility might cause lymphedema, which occurred only in a part of the patients after the same tumor resection. In addition, cold or fever could often be traced as an inducing factor in many patients with primary lymphedema. Therefore, the dividing line between primary and secondary lymphedema was not so clear as traditional classification. It could be inferred that lymphedema was the common actions of primary factors within the body and external factors. Consequently, with an effort to reduce the external factors, screening of primary factors would be the key for the prevention and cure of lymphedema. It was found that there were a close relationship between VEGF-C and Milroys disease[1,2], FOXC2 and LD syndrome[3,4].

In our study, there were 20 genes showed low expression in the affected limbs as No.1 to No.20 in tab. 1. The function of some genes might be related to development of the lymphedema. (1) PRDX3 encoded mitochondrial protein, which took part in the process of proliferation, transformation and death, took part in the oxidation-stress reaction and kept stability of the internal circumstance [7,8]. The low expression of PRDX3, MGST1(which had the function of modulating the oxidation-stress reaction too[9,10]) and SLC25A5 might be related with the longer path of oxygen diffusion and difficulty of oxygenic metabolism. Lately, it was proved by Siems[11] that the oxidation-stress reaction was improved in the body of patients with lymphedema, and it might be related with PRDX3 and MGST1 by labeling the product of oxidation-stress quantitatively. The result was identical with that of this paper. (2) The protein encoded by ANXA1 was a conjugated protein by calcium with anti-inflammation character, which was rich in the leukocytes in peripheral blood and was able to modulate the role of anti-inflammation. The low expression of ANXA1 and APT6M8-9 might be associated with the decrease of the ability of anti-inflammation. (3) APT6M8-9 was an X-linked gene located at Xp11.4, which was considered by Demirci[14] as important pathogenic gene of diseases located at the area. Therefore variation of that gene might be related with pathogenesis of lymphedema, thinking of that female patients were much more than male ones of lymphedema clinically. (4) The protein encoded by SRP[15,16] was a kind of ribose nucleoprotein in cytoplasm which played an important role in sorting the protein. It recognized the signal sequence of original peptides and located the complex of original ribosome and SRP chain to rough endoplasmic reticulum. In addition, SRP9 and SRP14 formed an isomeric dipolymer which in charged of the rupture of translation. Therefore low expression of SRP9 and SRP18 might be connected with the decrease of the synthesis of protein and the repairing ability in the affected tissue.

There were 7 genes showed high expression in the affected limbs as No.21 to No.27 in tab.1, which would also provide basis and evidence for further research.

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