

Original Article

Microarray analysis of potential genes in the pathogenesis of recurrent oral ulcer

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Abstract: Recurrent oral ulcer seriously threatens patients' daily life and health. This study investigated potential genes and pathways that participate in the pathogenesis of recurrent oral ulcer by high throughput bioinformatic analysis. RT-PCR and Western blot were applied to further verify screened interleukins effect. Recurrent oral ulcer related genes were collected from websites and papers, and further found out from Human Genome 280 6.0 microarray data. Each pathway of recurrent oral ulcer related genes were got through chip hybridization. RT-PCR was applied to test four recurrent oral ulcer related genes to verify the microarray data. Data transformation, scatter plot, clustering analysis, and expression pattern analysis were used to analyze recurrent oral ulcer related gene expression changes. Recurrent oral ulcer gene microarray was successfully established. Microarray showed that 551 genes involved in recurrent oral ulcer activity and 196 genes were recurrent oral ulcer related genes. Of them, 76 genes up-regulated, 62 genes down-regulated, and 58 genes up-/down-regulated. Total expression level up-regulated 752 times (60%) and down-regulated 485 times (40%). IL-2 plays an important role in the occurrence, development and recurrence of recurrent oral ulcer on the mRNA and protein levels. Gene microarray can be used to analyze potential genes and pathways in recurrent oral ulcer. IL-2 may be involved in the pathogenesis of recurrent oral ulcer.

Keywords: Recurrent oral ulcer, gene expression analysis, gene microarray, gene, pathway, interleukin

Introduction

Recurrent oral ulcer is a type of oral mucosal disease with high incidence [1]. It could occur on lingual margin, lips, and inside of the cheeks, and its related disease severely threatened patients' life and health [2]. Current studies suggested that recurrent oral ulcer was related to immune, genetic, psychological and physiological conditions [3]. However, it was still unclear about the molecular mechanism of recurrent oral ulcer occurrence, development and recurrence [4]. Particularly, related genes and signaling pathways were not studied systematically [5]. More importantly, the latest research suggested that recurrent oral ulcer is a kind of oral disease induced by multiple factors [6]. Therefore, it is necessary to study gene expression patterns in recurrent oral ulcer occurrence, development and recurrence.

Recurrent oral ulcer was regulated by many genes [7]. Recurrent oral ulcer involved in the interaction and mutual regulation of genes

related to occurrence, development and recurrence. These physiological activity related genes made up a complex regulatory network, and determines disease status by the cascade amplification function [8]. High throughput, high efficient, accurate and automatic test method is needed to study hundreds of genes role involved in recurrent oral ulcer occurrence, development and recurrence [9]. Thus, we selected Human Genome 280 microarray 6.0 produced by Affymetrix to study genes role in recurrent oral ulcer [10].

The aim of this study is to reveal the potential genes and pathways that play a role in recurrent oral ulcer from the perspective of bioinformatics and systems biology. Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Western blot were applied to test the screened interleukins expression in recurrent oral ulcer, to provide useful information for the treatment and prognosis of recurrent oral ulcer in the future.

Materials and methods

Candidate gene screening method and strategy

BIOCARTA, GENMAPP, KEGG, NCBI, RGD databases and related papers were searched to get the recurrent oral ulcer related genes [11]. Then, these genes were further consulted in Human Genome 280 6.0 microarray data. Microarray hybridization was performed to get each pathway's recurrent oral ulcer related genes [12]. RT-PCR was applied to test four genes related to recurrent oral ulcer to verify the reliability of microarray results. Data transformation, scatter plot, clustering analysis, and expression pattern analysis were used to analyze recurrent oral ulcer related gene expression changes and further investigate their role in recurrent oral ulcer.

Ulcer tissue selection

Recurrent oral ulcer tissue preparation: recurrent oral ulcer patients and healthy controls were included with 6 subjects in each group. Inclusion and exclusion criteria were in accordance with the literature reported [13]. Mucosal tissue was scratched from the patients [14], when the symptoms appeared [15]. Normal tissue was got from the corresponding part in healthy controls [16].

Ulcer tissue sampling time selection

Multiple studies confirmed that recurrent oral ulcer process was regulated by genes strictly [17]. We determined the process by cell cycle [18]. For example, recurrent oral ulcer activated at 0.5-4 h with large amount genes expression such as c-fos, c-myc, and c-jun [19]. It was an important period and we chose four time points as 0.5, 1, 2, and 4 h. G1 phase of the first cell cycle of recurrent oral ulcer was at 6-12 h, and we chose 6, 8, and 12 h time points. S and M phase of the first cell cycle was at 16-36 h, thus we chose 16, 18, 24, 30, and 36 h time points. The second cell cycle was at 36-66 h, and we selected 42, 54, and 66 h time points. Recurrent oral ulcer became mature and complete till 168 h, and the lost oral ulcer tissue has been compensated [20]. Generally, the time points for sampling in both experiment group and healthy control were 0.5, 1, 2, 4, 6, 8, 12, 16, 18, 24, 30, 36, 42, 54, 66, 96, 120, 144, and 168 h.

Microarray hybridization

Total RNA was extracted and then reverse transcript to cDNA [21]. cDNA was synthesized to biotin labeled cRNA by in vitro transcription (IVT). After fragmentation, cRNA was hybridized with Human Genome 280 6.0 microarray. Gene expression signal was read after elution and scanning [22].

In order to reduce the microarray analysis error, each point on the Human Genome 280 6.0 microarray was tested for three times. Gene microarray hybridization was performed at triplication and the mean value was used for further analysis. The main instruments included chip hybridization oven, automatic washing workstation, control system GCOS (GeneChip Operating Software), and high resolution scanner (2.5 microns).

Recurrent oral ulcer related gene confirmation

Identification principle of recurrent oral ulcer related genes [23] is: 1, gene expression up-regulated or down-regulated more than two times at least in one time point; 2, similar results in the two or three times of replication; 3, significant difference existed between experimental group and control group (F test was applied and $P < 0.05$ was considered for significant differences).

RT-PCR and western blot

RT-PCR was applied to test the genes showed significant differences in microarray analysis [24]. Western blot was used to test the protein with obvious difference in microarray.

Statistical analysis

All data store and statistical analyses were performed using SPSS16.0 software (Chicago, IL). Numerical data were presented as means and standard deviation (\pm SD). Differences between groups were analyzed by F tests. $P < 0.05$ was considered as significant difference.

Results

Differentiated expression genes related to recurrent oral ulcer

Microarray hybridization results were shown in **Table 1**. Recurrent oral ulcer related genes in each pathway were obtained by the hybridiza-

Microarray in recurrent oral ulcer

Table 1. Differential expression gene list

Gene Abbr	Associated to	Fold difference	Gene Abbr	Associated to	Fold difference	Gene Abbr	Associated to	Fold difference	Gene Abbr	Associated to	Fold difference
Bmp15	1	0.4, 2.8	Ctsq	2	0.3, 2.2	Nfkb1	2	0.4, 2.3	TP533	2	2.9
EPOR	1	0.3	Ctsr	2	0	Nppa	2	0.3, 3.2	TRO	2	0.3
FGF1	1	0.3	Cuzd1	2	0.4	Npy1r	2	0.4, 8.6	TTPA	2	0.2, 2.5
HPSE	1	0.3, 6.3	CXCL12	2	0.2	NR3C2	2	0.3	UCN	2	0.2, 2.5
INHBB	1	0.4, 5.8	*Cyp4a14	2	2.2	OK138	2	3.2	Crhbp	2, 3	0.2, 4.3
KITL	1	2.5	Dtprp	2	3.5	Pde5a	2	0.5, 2.6	Crhr1	2, 3	0.5, 3.8
VGF	1	7.5	EDN1	2	0.4, 2.6	PLAU	2	0.4, 3	CYP19A1	2, 3	0.2, 6.5
ACE	1	0.5	Epd4.113	2	0.3, 2.5	Prf1	2	0.2	HPGD	2, 3	4.2
Adm	1	8	Erb2	2	0.1	PRKAR2A	2	2.7	IL1B	2, 3	0.4
PLAT	1	0.4, 4.9	Esrra	2	0.2	*Prkca	2	4.6	*IL6	2, 3	0.3, 6.1
MIF	1	3.2	F2	2	0.3	*Prkcb1	2	8.2	MAFF	2, 3	0.1, 16
*AFP	1.2	0.1, 3.7	F3	2	0.1, 2	Pr1pb	2	14	Ptgs2	2, 3	0.1, 2.1
CX3CR1	1.2	0.4, 6.8	F5	2	0.5	Pr1pc2	2	0.1	Rgs2	2, 3	6
ESR1	1.2	6.1	FAAH	2	4.2	Pr1pf	2	2.5	Oxt	2, 3, 4	0.2, 3.2
*ESR2	1.2	0.5, 3.5	FABP7	2	0.5, 15	Pr1pi	2	0.4	Oxtr	2, 3, 4	0.4, 2.1
FSHB	1.2	0.2	FKBP4	2	0.3	Pr1pk	2	0.3, 2.8	Agtrla	2, 4	0.4
INHA	1.2	0.2	*Flt1	2	2.3	Proc	2	0.3	A1doc	2, 4	2.3
LHCGR	1.2	2.4	Fos	2	28	PROCR	2	6.5	Birc4	2, 4	4.9
MMP9	1.2	0.5, 9.5	Fstl1	2	2.5	PROS1	2	2.1	Irs1	2, 4	0.4
NOS3	1.2	0.3, 2.1	Ga1	2	11	Psgb1	2	2.5	Jak2	2, 4	6.5
SELL	1.2	0.3, 3	Ghr1	2	4	Pzp	2	0.3	Nfic	2, 4	3.2
*TIMP1	1.2	8.6	GNB3	2	3.2	Sdc4	2	2.9	Pmch	2, 4	0.1
MUC1	2	0.2, 6.8	GSTM1	2	2.2	Se1e	2	13	Pr1r	2, 4	0
RBP2	2	0.1, 2.5	GSTT1	2	3.2	SERPIND1	2	0.1	Retn	2, 4	0.2, 2.2
Nme1	2	4	HADHA	2	0.5	Sfrp4	2	0.4	Stat5a	2, 4	0.2
Ppargcla	2	0.2, 2.5	*HGF	2	0.4	SHBG	2	0.1, 2.8	Stat5b	2, 4	0.3
Spp1	2	0.5, 2.7	*Hmox2	2	0.4	*S1c10a1	2	0.3	Trh	2, 4	9.8

*Fold difference in sample with empirical p-value < 0.05.

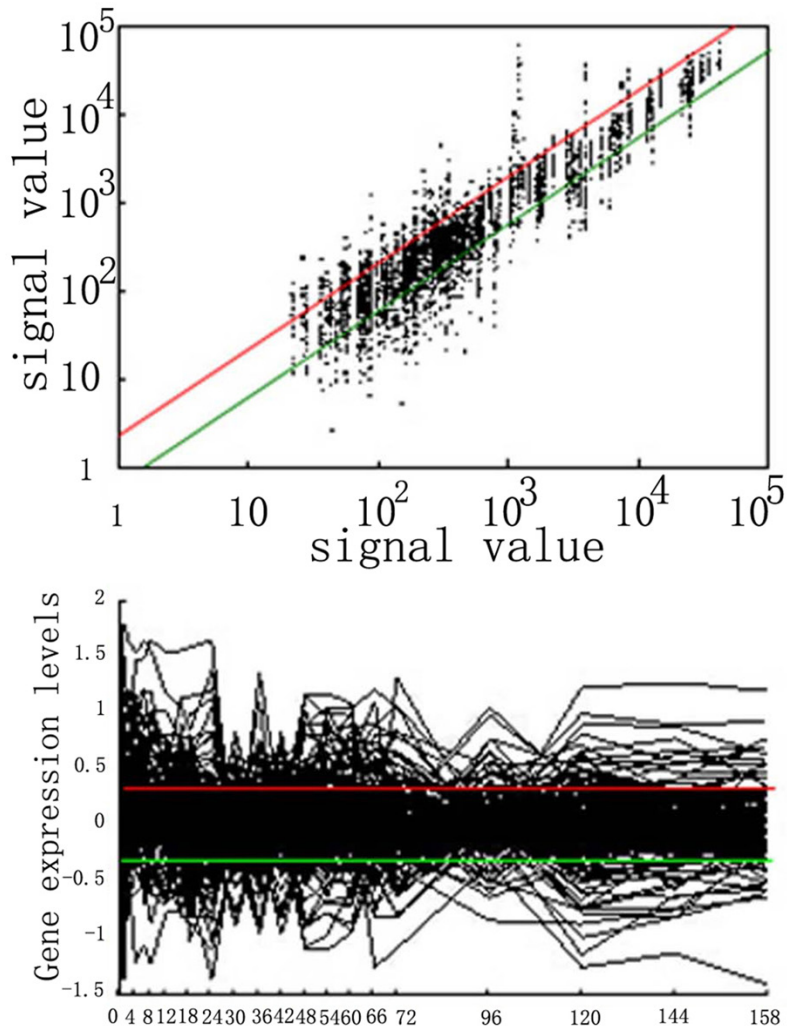


Figure 1. Related gene expression frequency, abundance, and dynamic in recurrent oral ulcer. 551 genes involved in recurrent oral ulcer activity and 196 genes were recurrent oral ulcer related genes. Of them, 76 genes up-regulated, 62 genes down-regulated, and 58 genes up-/down-regulated. Total expression level up-regulated 752 times (60%) and down-regulated 485 times (40%). The up-regulation scope was 2-65 times and the down-regulation scope was 2-26 times.

tion. Most of the genes were related to immune, suggesting that immune related genes played an important role in recurrent oral ulcer occurrence, development and recurrence.

Related gene expression frequency, abundance, and dynamic in recurrent oral ulcer

Figure 1 represented the frequency, abundance, and dynamic of recurrent oral ulcer related genes. It was the scatter plot composed of the mRNA content comparison between experimental and control group. X axis means the signal value in control, and Y axis represents the signal value in the experimental

group. Each dot represents one gene expression in one time point. The oblique lines means the differentiated expression times reached two and the dots between them represent expression changes less than twice. The following figure means the gene expression dynamic graph. X axis was the recovery time after the recurrent oral ulcer, while Y axis was gene expression changes in value. Red and green lines represent twice changes between two groups.

Related gene starting and total expression in recurrent oral ulcer

Figure 2 represented starting and total expression of related genes in recurrent oral ulcer. Non-dot column showed the genes expressed in the start; Dot column showed the genes expressed totally. Grey column represented the up-regulated genes, while white column mean the down-regulated genes. Genes began to express mainly at 0.5-48 h after the ulcer onset, and only a few genes start express at other time. Gene expression changes throughout the whole process,

and up-regulated genes were in the ascendant.

Cluster analysis

Recurrent oral ulcer related genes promoted cell proliferation and inhibited apoptosis. Red represented up-regulated genes, green mean down-regulated genes, and black were for no significant changes genes. Up tree was the cluster of similar expression. They were divided into 5 groups and each group may have the similar function. Right tree clustered the correlated time, and cells at the clustered times may have the similar cell physiological activities.

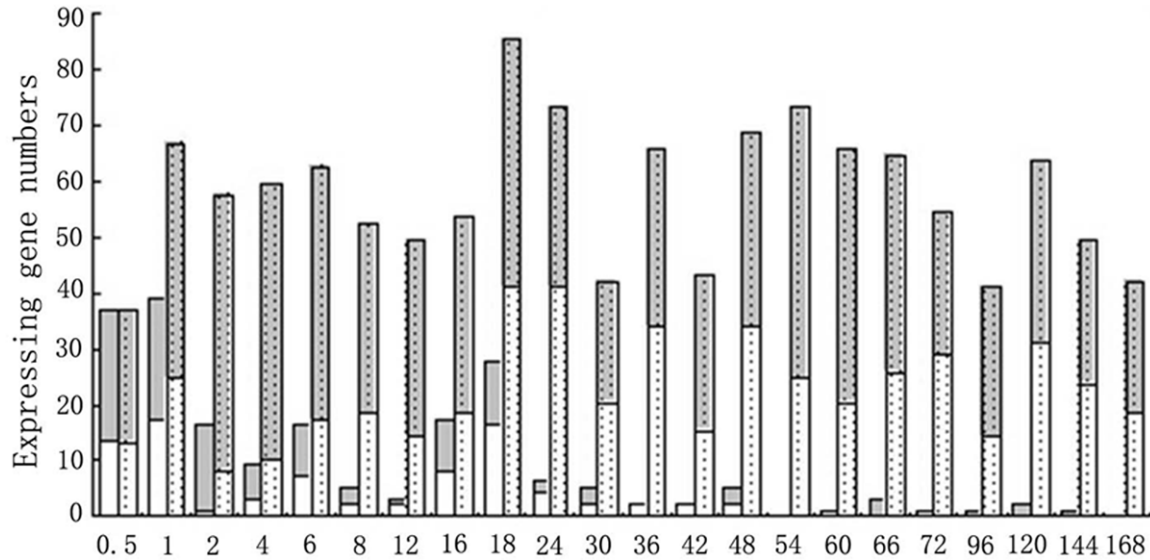


Figure 2. Related gene starting and total expression in recurrent oral ulcer.

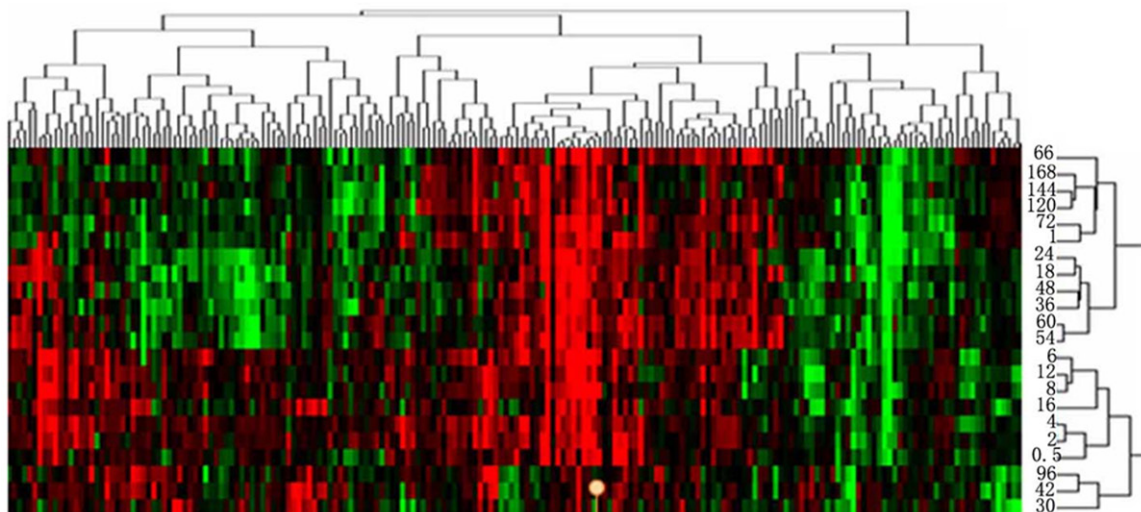


Figure 3. Cluster analysis of recurrent oral ulcer related genes.

More than 70% of the total genes were related to cell proliferation and apoptosis, indicating that recurrent oral ulcer related genes may promote cell proliferation and suppress apoptosis. **Figure 3** showed that IL-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 had similar changes rule at recurrent oral ulcer.

Recurrent oral ulcer related genes expression pattern

IL-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 expression pattern in recurrent oral ulcer were shown in **Figure 4**. Four genes (IL-2, 3, 10, and 11)

showed significant changes in recurrent oral ulcer. Of them, IL-2 presented the most obvious changes.

IL-2 expression analysis in recurrent oral ulcer

Figure 5 showed that IL-2 participated in the occurrence, development and recurrence processes of recurrent oral ulcer both in mRNA and protein levels.

Discussion

In this paper, we established the method to analyze gene expression in recurrent oral ulcer

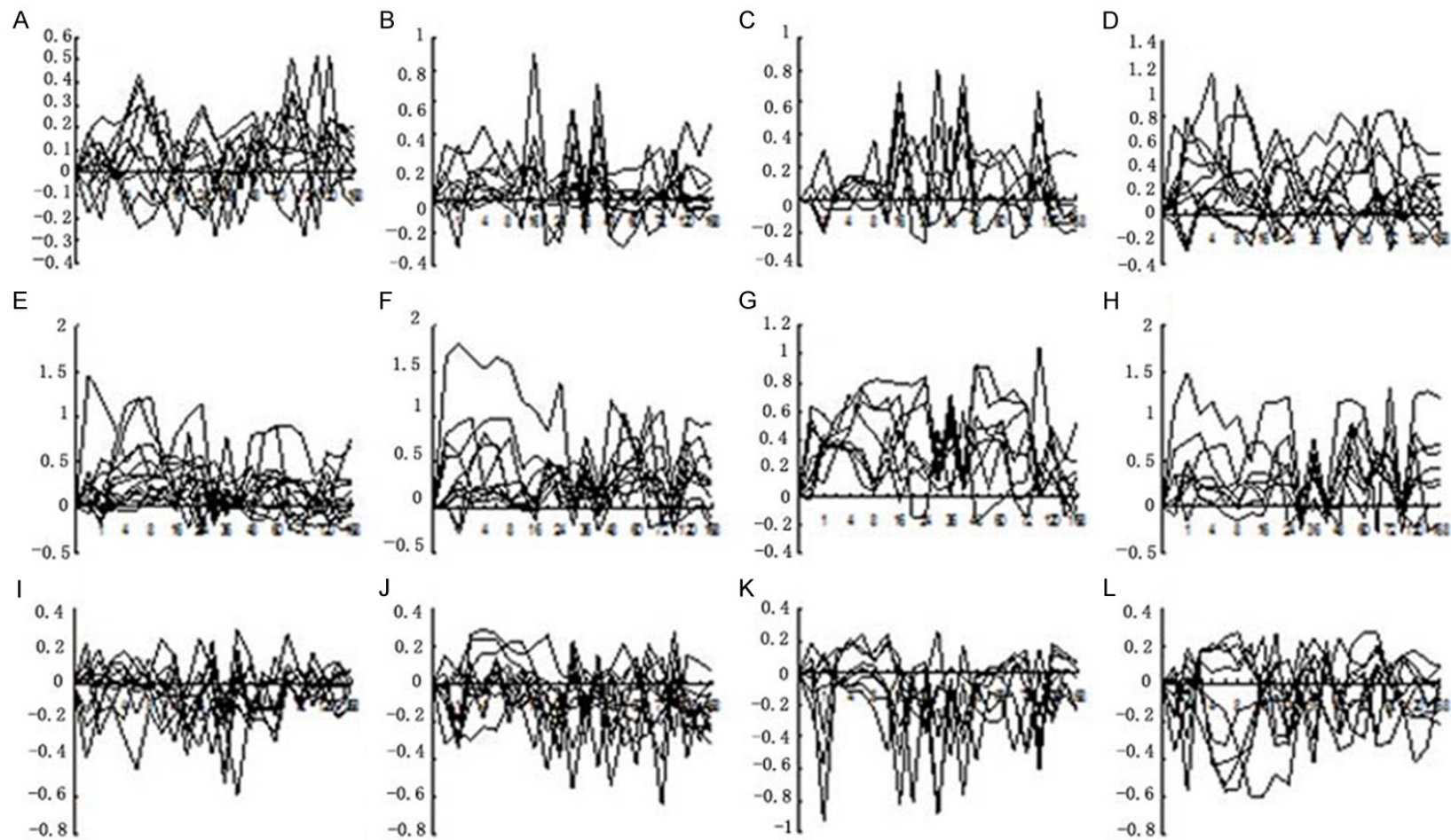


Figure 4. Recurrent oral ulcer related genes expression pattern.

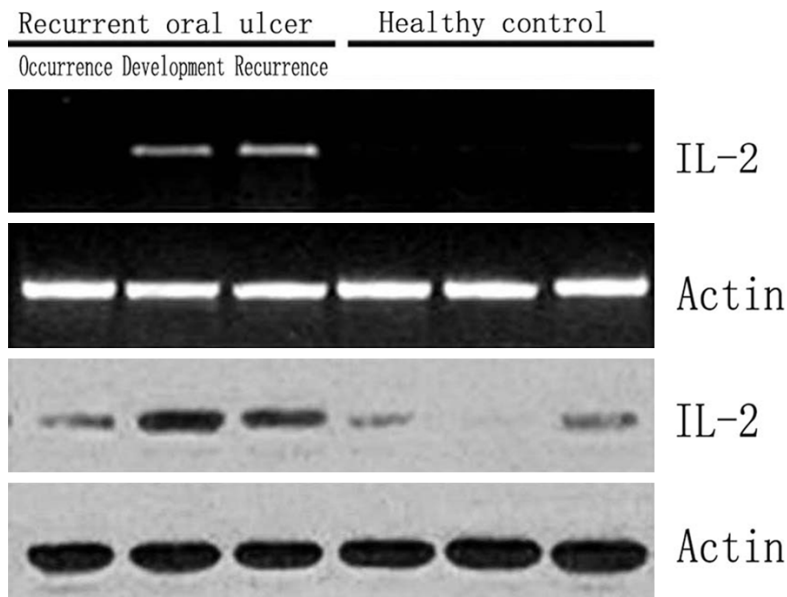


Figure 5. IL-2 expression analysis in recurrent oral ulcer.

dynamic, mode and effect by microarray detection and bioinformation. Bioinformatics and systemic biology approaches confirmed that immune, apoptosis and cell cycle were associated with recurrent oral ulcer [25].

In this paper, we detected recurrent oral ulcer related gene expression by high throughput method at a long time from 0.5 h to 7 d and multiple time points [23]. 196 recurrent oral ulcer related genes were closely related to physiological and biochemical activities. Immune, apoptosis and cell cycle had 21, 173, 16, and 33 genes associated with recurrent oral ulcer, respectively. Among the 196 genes, it showed five kinds of expression trend, 14 groups of time correlation and 20 kinds of expression mode. Four genes (IL-2, 3, 10, and 11) from the microarray results played an important role in recurrent oral ulcer. Of them, IL-2 showed the most significant changes both in mRNA and protein levels.

Human Genome 280 6.0 microarray was a useful tool for gene transcription analysis [26]. Recurrent oral ulcer related genes promoted cell proliferation and inhibited apoptosis. However, gene → mRNA → protein → functions were affected by multiple factors. We would analyze the abovementioned results by Northern blot, protein microarray, RNA interference, and protein interaction.

Our innovation point was to investigate recurrent oral ulcer related gene expression dynamic, mode, and function by using high-throughput microarray technology and bioinformatics analysis. Bioinformatics and systemic biology approach confirmed that recurrent oral ulcer related genes were associated with recurrent oral ulcer occurrence, development and recurrence.

We used microarray to analyze recurrent oral ulcer related genes mRNA expression in recurrent oral ulcer to clarify dynamic gene expression changes. mRNA → protein → functions were affected

by numerous factors. Only study on gene transcription level was not enough, and protein expression analysis was further needed.

In brief, Gene microarray can be used to analyze potential genes and pathways in recurrent oral ulcer. IL-2 may be involved in the pathogenesis of recurrent oral ulcer.

Disclosure of conflict of interest

None.

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