Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/burns

Time series analysis of gene changes and processes after burn with human gene expression profiles

Dan Wu [a,1,*], Ming Zhou [b,1], Liang Li [a], Xiangfeng Leng [c], Zheng Zhang [a], Ning Wang [a], Yanwei Sun [a]

[a] Department of Burn and Plastic Surgery, Zibo Central Hospital, Zibo, Shandong, 255036, China
[b] Department of Joint Surgery, Zibo Central Hospital, Zibo, Shandong, 255036, China
[c] Department of Plastic Surgery, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China

ARTICLE INFO

Article history:
Accepted 2 August 2018

Keywords:
Burn
GEO
WGCNA
Time series
Temporal expression

ABSTRACT

Severe burns might be followed by severe infection associated with high mortality. In this study, we aimed to identify changes in genes and processes across time points after burn via analyzing time series gene expression profiles in burn patients and control from the Gene Expression Omnibus (GEO). Patients were classified into four groups according to time after burn and weighted gene co-expression network analysis (WGCNA) obtained three gene modules including magenta, yellow and greenyellow modules that significantly correlated positively with time after burn. We also identified four groups of differentially expressed genes (DEGs) in samples at 0-1d, 1-2d, 2-4d, and 4-7d after burn compared with controls. Functional enrichment analysis of those DEGs indicated significant enrichment of inflammatory/immune related processes throughout time points after burn, while, samples at later time points were also closely associated with cell activation regulation related processes. Short time series expression analysis of overlapping genes among the four lists of DEGs screened out two temporal gene expression profiles that exhibited decreasing and increasing expression trend across times after burn, and genes contained in those two profiles might be related to pathologic changes after severe burn.

1. Introduction

Burns, especially large area of burn injury not only give rise to damage of the local skin and deep tissue, but can cause obvious changes of the body's internal organs and the system function, metabolism and morphology [1,2]. Systematic researches have shown that serious burn injury not only induce immune function and metabolism disorder, at the same time can cause DNA damage, apoptosis, and a series of pathophysiological changes [3–6]. The hypermetabolic response begins on the fifth day of post-injury, while the inflammatory response starts immediately after burn and persists for up to several months [7]. Post burn immune dysfunction (PID) is considered to cause severe infection, multiple organ system dysfunction and even the principal cause of death.

Recently, a bioinformatics method was developed to analyze the gene expression profiles of primary immune cells in mice to build a network of protein interactions. Gene chip

* *Corresponding author at*: Department of Burn and Plastic Surgery, Zibo Central Hospital, Gongqingtuanxi Road, Zibo, Shandong, 255036, China.
E-mail address: wudan17214@outlook.com (D. Wu).
[1] Indicates equal contribution.
https://doi.org/10.1016/j.burns.2018.08.022
0305-4179/

technology, characterized by high sensitivity and throughput, could detect multiple gene expression at the same time which provides a platform for the study of multiple genes influenced by PID. Previous studies have used gene chip technology to preliminarily explore the sequential changes of mouse and human immune cell genome, and found abundant subversive information. However, these studies were mainly concentrated on the contrast between the burn injury and other wounds, but fewer on in-depth studies and analysis of gene transcription level after burn [8,9]. Lars H. Evers proposed that gene chips should start at the level of the whole cell genome to provide new trains of thought and therapeutic targets for the research of PID mechanism [10].

Burn is a complicated pathophysiological process that can cause changes in gene expression [11,12]. Bioinformatics analysis of gene chip expression profiles can reflect the molecular biological processes of immune cells after burning at the transcriptional level, contributed to fully clarify the mechanism of PID [13]. Murine models have been extensively used to identify the gene expression after burn, while it is not clear how gene expression is expressed in human condition, especially for inflammatory diseases [14]. The aim of our study is to reveal the difference of genes expression between burn patients and healthy people by the gene chip data analysis and further to show the biological characteristics and metabolic pathways involved in differential genes. Thus the fully understanding of the molecular mechanism of immune cells in the process of early burning stimulation will provide a new direction for clinical outcome and treatment options of burn.

2. Materials and methods

2.1. Study population

The dataset of severe burn injury was downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) with the accession number of GSE37069 (mRNA profiling data based on Affymetrix Human Genome U133 Plus 2.0 Array), which were derived from the blood sample of 533 severely burned patients and 37 healthy persons. A total of 271

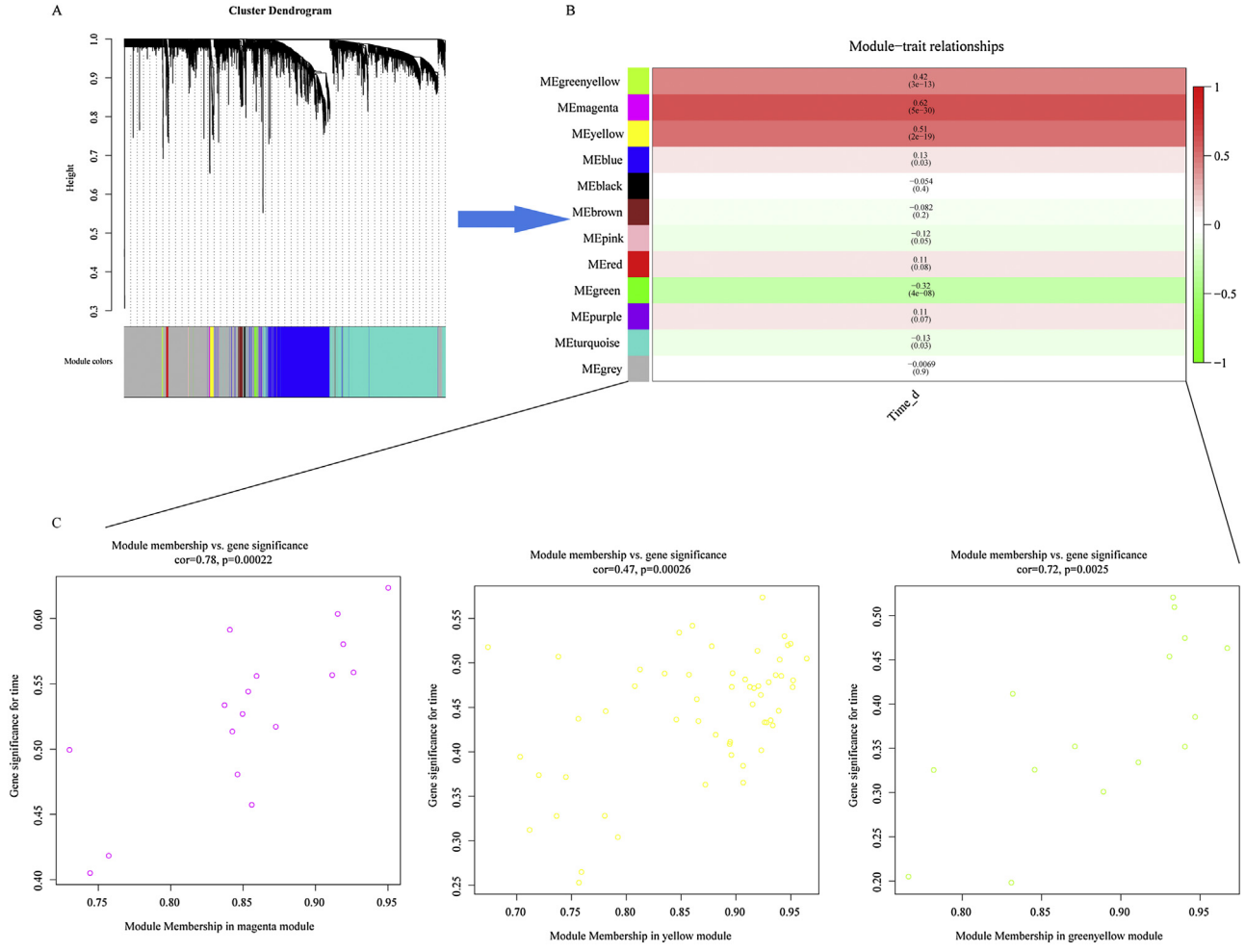

Fig. 1 – Weighted gene co-expression analysis. (A) Gene cluster dendrogram with each cluster was designated as a color. (B) Correlation analysis between gene cluster profiles and post-burn time. Number in and outside the bracket represents *P*-value and Pearson coefficient, respectively. (C) Correlation analysis between genes' module membership and gene significance in magenta, yellow and greenyellow modules.

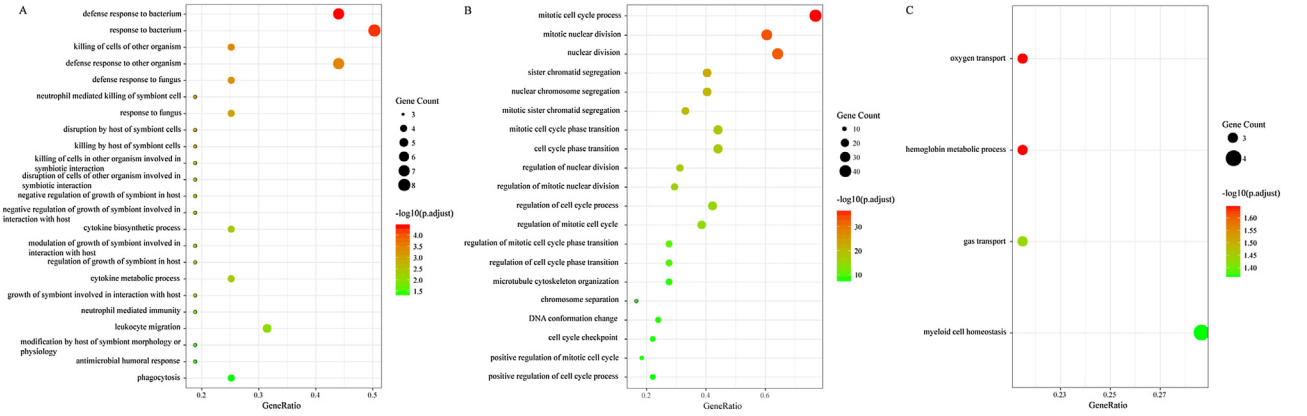

Fig. 2 – GO enrichment analysis of genes in post-burn time related modules. (A) and (B) is the top 20 most significantly enriched GO terms of genes in magenta and yellow module, respectively. (C) is the full list of significantly enriched GO terms of genes in greenyellow module.

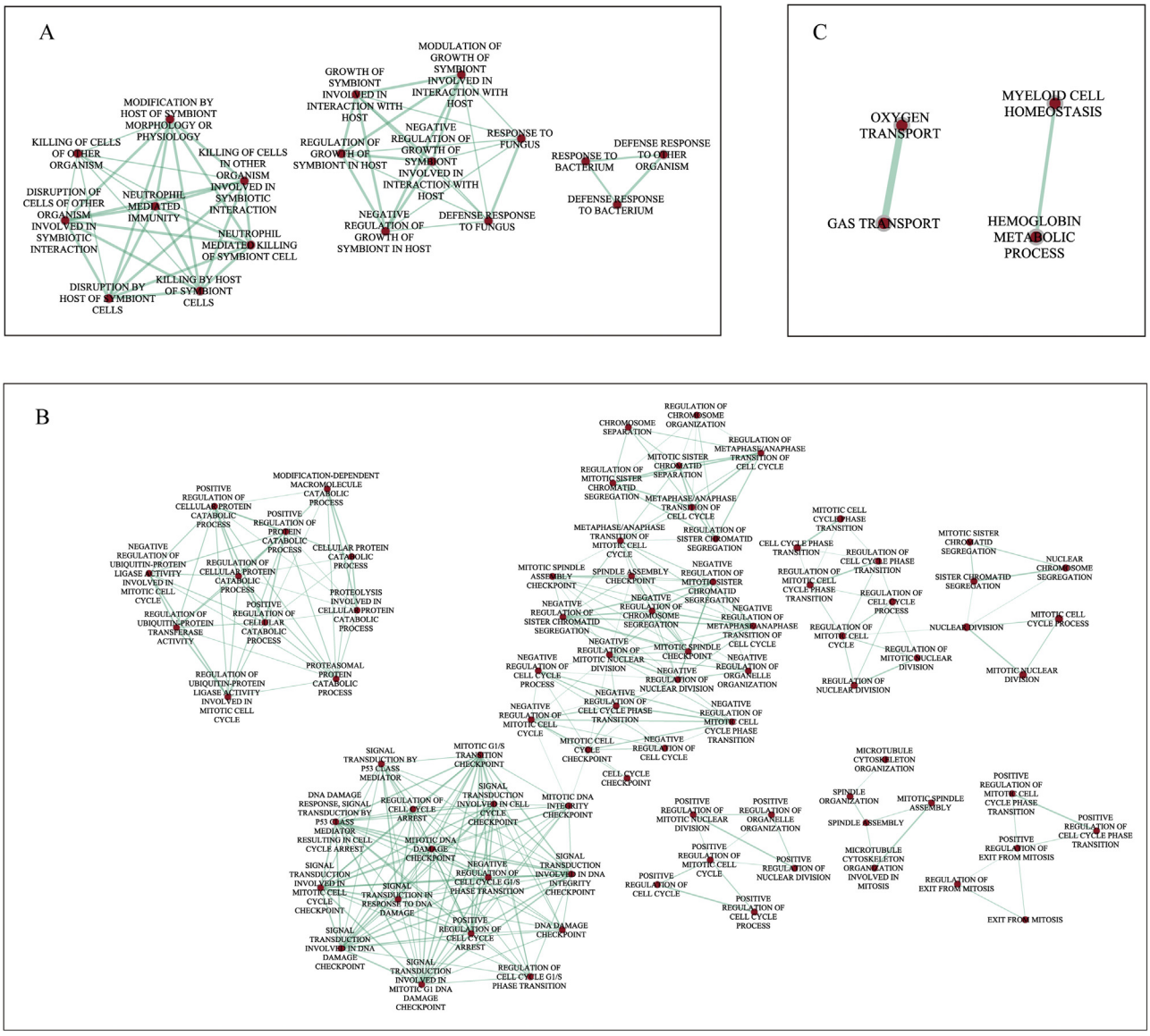

Fig. 3 – Interaction analysis of GO terms with significant enrichment in genes of magenta (A), yellow (B) and greenyellow (C) modules. Node and edge represents GO term and interaction between two GO terms if there is any overlapping gene between them, respectively. Thicker edge indicates more overlapping genes between the two GO terms.

Table 1 – Magenta module-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa05202	Transcriptional misregulation in cancer	6.21E-05	8.70E-04
hsa05322	Systemic lupus erythematosus	1.57E-03	9.45E-03
hsa04145	Phagosome	2.02E-03	9.45E-03
hsa04614	Renin-angiotensin system	1.02E-02	3.57E-02
hsa05310	Asthma	1.40E-02	3.92E-02

Table 2 – Yellow module-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa04110	Cell cycle	3.06E-08	1.16E-06
hsa04114	Oocyte meiosis	1.06E-06	2.02E-05
hsa04115	p53 signaling pathway	3.55E-06	4.50E-05
hsa00790	Folate biosynthesis	2.30E-04	2.19E-03
hsa00240	Pyrimidine metabolism	4.85E-04	3.68E-03
hsa05203	Viral carcinogenesis	3.18E-03	2.01E-02
hsa01524	Platinum drug resistance	5.31E-03	2.87E-02
hsa05166	HTLV-I infection	6.04E-03	2.87E-02
hsa04914	Progesterone-mediated oocyte maturation	8.80E-03	3.72E-02
hsa00750	Vitamin B6 metabolism	9.80E-03	3.73E-02

Table 3 – Greenyellow module-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa00910	Nitrogen metabolism	6.77E-03	2.99E-02
hsa04966	Collecting duct acid secretion	1.05E-02	2.99E-02
hsa00260	Glycine, serine and threonine metabolism	1.54E-02	2.99E-02
hsa00860	Porphyrin and chlorophyll metabolism	1.61E-02	2.99E-02
hsa05144	Malaria	1.87E-02	2.99E-02

samples within 7 days after burn were selected for this study, and which were divided into four groups of 0–1d (74 samples), 1–2d (72 samples), 2–4d (93 samples) and 4–7d (32 samples) according to the time after burn.

2.2. *Construction of weighted gene co-expression network and identification of significant modules*

To identify gene co-expression modules (CEMs) associated with burn injury, the weighted gene co-expression network analysis (WGCNA) was conducted. The remarkable characteristics of WGCNA is to find clusters (modules) of highly correlated genes, and summarize such clusters using the module eigengene for relating modules to external sample traits. Firstly, the average expression value of each gene of 271 severe burn injury samples and 37 control samples were calculated. Then, the top 5000 genes were selected for WGCNA according to the absolute value by subtracting of the average expression value of the two groups. The network and the modules were constructed and detected by WGCNA. The name of CEMs were named as the color assigned by WGCNA.

2.3. *Functional enrichment analysis of CEMs*

Gene Ontology (GO) enrichment analysis was performed by the Database for Annotation, Visualization and Integrated Discovery (DAVID). Biologic Process (BP) terms of GO terms with P_{BH} (*P* value by Benjamini and Hochberg) <0.05 were filtered out as the final results and further clustered through the enrichmentMap plug-in of Cytoscape. The enrichKEGG was used for the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. KEGG terms with $P_{BH}<0.05$ were considered as significantly enriched.

2.4. *Differential expression analysis*

To identify genes dysregulated (DEGs) in burn samples at 0–1d, 1–2d, 2–4d and 4–7d post-burn compared with the controls, we conducted differential expression analysis using limma R package. Genes with absolute log2 (fold change) >1 and FDR adjusted p values <0.05 were considered as significant.

2.5. *Functional enrichment analysis of DEGs*

Upon the DEGs we obtained from the four groups according to the time point, we performed the GO enrichment analysis via DAVID. We considered BP terms with $P_{BH}<0.05$ as significantly enriched, which further clustered through the enrichmentMap plug-in of Cytoscape. We performed KOBAS for the KEGG analysis of the DEGs. Enriched KEGG terms were identified with a threshold of $P_{BH}<0.05$.

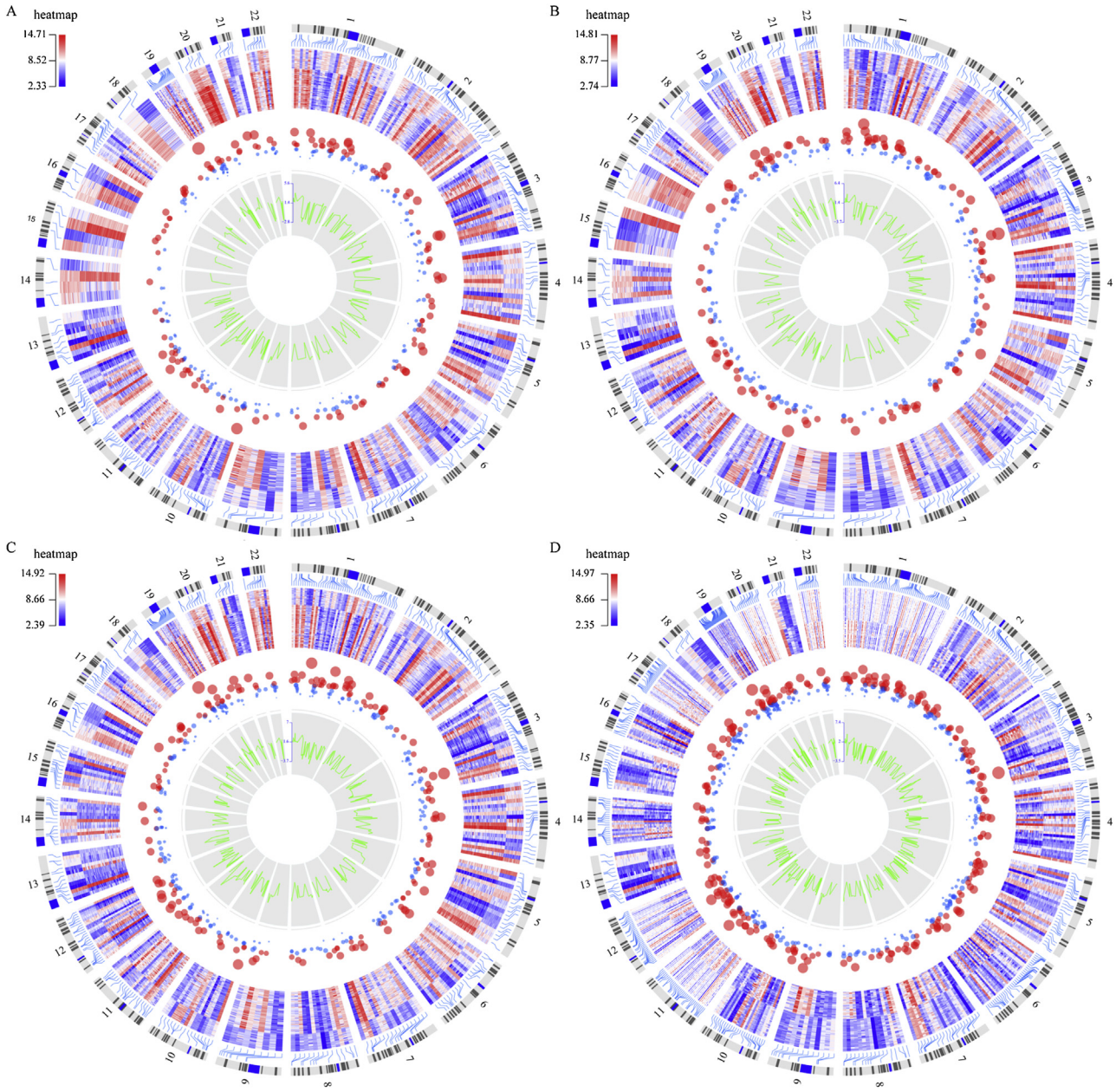

Fig. 4 – Circos plot of DEGs in burn samples at 0-1d (A), 1-2d (B), 2-4d (C) and 4-7d (D) compared with controls. Tracks from outside to inside represents genomic location, expression heatmap, differential expression *P*-value and log2 (fold change), respectively. Down- and up-regulated genes were separated with different colors in the third track with larger dot size indicate more significant, i.e. smaller *P*-value.

2.6. *Time series regression analysis*

To identify the genes or gene sets co-regulated time dependently (temporal expression profiles), software program "Short Time-series Expression Miner" (STEM, version 1.3.11, http://www.cs.cmu.edu/~jernst/stem/) was used. The main advantage of STEM is that it implements a novel method for clustering short time series expression data, which can distinguish between real and random patterns. To reduce the redundancy, the overlapped genes among the DEGs at 0-1d, 1-2d, 2-4d and 4-7d were used in this process. Group was defined as temporal expression profiles n (n=1,2,3 . . . n) according to the corresponding *P* values. Genes belong to the profile n were fellow named temporal expression profiles gene n (TEPGs).

3. Results

3.1. *Weighted gene co-expression module*

Weighted gene co-expression network analysis was applied to the top 5000 genes with largest coefficient of variation in expression values between burn and control samples and by which 12 CEMs were identified that named by different colors.

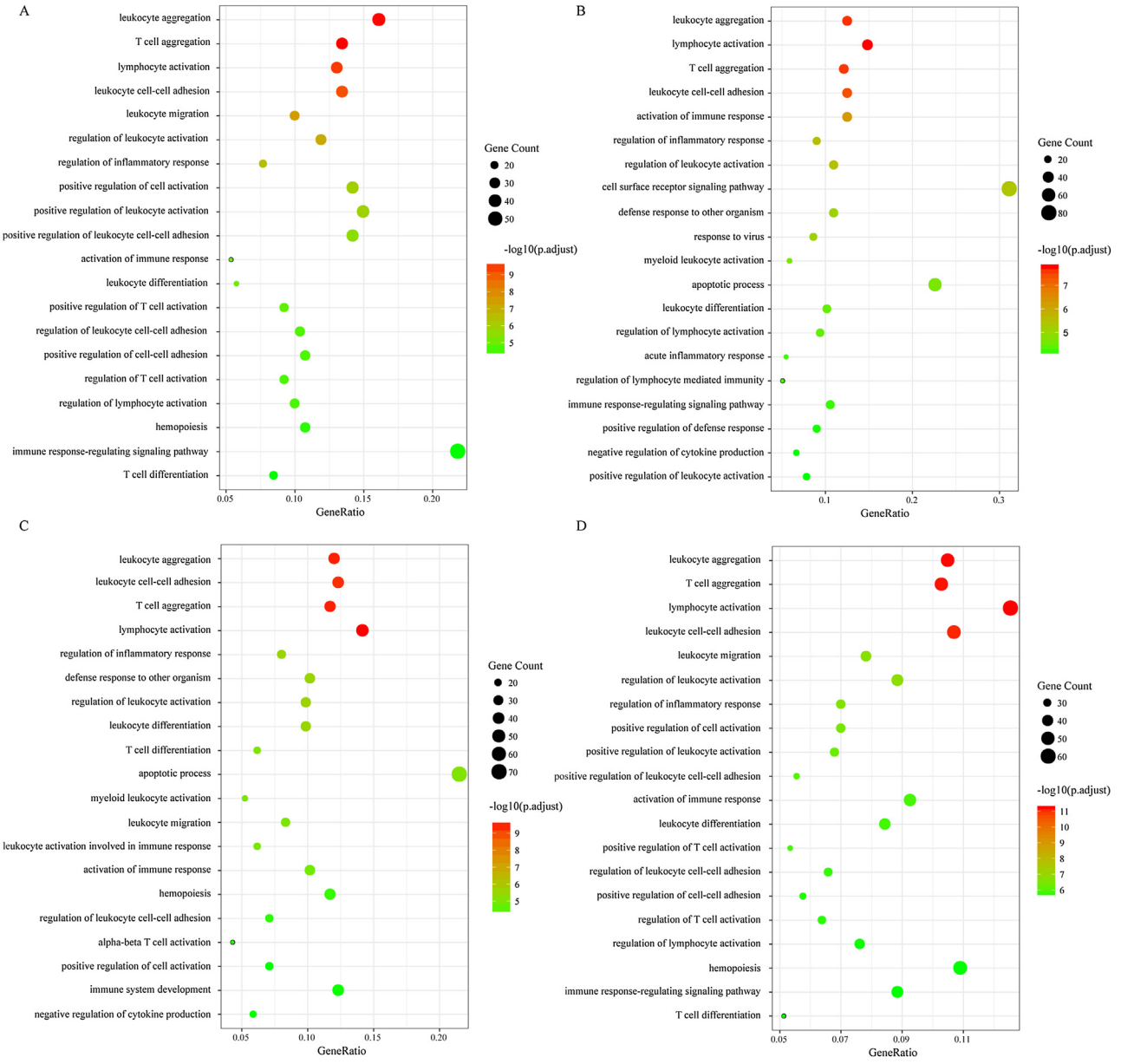

Fig. 5 – GO enrichment analysis of DEGs in burn samples at 0-1d (A), 1-2d (B), 2-4d (C) and 4-7d (D) post-burn compared with controls, respectively.

Fig. 1A shows the mapping relations between the 5000 genes 12 CEMs. We furtherly calculated the correlation between every CEM and post-burn time. As a result, three CEMs including magenta (P-value=5×10^{-30}), yellow (P-value=2×10^{-19}) and greenyellow (P-value=3×10^{-13}) were found to be significantly positive correlated with post-burn time (Fig. 1B). Besides, in the three CEMs, genes' module memberships (MMs) were all significantly positive correlated with their gene significances (GSs) as shown in Fig. 1C.

3.2. *Significantly enriched functions of the three CEMs*

Significantly positive correlations between the three CEMs and time after burn, as well as between CEM genes' MMs and GSs, all indicated potential important roles of those CEMs in biological processes post-burn. Therefore, functional enrichment analysis was performed to identify GO terms and KEGG pathways associated with genes contained in the three CEMs. As a result, 23, 114 and four GO terms were significantly enriched in magenta, yellow and greenyellow modules, and Fig. 2A–C illustrated the top 20 most significantly enriched ones for magenta and yellow modules and all for greenyellow module, respectively. The enriched GO terms were furtherly visualized as an interaction network on the basis of overlapping genes between any two GO terms (Fig. 3A–C). Several function modules related to immune response, cell cycle regulation, and metabolic were obtained. In addition, we also identified several KEGG pathways related to immune/inflammation response were significantly enriched in magenta (Table 1), yellow (Table 2) and greenyellow (Table 3) modules.

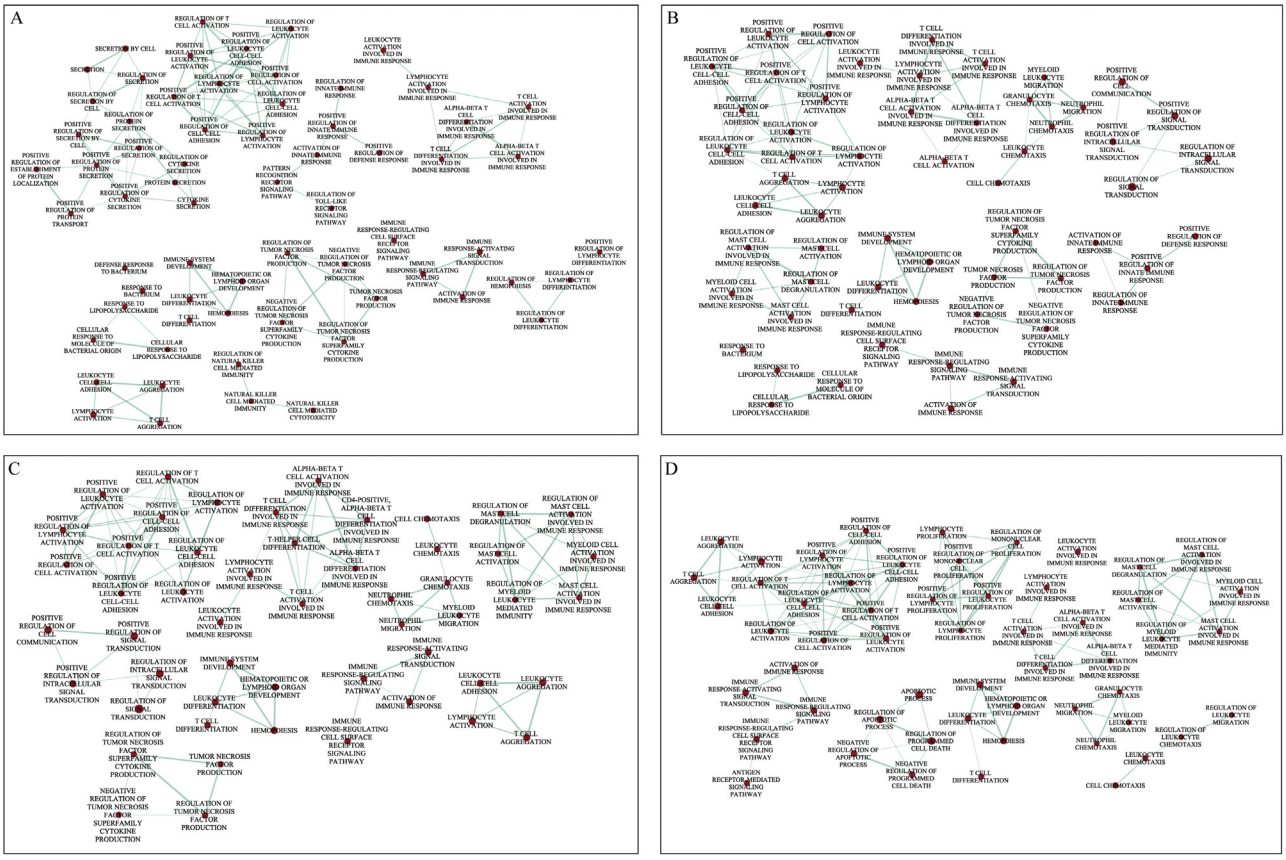

Fig. 6 – Interaction analysis of GO terms with significant enrichment in DEGs in burn samples at 0-1d (A), 1-2d (B), 2-4d (C) and 4-7d (D) post-burn compared with controls, respectively. Node and edge represents GO term and interaction between two GO terms if there is any overlapping gene between them, respectively. Thicker edge indicates more overlapping genes between the two GO terms.

Table 4 – Deg_G1-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa04640	Hematopoietic cell lineage	3.55E-10	8.63E-09
hsa05321	Inflammatory bowel disease (IBD)	1.67E-06	2.07E-05
hsa05202	Transcriptional misregulation in cancer	1.88E-05	1.86E-04
hsa04060	Cytokine-cytokine receptor interaction	6.51E-05	5.78E-04
hsa05200	Pathways in cancer	9.77E-05	8.29E-04
hsa04660	T cell receptor signaling pathway	2.38E-04	1.74E-03
hsa04630	Jak-STAT signaling pathway	3.24E-04	2.26E-03
hsa04670	Leukocyte transendothelial migration	4.32E-04	2.89E-03
hsa04610	Complement and coagulation cascades	5.10E-04	3.34E-03
hsa04014	Ras signaling pathway	5.54E-04	3.59E-03
hsa05152	Tuberculosis	6.62E-04	4.20E-03
hsa04380	Osteoclast differentiation	7.59E-04	4.73E-03
hsa04062	Chemokine signaling pathway	8.48E-04	5.20E-03
hsa01100	Metabolic pathways	9.82E-04	5.87E-03
hsa04210	Apoptosis	1.02E-03	6.06E-03
hsa04010	MAPK signaling pathway	1.12E-03	6.58E-03
hsa05134	Legionellosis	1.16E-03	6.81E-03
hsa05166	HTLV-I infection	1.23E-03	7.03E-03
hsa00500	Starch and sucrose metabolism	1.31E-03	7.41E-03
hsa04151	PI3K-Akt signaling pathway	1.82E-03	9.72E-03

3.3. *Identification of differential expression genes*

It should be helpful for understanding molecule changes post-burn to identify DEGs in burn samples at different time points after burn compared with control samples. In this study, we identified a total of 315, 301, 398 and 576 DEGs in burn samples at 0–1d, 1–2d, 2–4d and 4–7d after burn compared with normal

Table 5 – Deg_G2-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa04640	Hematopoietic cell lineage	1.30E-11	2.36E-09
hsa04610	Complement and coagulation cascades	1.31E-09	1.19E-07
hsa04060	Cytokine–cytokine receptor interaction	1.96E-07	1.19E-05
hsa05321	Inflammatory bowel disease (IBD)	1.23E-06	5.60E-05
hsa05202	Transcriptional misregulation in cancer	1.77E-06	6.15E-05
hsa05142	Chagas disease (American trypanosomiasis)	2.03E-06	6.15E-05
hsa05150	Staphylococcus aureus infection	9.92E-05	2.20E-03
hsa04621	NOD-like receptor signaling pathway	9.92E-05	2.20E-03
hsa04062	Chemokine signaling pathway	1.13E-04	2.20E-03
hsa04210	Apoptosis	1.21E-04	2.20E-03
hsa05020	Prion diseases	1.99E-04	3.28E-03
hsa01100	Metabolic pathways	2.18E-04	3.30E-03
hsa04668	TNF signaling pathway	2.37E-04	3.31E-03
hsa05133	Pertussis	3.31E-04	4.30E-03
hsa04650	Natural killer cell mediated cytotoxicity	6.70E-04	8.13E-03
hsa04010	MAPK signaling pathway	8.33E-04	9.47E-03

Table 6 – Deg_G3-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa04640	Hematopoietic cell lineage	2.40E-11	4.93E-09
hsa04610	Complement and coagulation cascades	1.89E-08	1.93E-06
hsa05202	Transcriptional misregulation in cancer	7.16E-08	4.89E-06
hsa05321	Inflammatory bowel disease (IBD)	6.86E-07	3.51E-05
hsa05150	Staphylococcus aureus infection	3.19E-06	1.31E-04
hsa04060	Cytokine–cytokine receptor interaction	4.43E-06	1.51E-04
hsa05142	Chagas disease (American trypanosomiasis)	1.57E-05	4.58E-04
hsa04010	MAPK signaling pathway	7.30E-05	1.77E-03
hsa04380	Osteoclast differentiation	7.75E-05	1.77E-03
hsa04062	Chemokine signaling pathway	1.50E-04	3.04E-03
hsa04668	TNF signaling pathway	1.63E-04	3.04E-03
hsa04621	NOD-like receptor signaling pathway	3.63E-04	6.19E-03
hsa05310	Asthma	4.20E-04	6.62E-03
hsa05020	Prion diseases	5.72E-04	8.38E-03
hsa04210	Apoptosis	6.52E-04	8.92E-03
hsa04664	Fc epsilon RI signaling pathway	7.71E-04	9.25E-03
hsa05200	Pathways in cancer	8.10E-04	9.25E-03
hsa01100	Metabolic pathways	8.57E-04	9.25E-03
hsa04620	Toll-like receptor signaling pathway	8.58E-04	9.25E-03

samples, respectively. Circos plot in Fig. 4A–D illustrated expression values, log2 (fold change) and adjusted P-value of the four lists of DEGs.

3.4. *Significantly enriched functions of the four lists of DEGs*

There might be largely different in pathologic changes in different stage post-burn. Here we investigated GO terms and KEGG pathways associated with the four lists of DEGs to explore pathologic differences in different time points post-burn. Fig. 5A–D showed the top 20 most significantly enriched GO terms and Fig. 6A–D represented the interaction relations among GO terms based on the overlapping genes between any two GO terms. It could be obviously concluded that inflammatory and immune related processes, such as leukocyte aggregation, T cell aggregation, went throughout the whole process after burn, while, several processes related to cell activation regulation were found to be only significantly enriched in samples at 4–7d after burn. Tables 4–7 provided the full lists of KEGG pathways significantly enriched in the four lists of DEGs, which were consistent with the results of GO enrichment analysis.

3.5. *Gene expression time series analysis*

Identification of gene groups within which genes have consistent expression trend across time points post-burn should be helpful for exploring pathologic changes after burn injury. Here we performed short time series expression analysis for the 219 overlapping genes among the four lists of DEGs (Fig. 7A) and identified a total of 50 temporal expression profiles named as profile1 to profile50 (Fig. 7B), out of which profile2 and profile38 showed statistical significant. As shown in Fig. 7C and D, profile2 exhibited significantly decreasing expression profiles from 0 to 7d after burn; on the

Table 7 – Deg_G4-enriched KEGG pathways.

Pathway ID	Description	P-value	Corrected P-value
hsa04640	Hematopoietic cell lineage	1.78E-12	4.14E-10
hsa05321	Inflammatory bowel disease (IBD)	9.02E-11	1.05E-08
hsa05310	Asthma	5.03E-09	3.89E-07
hsa05202	Transcriptional misregulation in cancer	2.46E-08	1.42E-06
hsa05150	Staphylococcus aureus infection	3.60E-08	1.49E-06
hsa05332	Graft-versus-host disease	3.85E-08	1.49E-06
hsa04062	Chemokine signaling pathway	2.31E-07	6.96E-06
hsa01100	Metabolic pathways	2.40E-07	6.96E-06
hsa05330	Allograft rejection	2.98E-07	7.50E-06
hsa05140	Leishmaniasis	3.23E-07	7.50E-06
hsa05416	Viral myocarditis	5.72E-07	1.21E-05
hsa04940	Type I diabetes mellitus	6.69E-07	1.29E-05
hsa04145	Phagosome	9.22E-07	1.64E-05
hsa05322	Systemic lupus erythematosus	1.47E-06	2.44E-05
hsa05320	Autoimmune thyroid disease	2.66E-06	4.11E-05
hsa04612	Antigen processing and presentation	4.13E-06	5.99E-05
hsa04380	Osteoclast differentiation	6.68E-06	9.12E-05
hsa05166	HTLV-I infection	1.02E-05	1.32E-04
hsa05323	Rheumatoid arthritis	1.30E-05	1.53E-04
hsa04060	Cytokine-cytokine receptor interaction	1.32E-05	1.53E-04
hsa05145	Toxoplasmosis	1.63E-05	1.80E-04
hsa05152	Tuberculosis	2.02E-05	2.13E-04
hsa04015	Rap1 signaling pathway	2.14E-05	2.16E-04
hsa05200	Pathways in cancer	3.25E-05	3.13E-04
hsa04610	Complement and coagulation cascades	3.37E-05	3.13E-04
hsa05169	Epstein-Barr virus infection	6.71E-05	5.98E-04
hsa04672	Intestinal immune network for IgA production	1.40E-04	1.20E-03
hsa04660	T cell receptor signaling pathway	2.14E-04	1.77E-03
hsa00500	Starch and sucrose metabolism	2.69E-04	2.08E-03
hsa04621	NOD-like receptor signaling pathway	2.69E-04	2.08E-03
hsa04668	TNF signaling pathway	2.88E-04	2.16E-03
hsa05164	Influenza A	3.47E-04	2.51E-03
hsa04514	Cell adhesion molecules (CAMs)	3.88E-04	2.73E-03
hsa04670	Leukocyte transendothelial migration	4.48E-04	3.06E-03
hsa04010	MAPK signaling pathway	4.75E-04	3.15E-03
hsa05168	Herpes simplex infection	5.23E-04	3.37E-03
hsa04611	Platelet activation	5.52E-04	3.46E-03
hsa00480	Glutathione metabolism	1.28E-03	7.64E-03
hsa04210	Apoptosis	1.28E-03	7.64E-03

contrary, genes in profile38 exhibited significantly increasing expression values from 0 to 7d post-burn. So, genes in profile 2 including DDX24, GLO1, NARS, NPM1, PPP1CC, RPL10A, RPL14, RPL24, RPS7, SRSF11, TOMM20 and ZNF146, and in profile38 including SNX3, AP2B1, PFN1, NUCB1, LDHA, P4HB, CD63, FKBP1A, OS9, ACTR1A, PGK1 and CTSD should play important roles in post-burn progression. In addition, we used another gene expression dataset with the accession number of GSE19743 that contained114 burn samples and 63 normal samples as the validation dataset to validate the time series result. As a result, we found 10 genes that contained in profile2 or profile38 were also aberrantly expressed in burn samples of the validation dataset and the expression changes were consistent between the two datasets (Fig. S1).

4. Discussion

Gene expression chip is widely used for gene analysis, which is a cutting-edge technology developed in recent years [15]. It can compare the expression of thousands of genes at the same time, screening of all relevant genes for a phenotype or a disease comprehensively. It can also reveal the interrelationships among different genes [16,17].

At present, gene chip technology has been widely used in burn. However, most of the studies were conducted to analyze the gene expression of a certain cytokine, and large amount of data produced by gene chip was not used well. The data processing was still in the primary stage of functional verification analysis. Therefore, it is necessary to excavate and analyze the data more deeply on the large amount of data generated by the gene chip to search for related biological targets for burn diagnosis and treatment [18,19].

In the present study, gene expression was analyzed by a bioinformatics method in the blood of severely burn patients and health people as controls. As burns mostly happen without intent, the burn patients were divided by 0-1d, 1-2d, 2-4d, 4-7d after severe burn. Compared with the control group, gene expression was significantly correlated with the time after burn. The finding is consistent with the finding that most

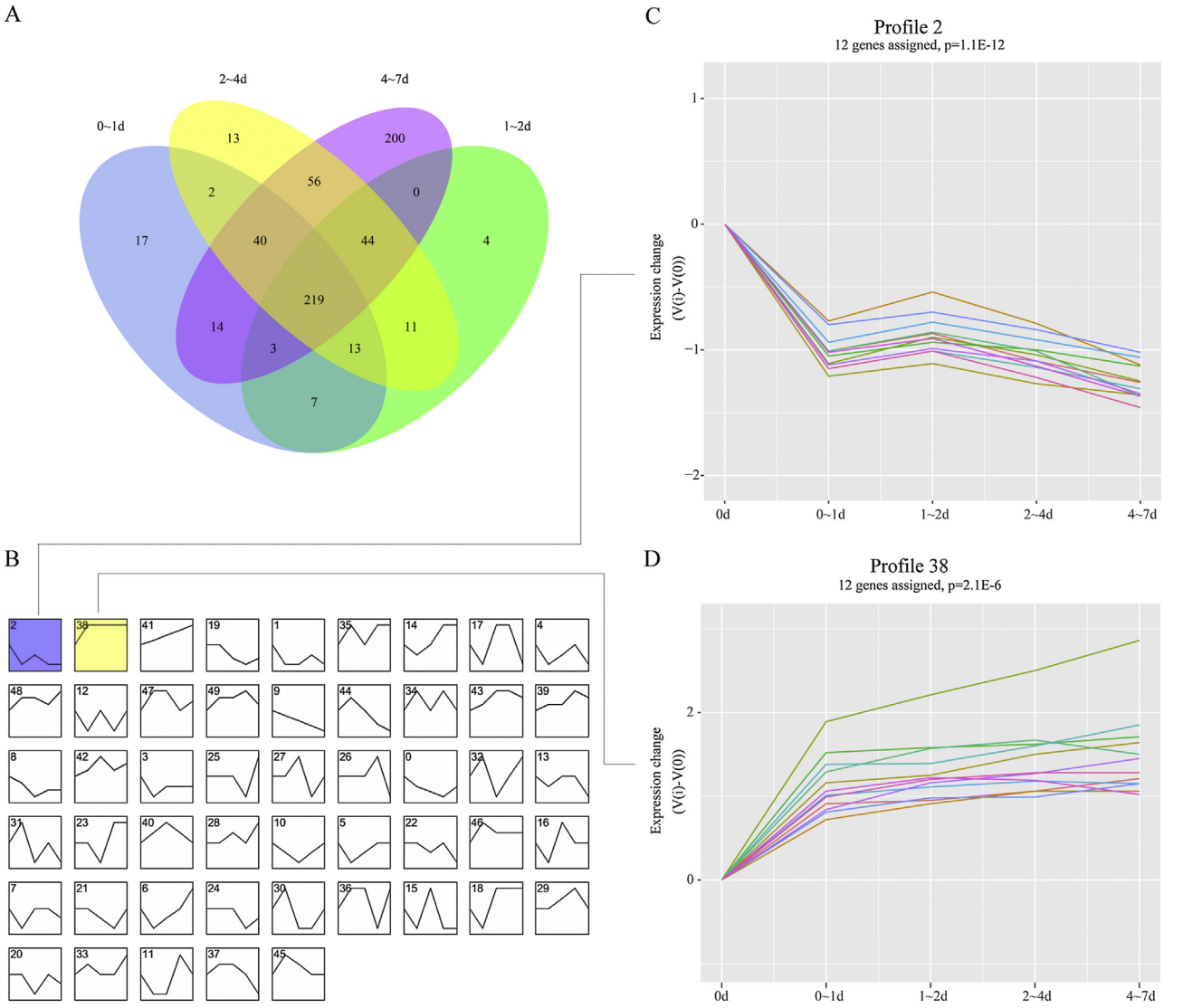

Fig. 7 – Short time expression analysis of overlapping DEGs among the four lists of DEGs. (A) Venndiagram indicates relations among the four lists of DEGs. (B) Short time expression analysis identified 50 gene expression profiles with profile2 and profile38 exhibited statistical significant. (C) and (D) show changes of genes' expression in profile2 and profile38 across time points post-burn, respectively.

pathological changes during 1–2 weeks after the sustaining the burn [20], while most common period of survival was 4–7 day [21]. We calculated the difference of gene expression in the four time point groups compared to control group, and obtain 315, 301, 398 and 576 differentially expressed genes, respectively, with 219 overlapping ones. So we consider that these 219 genes should play a major role in the healing of the burn. Through the GO function enrichment analysis, it was found that these differentially expressed genes were up-regulated in the biological processes such as cell process, metabolic process, biological regulation, stimulus response and immune system process. The increase was even more pronounced after the burning, suggesting that we can strengthen the process of immune cell metabolism respond to external stimuli cell process and research the regulation and mechanism of immune function after burn in the study of gene function after burn.

Through KEGG pathway analysis, it was found that the up-regulated genes were principally involved in pathways related to the immune system, cell growth, death including signal transduction pathways and lipid metabolism pathways. The toll-like receptor signaling pathway is the most significant pathway for up-regulated gene [22]. Toll-like receptors are membrane-bound receptors expressed on the surface of the innate immune cells such as macrophages and dendritic cells, which are the pathogen recognition receptors that quickly excite the innate immune system by producing proinflammatory cytokines and up-regulation of co-stimulators [23,24]. A large number of studies have shown that thermal damage can enhance the response of the innate immune system to release of excessive inflammatory mediators and improve the response of toll-like receptors [25]. It was reported that after burn, toll-like receptors were activated by MyD88 dependent pathway,

leading to the rapid activation of the MAPK signaling pathway, enhancement of innate immune response and increase in proinflammatory cytokines and the total stimulus [26].

In this research through analyzing the data of immune cell gene chip after burning it was found that the immune cells at the transcription level could change the immune function. At the same time, it was closely related to cellular processes, metabolic processes, response to the stimulation and cell biological regulation and apoptosis. Despite the results of chip analysis have certain limitations, they can also provide new view for follow-up research on clinical diagnosis and treatment to improve outcome of severely burned patients.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.burns.2018.08.022.

REFERENCES

[1] Greco 3rd JA, Pollins AC, Boone BE, Levy SE, Nanney LB. A microarray analysis of temporal gene expression profiles in thermally injured human skin. Burns 2010;36:192–204.
[2] Matuszczak E, Tylicka M, Debek W, Hermanowicz A, Ostrowska H. The comparison of C-proteasome activity in the plasma of children after burn injury, mild head injury and blunt abdominal trauma. Adv Med Sci 2015;60:253–8.
[3] Park MS, Salinas J, Wade CE, Wang J, Martini W, Pusateri AE, et al. Combining early coagulation and inflammatory status improves prediction of mortality in burned and nonburned trauma patients. J Trauma 2008;64:S188–94.
[4] Zhang W, Xie Y, Liu W, Xu X, Chen X, Liu H, et al. Role of metallothionein in post-burn inflammation. Inflammation 2016;39:768–74.
[5] Schwacha MG. Macrophages and post-burn immune dysfunction. Burns 2003;29:1–14.
[6] Yao CL, Somero GN. Thermal stress and cellular signaling processes in hemocytes of native (*Mytilus californianus*) and invasive (*M. galloprovincialis*) mussels: cell cycle regulation and DNA repair. Comp Biochem Physiol A Mol Integr Physiol 2013;165:159–68.
[7] Finnerty CC, Herndon DN, Przkora R, Pereira CT, Oliveira HM, Queiroz DM, et al. Cytokine expression profile over time in severely burned pediatric patients. Shock 2006;26:13–9.
[8] Lederer JA, Brownstein BH, Lopez MC, Macmillan S, Delisle AJ, Macconmara MP, et al. Comparison of longitudinal leukocyte gene expression after burn injury or trauma-hemorrhage in mice. Physiol Genomics 2008;32:299–310.
[9] Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, et al. A genomic storm in critically injured humans. J Exp Med 2011;208:2581–90.
[10] Vasconcellos P, Noia MP, De Castro ICV, Dos Santos JN, Pinheiro ALB, Marques AMC, et al. Influence of laser therapy on the dynamic formation of extracellular matrix in standard second degree burns treated with bacterial cellulose membrane. J Photochem Photobiol B Biol 2018;182:1–8.
[11] Zou Q, Gao YB, Jin H, Lu ZY, Shi PW, Yang L. Screening of biomarkers related with leukocyte responses early after burn injury in mice by differential gene expression profiling. Nan fang yi ke da xue xue bao — J South Med Univ 2017;37:767–73.
[12] Jin H, Gao Y, Lu Z, Zhou Q, Shi P, Yang L. Screening genes related with leukocyte responses early after burn injury: analysis of differentially gene expression profiling data in mice. Nan fang yi ke da xue xue bao — J South Med Univ 2015;35:1775–81.
[13] Gao Y, Nai W, Yang L, Lu Z, Shi P, Jin H, et al. Construction of an immunorelated protein-protein interaction network for clarifying the mechanism of burn. Burns 2016;42:405–13.
[14] Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2013;110:3507–12.
[15] Marshall A, Hodgson J. DNA chips: an array of possibilities. Nat Biotechnol 1998;16:27–31.
[16] Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, et al. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res 2002;30:e15.
[17] Smyth GK, Michaud J, Scott HS. Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 2005;21:2067–75.
[18] Wang J, Zhou X, Zhu J, Gu Y, Zhao W, Zou J, et al. GO-function: deriving biologically relevant functions from statistically significant functions. Briefings Bioinf 2012;13:216–27.
[19] Dennis Jr. G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 2003;4:P3.
[20] Zanzad NP, Godbole HV. Study of fatal burn cases in medico-legal autopsies. J Indian Acad Forensic Med 2007;29(3):42–9.
[21] Gadge SJ, Meshram RD, Shrigiriwar MB, Kuchekar SV. Epidemiological study of fatal burns cases in SVN government medical college. J Acad Ind Res 2014;2(10):552–5.
[22] Liu T, Ji RR. Toll-like receptors and itch. In: Carstens E, Akiyama T, editors. Itch: mechanisms and treatment. Boca Raton (FL).
[23] Brennan JJ, Gilmore TD. Evolutionary origins of toll-like receptor signaling. Mol Biol Evol 2018;35:1576–87.
[24] Gardner JC, Noel JG, Nikolaidis NM, Karns R, Aronow BJ, Ogle CK, et al. G-CSF drives a posttraumatic immune program that protects the host from infection. J Immunol 2014;192:2405–17.
[25] Oppeltz RF, Rani M, Zhang Q, Schwacha MG. Burn-induced alterations in toll-like receptor-mediated responses by bronchoalveolar lavage cells. Cytokine 2011;55:396–401.
[26] Li X, Rendon JL, Akhtar S, Choudhry MA. Activation of toll-like receptor 2 prevents suppression of T-cell interferon gamma production by modulating p38/extracellular signal-regulated kinase pathways following alcohol and burn injury. Mol Med 2012;18:982–91.