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ORIGINAL ARTICLE

Severe Burn Injury Progression and Phasic Changes of Gene Expression in Mouse Model

Dan Wu,[1,4] Ming Zhou,[2] Liang Li,[1] Xiangfeng Leng,[3] Zheng Zhang,[1] Ning Wang,[1] and Yanwei Sun[1]

Abstract— Patients with severe burns are susceptible to infectious complications including burn-site infections and sepsis. The purpose of this study was to explore the pathologic development of burn injury in a mouse model and to screen genes dysregulated at different time points on the basis of gene expression microarrays. Differential expression analysis identified a total 223 genes that related to only time progression independent of burn injury and 214 genes with aberrant expression due to burn injury. Weighted gene co-expression network analysis (WGCNA) of the 214 genes obtained seven gene modules which named as red, blue, turquoise, green, brown, yellow, and gray module, and the blue module was found to be significantly associated with severe burn injury progression, and in which several genes were previously reported being associated with inflammation and immune response, such as interleukin *IL-6*, *IL-8*, and *IL-1b*. Functional enrichment analysis indicated significant enrichment of biological processes that related to metabolism and catabolism, and pathways of proteasome, notch signaling and cell cycle. This result supports a phase progression of severe burn with gene expression changes and interpretation of biological processes in mouse.

KEY WORDS: Severe burn; WGCNA; Gene expression; Pathway.

INTRODUCTION

Burn is a complex traumatic tissue injury which can cause a range of fatal complications, including shock, infection, electrolyte imbalance, and respiratory failure [1, 2]. According to World Health Organization data, the worldwide incidence of burns is about 1%, and as many as 195,000 people die from burn injury each year [3, 4]. Despite a greater understanding of pathophysiological changes and a more sophisticated monitoring of clinical indicators, it is still impossible to accurately predict the post-burn process of different patients [5]. Multisystem organ failure is regarded as the most important symptom of burn injury, and 93% of these patients had systemic inflammatory response syndrome at time of death [6]. Thus, infections seemed to be responsible for the fatal clinical deterioration.

Dan Wu and Ming Zhou contributed equally to this work.

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[1] Department of Burn and Plastic Surgery, Zibo Central Hospital, Gongqingtuanxi Road, Zhangdian District, Zibo, 255036, Shandong, China
[2] Department of Joint Surgery, Zibo Central Hospital, Zibo, 255036, Shandong, China
[3] Department of Plastic Surgery, The Affiliated Hospital of Qingdao University, Qingdao, 266003, Shandong, China
[4] To whom correspondence should be addressed at Department of Burn and Plastic Surgery, Zibo Central Hospital, Gongqingtuanxi Road, Zhangdian District, Zibo, 255036, Shandong, China. E-mail: wudan17214@outlook.com

Whatever the location and depth of burn, burn victims will all experience different degrees of secondary infection [7]. The immunologic response to severe burn injury is pro-inflammatory during the first few days after burn injury. There is also a synergistic response between inflammation and coagulation systems [8]. Later, the anti-inflammatory response predominately restores normal physiology and maintains homeostasis [9]. Burn injury would lead to sustaining changes in the response, including serum cytokine induction, whole-blood lymphocyte populations, dendritic cell function/phenotype, and the ensuing adaptive immune responses of CD4 and CD8 T cell populations [10]. The bacterial translocation from the intestine has been shown to be the primary pathogenesis of most enterogenic infections and, in burns, may contribute to the development of sepsis. The risk of post-burn infection could be reduced in burn patients who are supported by earliest possible enteral nutrition [11]. Nowadays, most of the research on the immune reaction after burn injury were concentrated on the response of immune cells and immune factors [12]. However, recent reports have indicated that, genetic variation may also influence the risk for serious infections following burn injury [13, 14]. Huo's group identified *GNB2*, *LILRA2*, *ARRB2*, and *ARHGEF2* as potential key genes involved in the treatment of major burn injuries and prevention of complications [15]. Ou et al. confirmed that interleukins (*IL-6*, *IL-8*, and *IL-1b*) are continually differentially expressed throughout the time course post-burn injury by DNA microarray [16]. Therefore, the identification of differential gene expression would improve the prediction of biological processes during treatment of severe burn patients.

The mouse models have served as powerful means to explore basic pathophysiological mechanisms and evaluate new therapeutic approaches. Furthermore, it was suggested that gene expression patterns in mouse models closely recapitulate those in human inflammatory conditions [17]. Yin et al. identified the cholecystokinin 2 receptor as an analgesic target by transcriptomic analyses with a burn injury mouse model [18]. Nakazawa et al. found iNOS as one of the drivers of inflammation and apoptosis in mouse after burn injury [19]. It has been confirmed that, the orthologs of the injury-affected mouse leukocyte genes, which are related in pathways of immune response, anti-apoptosis, cell cycle control, chromosome segregation, DNA replication, chromosome condensation, and pyrimidine metabolism, shown to be similarly altered in sequential studies of gene expression obtained from human burn patients [20]. These reports indicated that the analysis of progression and phasic changes in burn injury mouse model would contribute to make go or no-go decisions to carry new drug candidates for human burn treatment forward into clinical trials. Thus in this study, gene expression profiles of burned mice from the Gene Expression Omnibus (GEO) database were analyzed to explore the relationship between the sequential changes of global gene expressions in the blood samples and pathophysiological process after burn injury. Weighted gene co-expression analysis (WGCNA) identified gene modules that significantly associated with the time after burn injury. Functional enrichment analysis of those modules indicated several biological processes that might be involved in burn injury processes. These results could shed light on the inflammatory progression post-burn injury and potentially improving the clinical outcome of burn injury.

MATERIALS AND METHODS

Study Population

The dataset of burn injury was downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo), with the accession number of GSE7404. C57BL/6J mice that purchased from the Jackson Laboratory (Bar Harbor, ME) were used in the raw dataset. Burn injury model in mice was induced by 25% total body surface area scald burn following inhalation anesthesia [20]. A total of 32 samples, including 16 blood samples of scald induced burn injury mice and 16 blood samples of mice underwent sham burn, were selected for this study. Gene expression profiles were detected at four time points, *i.e.*, 2 h, 1d, 2d, and 7d post-burn with four samples at each time point based on Affymetrix Mouse Genome 430 2.0 Array, for burn and sham group.

Microarray Preprocessing

The raw data of GSE7404 stored as the CEL file was normalized *via* the robust multi-array average (RMA) method by using the affy package. Probe names were converted to gene symbols according to the microarray annotation information. Logarithm transformation was applied to the normalized expression values for their normal distribution.

Differential Expression Analysis

Relative gene expression in the sham group was calculated to identify the differentially expressed genes that changed along with time over the 7-day time course independent of burn injury. For this analysis, the average gene expression value of sham group at 2 h was used to ensure a consistent baseline, and each biological replicate of the

sham group was analyzed individually to allow biological consistency to be measured in the analysis. Ratios of expression values of every sample in sham group at 1d, 2d, and 7d relative to the baseline were calculated and ratio > 2 or < 0.5, *i.e.*, |log2 (fold change)| > 1 was used as the criteria for the screening of differential expression genes (DEGs).

Identification of DEGs that changed over time due to burn injury should be important. For this purpose, sham replicate signal intensities for each time point were averaged to ensure a consistent baseline for each replicate; then, the ratios of expression values of every sample in burn group relative to the average gene expression values of time-matched sham group were calculated (sham burn blood 2 h used as control for burn blood 2 h *etc.*), and |log2 (fold change)| > 1 (|log2 (FC)| > 1) was used as the criteria for the screening of DEGs. All of the differential expression analysis were performed in R version3.4.1 programming software. Detailed comparisons were listed in Table 1.

Table 1. Comparison Details in Differential Expression Analysis

Case	Control	Case	Control
Sham_1day_1	Sham_2hour	Burn_2hour_1	Sham_2hour
Sham_1day_2	Sham_2hour	Burn_2hour_2	Sham_2hour
Sham_1day_3	Sham_2hour	Burn_2hour_3	Sham_2hour
Sham_1day_4	Sham_2hour	Burn_2hour_4	Sham_2hour
Sham_2day_1	Sham_2hour	Burn_1day_1	Sham_1day
Sham_2day_2	Sham_2hour	Burn_1day_2	Sham_1day
Sham_2day_3	Sham_2hour	Burn_1day_3	Sham_1day
Sham_2day_4	Sham_2hour	Burn_1day_4	Sham_1day
Sham_7day_1	Sham_2hour	Burn_2day_1	Sham_2day
Sham_7day_2	Sham_2hour	Burn_2day_2	Sham_2day
Sham_7day_3	Sham_2hour	Burn_2day_3	Sham_2day
Sham_7day_4	Sham_2hour	Burn_2day_4	Sham_2day
		Burn_7day_1	Sham_2day
		Burn_7day_2	Sham_2day
		Burn_7day_3	Sham_2day
		Burn_7day_4	Sham_2day

Sham_timepoint_n ($n = 1, 2, 3, 4$), the nth sham sample at time point; Sham_timepoint, average expression value of sham samples at time point; Burn_timepoint_n ($n = 1, 2, 3, 4$), the nth burn sample at time point

Functional Enrichment Analysis

To identify functions involved in burn injury, pathway enrichment analysis was performed for DEGs by using the ToppGene database (toppgene.cchmc.org/). Pathways with FDR < 0.05 were screened out as the final results.

Construction of Weighted Gene Co-expression Network

To identify genes that associated with the progression after burn injury, the weighted gene co-expression network analysis (WGCNA) R package was used for screening gene modules significantly associated with time points after burn injury. The expression data of DEGs in burn group compared with sham group were imported into R and preliminarily filtered. Consistency of gene expression profiles among genes was measured by Pearson correlation, and power adjacent function was applied to the Pearson correlation matrix, which was transformed into scale-free weighted gene co-expression networks. Gene modules were identified by the thresholds of power = 6 and minModuleSize = 6. Besides, associations between each module and time progression after burn injury were evaluated through correlation analysis for identifying the most reliable co-expression gene module (CEM) for burn injury healing.

Functional Enrichment Analysis of CEMs

To investigate functions involved in genes contained in CEMs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted by the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) and KOBAS (http://kobas.cbi.pku.edu.cn/index.php) online tool respectively. Biologic progress (BP) terms of GO terms and KEGG pathways with P_{BH} (P value by Benjamini and Hochberg) < 0.05 were screened out. Besides, enrichmentMap plug-in of Cytoscape software was used for exploring associations among significantly enriched BP terms.

RESULTS

DEGs over Time in the Sham Time Course

To detect changes in gene expression along with the time period, we compared the gene expression profiles at 1d, 2d, and 7d post-burn to that 2 h post-burn in the sham group. As a result, we obtained a total of 223 genes that were significantly and consistently dysregulated over time in the sham burn blood. A heat map illustrated that the majority of differentially expressed genes in the sham time course were upregulated, while few of them were down-regulated over time (Fig. 1A). Figure 1B showed a unimodal $\log_2$(FC) distribution of the sham time course throughout the experiment. However, the peak of curves became toward the positive side of zero at 1d, 2d, and 7d in sham group, indicating a global upregulation of these genes over time.

DEGs Changed over Time Due to Burn Injury

The results for DEGs changed over time due to burn injury revealed 214 genes which were significantly and consistently dysregulated in burn group compared with sham group at the corresponding time points. Examination of the heat map of these genes (Fig. 2A) revealed a phasic pattern, and the number of upregulated DEGs at 2 h and 1d were significantly more than those of 3d and 7d. Figure 2B illustrated the $\log_2$(FC) distribution of every sample in burn group compared with the average expression value of sham group at each corresponding time point. It could find that more genes tended to exhibit non-differential expression in burn samples compared with sham burn samples on 2d and 7d, which might attribute that burn mices were gradually recovering. Additionally, we identified three overlapping genes including *Lipg*, *Klrc2*, and *Pclo* among the DEGs in burn samples at the four time points as shown in supplemental Fig. 1.

DEGs Related to Time and Burn Injury and Functional Enrichment Analysis

GO and KEGG analysis failed to identify pathway because of the small size of the data set. Thus the ToppGene was used for the functional enrichment analysis. A total of 32 genes were found to be commonly differentially expressed in burn group compared with the sham group at the corresponding time points (Fig. 3A). The heat map in Fig. 3B illustrated that the majority of 32 genes were upregulated in sham group at 1d, 2d, and 7d compared with samples at 2 h and in burn group compared with sham samples at corresponding time point. Besides, the fold changes in burn group were generally larger than that in sham group. Pathway enrichment analysis of those 32 genes identified a total of 28 significantly enriched pathways that related to neurodegenerative disease and drug metabolism. Table 2 showed the full list of the 28 significantly enriched pathways.

Weighted Gene Correlation Network Analysis

The 32 samples were firstly clustered based on the expression profiles of the 214 DEGs in burn group. WGCNA of the 214 genes obtained seven gene modules which named as red, blue, turquoise, green, brown, yellow, and gray module; and the blue module was found to be significantly associated with severe burn injury progression. As shown in Fig. 4A, the burn groups and sham groups were well separated with samples of the same point clustered together. Interestingly, the samples in burn blood 3d and burn blood 7d were also gathered together.

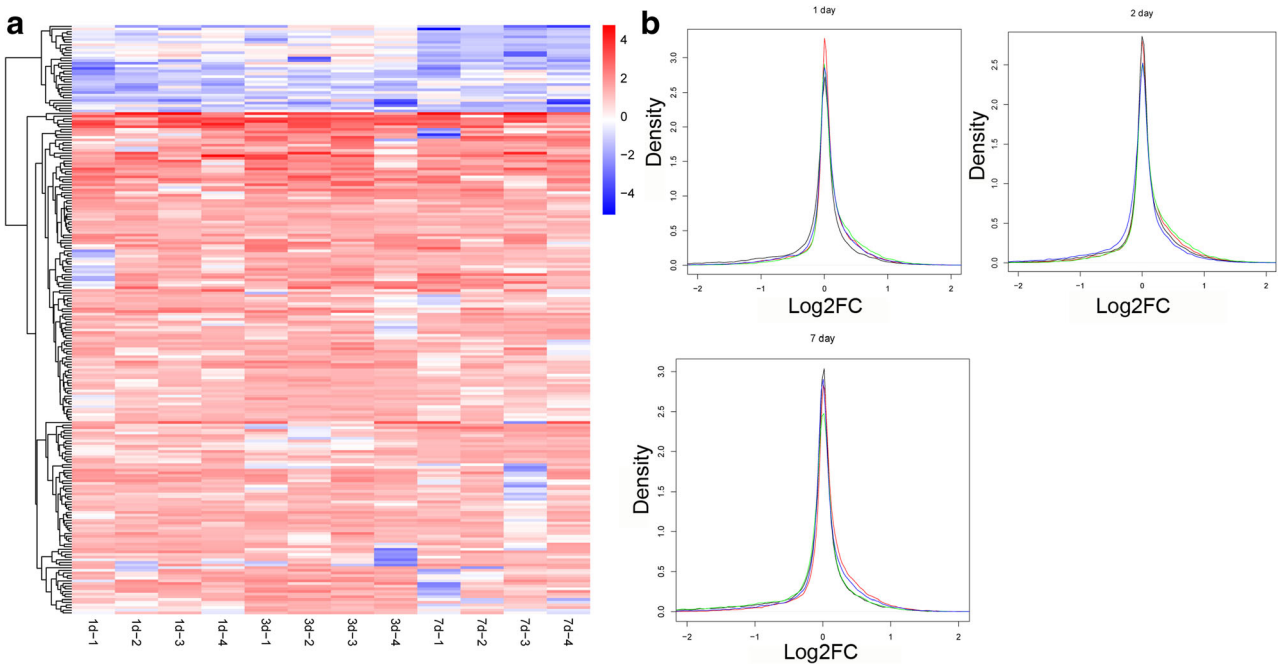

Fig. 1. Differential expression analysis among samples in sham group. (**a**) Heatmap shows the log2(FC) of the 223 expression values of DEGs in every sample at 1d, 3d, and 7d compared with the average expression value of samples at 2 h. (**b**) Distribution of the 223 log2(FC) of DEGs in every sample at 1d, 3d, and 7d compared with the average expression value of samples at 2 h. Samples were distinguished by lines with different colors.

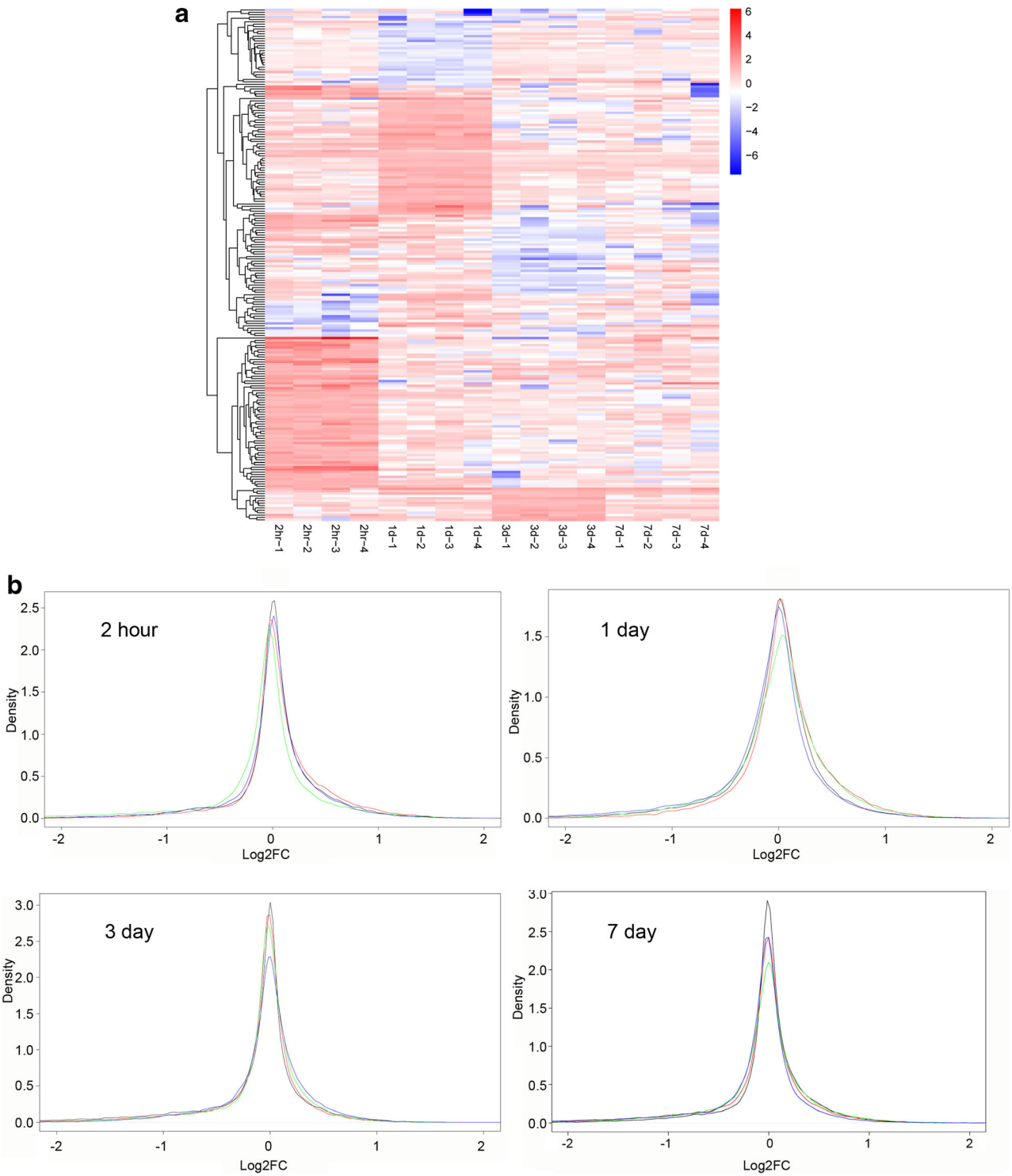

Fig. 2. Differential expression analysis between burn and sham samples. (**a**) Heatmap shows the log2(FC) of the 214 expression values of DEGs in every burn sample compared with the average expression value of samples at the corresponding time point in sham group. (**b**) Distribution of the 214 log2(FC) of DEGs in every burn group compared with the average expression value of samples at the corresponding time point in sham group. Samples were distinguished by lines with different colors.

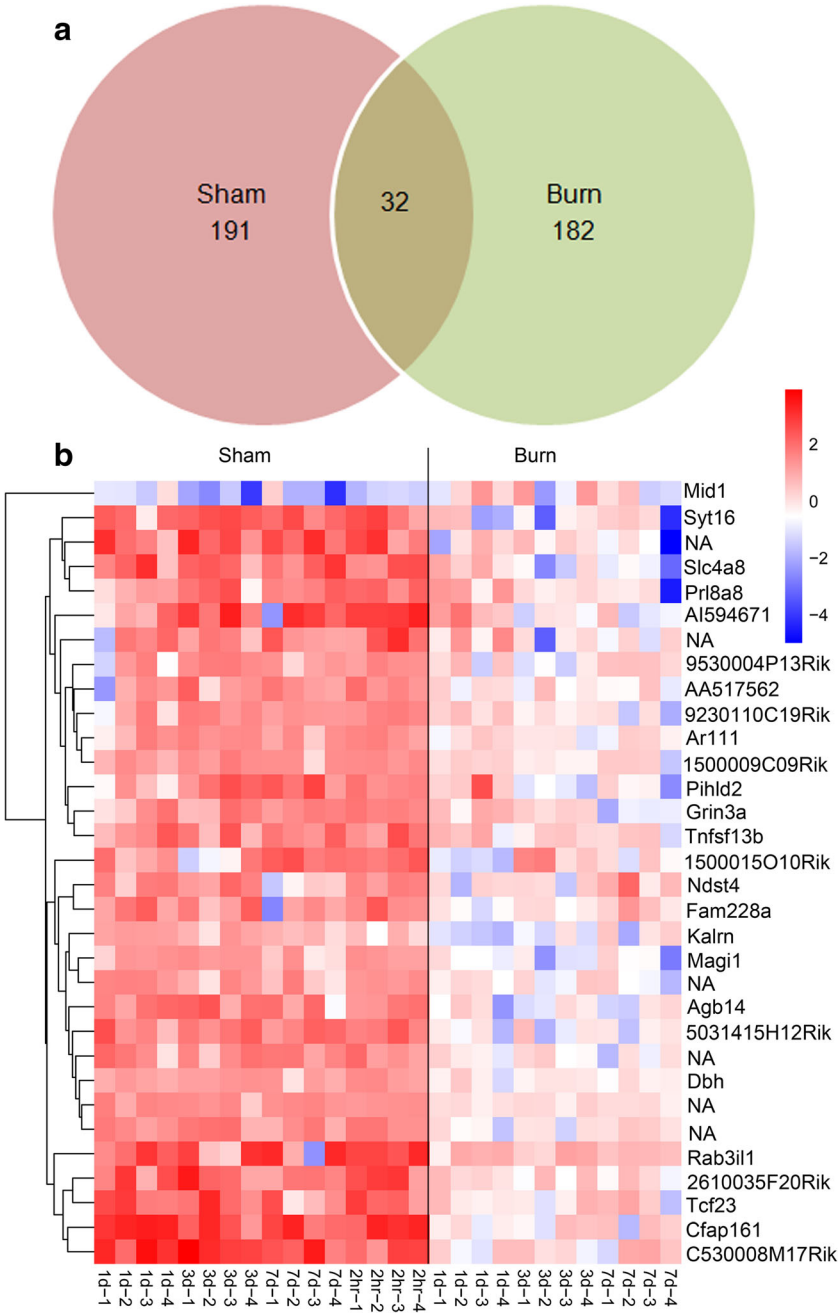

Fig. 3. Overlapping analysis between the two lists of DEGs. (**a**) Venn diagram indicates 32 overlapping genes between DEGs related to time progression and DEGs related to burn injury. (**b**) Heatmap shows the log2(FC) of the 32 overlapping genes in differential expression analysis among samples in sham group and between burn and sham samples.

WGCNA analysis obtained six modules that might be associated with the time duration post-burn injury, which colored turquoise, blue, brown, yellow, green, and red modules and contain 108, 40, 18, 17, 16, and 8 genes, respectively (Fig. 4B). The gray module represented unassigned genes. Genes contained in each module were provided in Supplemental Table 1. Figure 4C illustrated expression changes of genes in each module over time post-burn injury. Expression values of genes in turquoise, red, and blue modules peaked at 1d and decreased at later time points.

Table 2. Significantly Enriched Pathways of the 32 Overlapping Genes

ID	Name	Source	*p* value	P_{BH}	Count
1,270,176	Catecholamine biosynthesis	BioSystems: REACTOME	2.89E−03	4.76E−02	5
SMP00012	Catecholamine Biosynthesis	SMPDB	2.89E−03	4.76E−02	5
P00029	Huntington disease	PantherDB	3.33E−03	4.76E−02	4
413,357	Catecholamine biosynthesis, tyrosine ≥ dopamine ≥ noradrenaline ≥ adrenaline	BioSystems: KEGG	3.61E−03	4.76E−02	5
M4557	Biosynthesis of neurotransmitters	MSigDB C2 BIOCARTA (v6.0)	4.33E−03	4.76E−02	2
413,365	Glycosaminoglycan biosynthesis, heparan sulfate backbone	BioSystems: KEGG	7.21E−03	4.76E−02	3
1,269,909	Bicarbonate transporters	BioSystems: REACTOME	7.21E−03	4.76E−02	2
PW:0000455	Excitatory synaptic transmission	Pathway Ontology	7.21E−03	4.76E−02	2
138,038	Arf6 downstream pathway	BioSystems: Pathway interaction database	1.08E−02	4.76E−02	3
M12985	TACI and BCMA stimulation of B cell immune responses.	MSigDB C2 BIOCARTA (v6.0)	1.08E−02	4.76E−02	2
SMP00303	Nadolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00298	Atenolol pathway	SMPDB	1.22E−02	4.76E−02	3
SMP00302	Metoprolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00296	Acebutolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00300	Bisoprolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00299	Betaxolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00307	Propranolol Pathway	SMPDB	1.22E−02	4.76E−02	4
SMP00305	Penbutolol pathway	SMPDB	1.22E−02	4.76E−02	3
SMP00301	Esmolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00304	Oxprenolol pathway	SMPDB	1.22E−02	4.76E−02	3
SMP00366	Nebivolol pathway	SMPDB	1.22E−02	4.76E−02	5
SMP00297	Alprenolol pathway	SMPDB	1.22E−02	4.76E−02	2
SMP00306	Pindolol pathway	SMPDB	1.22E−02	4.76E−02	3
1,269,330	TNF receptor superfamily (TNFSF) members mediating non-canonical NF-kB pathway	BioSystems: REACTOME	1.29E−02	4.76E−02	2
1,270,175	Amine-derived hormones	BioSystems: REACTOME	1.29E−02	4.76E−02	2
545,274	Heparan sulfate biosynthesis (late stages)	BioSystems: BIOCYC	1.37E−02	4.83E−02	2
SMP00367	Carvedilol pathway	SMPDB	1.51E−02	4.96E−02	2
SMP00368	Labetalol pathway	SMPDB	1.51E−02	4.96E−02	2

For brown module, most of the genes reached the highest expression values at 2 h and decreased gradually with time progression. Yellow and green modules gradually decreased from 2 h to 2d and recovered from 2d to 7d. To further explore the correlation between CEMs and burn time, CEM time correlation analysis was performed. As shown in Fig. 5A, the blue module was the most significant module that correlated with the time post-burn injury (correlation coefficient = 0.89, the correlation test *p* value = 3e−06).

Functional Enrichment Analysis of Genes in Blue Module

GO enrichment analysis identified 21 BP terms that significantly associated with genes contained in blue module as shown in Fig. 5B. Crosstalk analysis of those BP terms obtained two main functional clusters, which related with protein localization and metabolism respectively (Fig. 5C). Besides, KEGG pathway analysis indicated significant enrichment of 9 pathways, including HTLV-I infection, Notch signaling pathway and so on, in the genes of blue module. Table 3 shows the full list of significantly enriched KEGG pathways.

DISCUSSION

There is growing evidence that early treatment strategy of burn injury is highly correlated with the long-term outcome especially of severely burned patients [21, 22]. The inflammatory response starts immediately after burn and persists for up to several months. Burn injury increases the macrophage activity, thereby increasing the productive capacity of the pro-inflammatory mediators [23]. Meanwhile, the epidermis of the skin becomes damaged,

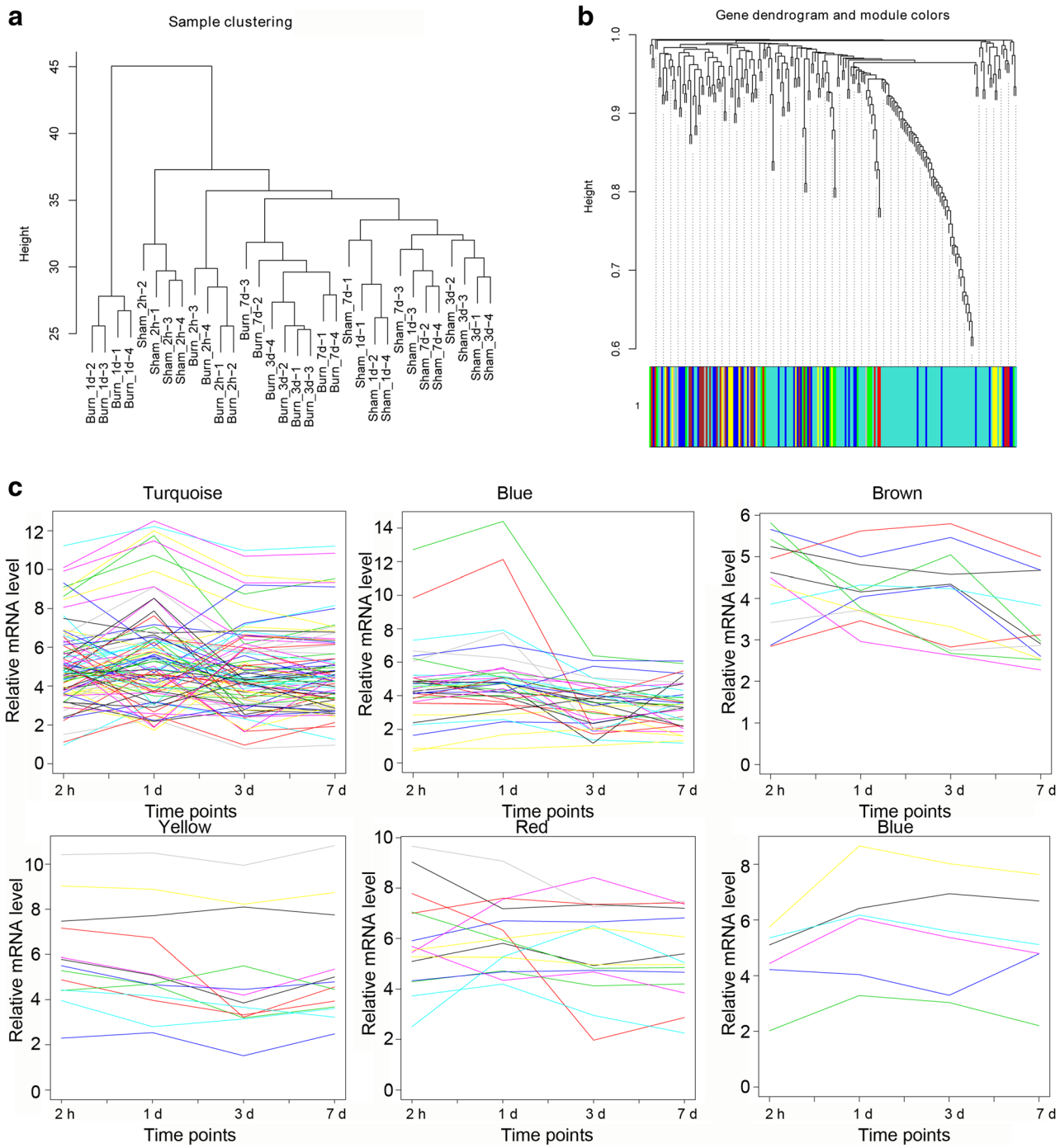

Fig. 4. Weighted gene co-expression network analysis. (**a**) Sample clustering analysis. (**b**) Weighted gene co-expression modules. Modules were distinguished by different colors. (**c**) Expression changes along with different time points of genes contained in every gene module.

allowing microbial invasion [24]. The total number of leukocyte cells begins to rise from 1 h after burn. At the same time, the percentage of neutrophils in the blood increased significantly and peaked at 12 h after injury [25]. Previous studies have reported a series of altered expressing cytokines, such as interleukin IL-1, IL-6, and

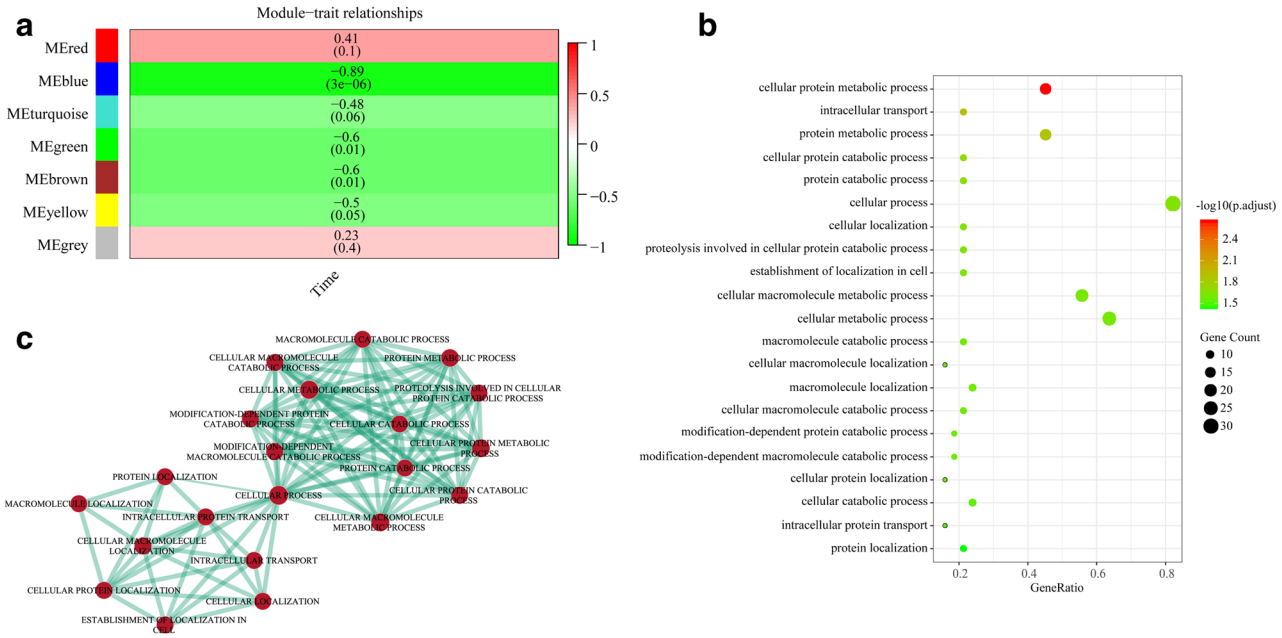

Fig. 5. Module-trait association and functional enrichment analysis. (**a**) Module-trait association analysis. Number contained in bracket indicates the *p* value and number outside the bracket represent the correlation coefficient between the gene module and time point after burn. (**b**) Significantly enriched BP terms of genes contained in the blue module. GeneRatio represents proportion of genes in blue module contained in the corresponding BP term in all of the genes contained the BP term. (**c**) Clustering analysis of the significantly enriched BP terms. Node indicates BP term and edge represents association between two BP terms if there any overlapping genes between the BP terms. Thicker line indicates more overlapping genes between two BP terms.

IL-8 [26–28], interferon-c [29, 30], and tumor necrosis factor alpha [31]. However, it was still necessary to achieve an overall understanding of gene expression changes after burn injury. Current efforts have advanced the research phase from "morphological" of wound repair to "molecular clues" of the temporal sequence of healing [32]. A study which focused on the changes of global gene expression patterns of the burn wound revealed an integrated upregulation of inflammatory and protease genes in acute time intervals, and a diminution of cytoskeleton and muscle contractile genes from 3 to 14 days after the injury [33]. Baron et al. confirmed the regulation of various biological processes simultaneously with the wound healing by microarray analysis, including cell proliferation, migration, inflammatory response, and immune response [34].

In this study, cluster analysis of the 32 samples by WGCNA showed that the burn groups and sham groups were well separated with samples of the same time point clustered together. Moreover, samples in burn blood 3d and burn blood 7d gathered together indicated that the expression pattern of these two time points is more similar. The Veen

Table 3. Significantly Enriched KEGG Pathways of Genes in the Blue Module

Name	ID	*p* value	P_{BH}	Count
Protein processing in endoplasmic reticulum	mmu04141	7.29E−07	2.99E−05	5
Ubiquitin mediated proteolysis	mmu04120	1.41E−05	2.88E−04	4
HTLV-I infection	mmu05166	2.09E−04	2.86E−03	4
Proteasome	mmu03050	9.87E−04	9.52E−03	2
Notch signaling pathway	mmu04330	1.16E−03	9.52E−03	2
Progesterone-mediated oocyte maturation	mmu04914	3.72E−03	2.54E−02	2
Oocyte meiosis	mmu04114	6.03E−03	3.17E−02	2
Lysosome	mmu04142	6.85E−03	3.17E−02	2
Cell cycle	mmu04110	6.95E−03	3.17E−02	2

diagram identified *Lipg*, *Klrc2*, and *Pclo* genes which were shared by the DEGs in burn samples at the four time points. *Lipg* is a protein coding gene, regulating the apelin signaling pathway and adipogenesis, which seemed to relate in the pro-inflammatory response of endothelial cells and immune-response of macrophages [35, 36]. *Klrc2* is one of the heterodimer encoders used by NK cells to sense HLA expression [37]. *Pclo* was mainly reported in nervous system diseases, and it also could be used to predict poor prognosis in several cancers [38, 39]. However, the role of these genes in the post-burn immune response remained unclear.

Thirty-two genes were found to commonly differentially express in sham as well as burn samples with the time extension. Also, the DEGs linked to impaired recovery from anesthesia were identified when comparing the sham 2 h to sham 1-7d groups. However, as the mouse in sham and burn groups all gone through the steps of anesthesia, we believed that the pre-treatment step of anesthesia would not affect the subsequent results analysis focused on burn injury, meaning the genetic differences obtained were still reliable. The functions of some of these genes were able to be annotated in previous studies. For example, *Syt16* have been reported associated in wound healing and development of motor neurons [40, 41]. *TNFsf13* was identified in the gene expressions of patients developing post-operative multiple organ failure [42]. Further functional analysis for the 32 genes indicated significant enrichment of TACI and BCMA stimulation of B cell immune responses and the TNF receptor superfamily (TNFSF) members mediating non-canonical NF-kB pathway processes, which were consistent with a severe inflammatory response of the burn injury. It has been clearly established that large burn injuries induce systemic immune dysfunction [43–47]. Besides, previous studies have also indicated that, different compositions existed in DEGs of different time points after burn injury, but the major biological pathways were associated with cell proliferation and inflammation [21].

Pathway enrichment analysis of those 32 genes identified a total of 28 significantly enriched pathways (Table 2). In the enriched pathways, the count value of the adrenaline-related pathways (ID:1270176, SMP00012, 413357) was higher than that of the immune response-related pathways (ID: M12985, 1269330). However, as we reported before, the focus of the time course pathological changes after burn injury in human were the regulation of immune response [48]. This may be because human actual burns occur in complex external environments, and the construction of animal models occurs more in cleaner laboratories. The infection suffered by humans after burns should be more serious and complicated than that of rats. In addition, a variety of drug metabolism-related pathways have also been enriched. Interestingly, the main effects of these drugs are regulation of heart rate, blood pressure, and adrenaline. We believe that this may be related to the self-regulation of the rodent's body under the severe pain caused by burns. In our previous study of human burn samples, these pathways were not enriched due to the use of pain relief and anesthetic drugs during treatment.

Dinh et al. found that the predominant gene groups upregulated initially after burn injury, included the heat shock proteins, growth arrest and repair genes, and DNA binding genes. These acute alterations generally had returned to baseline by 24 h post-burn [49]. The local and systemic inflammatory response to burn injury is extremely complex. Although the inflammation is initiated almost immediately after the burn injury, the systemic response progresses with time, usually peaking 5 to 7 days after the burn injury [50]. In this study, it was also found that burn day 1 should be a major outlier, which was consistent with other published findings. The consistent gene expressions in sham group from 1d to 7d indicated a stable physical state of mouse without burn injury. Besides, the gene expressions in burn blood 3d and burn blood 7d were also consistent with the pathophysiology of burns injury. However, the gene expression similarities between sham and burn at 2 h were found in Fig. 4A, which required further research to confirm.

An animal model research up to 2 weeks post-burn confirmed that the gene expressions were up-regulated at 2 h after the injury and returned to baseline by 3 days [30]. In this study, 40 genes in blue CEM were also identified as key genes associated with burn injury because of their strong negative correlation with time extension post-burn. The expressions of these 40 genes rose to a peak at 1d and then quickly decreased, indicating the expression of these genes matched with the wound healing process. These 40 genes shown in Supplemental Table 1 were all reported for the first time that significantly associated with burn injury. However, most of these genes could be found in the studies on stem cells (*e.g.*, *Pou4f3* [51]), cell proliferation, and differentiation (*e.g.*, *Tcf23* [52] and *EGFR* [53]), wound healing (*e.g.*, *Steap1* [54] and *Mt2* [55]), cell adhesion and inflammatory response (*e.g.*, *Vcam1* [56]) and chronic pain regulated by neuro-immune interaction (*e.g.*, *Adora2b* [57]). Moreover, the pathway analysis of the 40 genes in the blue CEM obtained a total of 9 significantly enriched pathways. Among the significantly enriched BP terms by functional analysis for the 40 genes in the blue CEM include those significantly associated with protein metabolic process, intracellular transport, cellular process, and cellular localization. As bury injury initiates systemic

inflammatory reactions producing burn toxins and oxygen radicals and finally leads to peroxidation in both local burn tissue damage and deleterious systemic effects on all other organ systems, other infection-related pathways were also enriched. Functional crosstalk analysis identified 2 clusters which closely associated with protein localization and intracellular transport, and cellular metabolic process respectively. These clusters were linked by cellular process, which was consistent with previous research [58, 59]. The proteasome, notch signaling pathway and cell cycle were the most significantly enriched ones. This finding was consistent with the participation of multiple biological steps in burn injury and repair, indicating protein breakdown [60], stem cell-related wound healing [61], and proliferation and activation of adult and immune cells [62], respectively.

CONCLUSION

In this study, we obtained the global gene expression changes in several time points after burn injury and analyzed the biological processes regulated by these genes. The results demonstrated a module contained 40 genes that significantly associated with burns time, and the genes in the module were proved to be key genes associated with burn injury. This study was critical for understanding burn injury and promote the development of clinical therapies.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

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