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Original Research

Role of AhR and Foxo1 in skin inflammation in burn animal model via MAPK signaling pathway

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Abstract: Burn generally refers to thermal damage, including tissue damage caused by hydrothermal (water, soup, oil, etc.), flame, steam, high-temperature gas, hot metal liquid or solid (such as molten steel, ingot). However, little is known about the pathogenesis and inducement of skin inflammation in burned rats. Therefore, this study has carried out an in-depth analysis of the related causes of skin inflammation in burned rats. We analyzed the gene expression and the differentially expressed genes co-expression in burned rats. Subsequently, a set of functional dysfunction modules about inflammation of skin tissue in burned rats were obtained by comprehensive enrichment analysis. In addition, based on related network prediction analysis, we identified a number of regulatory factors, such as endogenous genes, nCRNAs, and transcription factors that have potential monitoring effects on skin inflammation in burned rats. Firstly, we obtained 2679 differentially expressed genes and 7 disease-related dysfunction modules in burned rats. Secondly, we identified a series of regulators related to skin inflammation in burned rats, including 117 ncRNAs (including miR-17-5p, miR-122-5p, and miR-140-5p), 31 transcription factors (including AhR, Foxo1 and Sp1) and 10 endogenous genes (including Il5, Atp5d, and Cox4i1). Core transcription factors AhR and Foxo1 may induce skin inflammation in burned rats through the cascade of MAPK signals. According to the results of this study, we can show a new method for biologists and pharmacists to reveal the inducement of skin inflammation in burned rats and provide a valuable reference for different treatment options.

Key words: Burn; MAPK cascade; Gene expression disorder; Dysfunction module.

Introduction

Burns are serious injuries, which may be caused by thermal, radiation, chemical or electrical injuries (1-5). At the same time, electrical burn is an important cause of trauma in the world. Electrical burn mainly affects adult males who have occupational contact in the world (6). A chemical burn is also the main cause of corneal injury. Oxidative stress, inflammation and angiogenesis after chemical burn may aggravate the corneal injury and lead to a visual loss (7). Heat and chemical burns are the most common types of burns. Severe chemical burns can also cause redness, blistering, skin loss and swelling (8). Burns is not a single pathophysiological event, but a destructive injury that can lead to structural and functional deficiencies in many organ systems (1). Burns may cause physiological changes in numerous organ systems in the body. Burn mortality is usually attributed to pulmonary complications (9). Burns are reported to be one of the major causes of accidental injuries and deaths. In the United States, burns and inhalation injuries cause considerable mortality and morbidity.

Burn size and inhalation injuries are important predictors of post-burn deaths (10-12). Burns are a common form of injury in childhood, and the greatest risk is in infancy, so burns are also a major form of injury for children all over the world (13, 14). Severe physical and psychological complications caused by burns require comprehensive rehabilitation and coordination of acute burn care teams (15). Burns may induce inflammation in blood and wounds. Burn depth and burn size are key determinants in evaluating patients with burns (16, 17). Severe burns are more likely to constitute systemic diseases. Severe burns can lead to hypermetabolism, which increases the metabolism of muscles, bones and fat, making patients susceptible to multiple organ dysfunction and sepsis, and even death (18-27).

A severe burn is a serious injury with a global impact (28). Therefore, many doctors around the world have carried out in-depth research on the nursing and treatment of burns and severe burns. Firstly, scholars believe that it is very important to estimate the severity of burns correctly for patients to get proper treatment and avoid overwork (29). Effective burn care contributes to the burden of surgery, and burn care is also a considerable challenge to the health care system. The treatment of large-scale burn patients is related to social stability, life-saving and disability reduction (30-32). Healing burn wounds are closely related to wound depth and premature ejaculation, and burns usually require spe-

cial treatment at special burn centers (30, 33). The study found that burn treatment in a moist environment is more conducive to the recovery of patients (34). The survival of patients after severe burns largely depends on the size of the burn. The modern development of burn care has greatly improved survival and outcomes (35). Burnt patients not only suffer from physical, psychological, social, and spiritual effects but also undergo significant changes in their quality of life related to health, so patients must receive comprehensive treatment and care (36).

In this study, we conducted a series of comprehensive analysis based on the data of burned rats to explore the related factors of skin tissue inflammation in burned rats. Overall, our comprehensive strategy not only provides new insights into effective burn care and treatment but also provides abundant resources and guidance for biologists to design more experiments.

Materials and Methods

Differential expression analysis

The expression microarray data set collected of burn-related disease samples from NCBI Gene Expression Omnibus (GEO) database (37), numbered GSE802. The collected disease samples were analyzed by four groups of difference analysis (1 h burn control, 4 h burn control, 8 h burn control, 24 h burn control), using R language limma package (38).

Function modules related to co-expression analysis and recognition

Firstly, we use weighted gene co-expression network analysis (WGCNA) (39) to analyze the correlation phenotype of the expression profiles of these differentially expressed genes and find the gene module of co-expression. Second, using the Nth power of the correlation coefficient, the weighted value of the correlation coefficient was used to calculate the correlation coefficient (individual coefficient) between the two genes. The connection between genes in the network was pursued from a scale-free network, which makes this algorithm more biologically meaningful. The correlation coefficient between genes is then used to construct a hierarchical cluster tree. Different branches of the cluster tree show different gene modules and different colors show different modules.

Functional and Pathway Enrichment Analysis and Identification of Dysfunctional Modules

Studying the functions and pathways of gene signals is usually an effective tool for studying molecular mechanisms of diseases, and the functions and pathways involved in modular genes can determine the mechanism of modular dysfunction in disease processes.

Therefore, we applied R Language Cluster profiler package (40) to supplement and analyze the Go function (p-value Cutoff = 0.01, q value Cutoff = 0.01) and KEGG pathway (p-value Cutoff = 0.05, q value Cutoff = 0.2) respectively. Due to the function and pathway of the modular gene, it has been identified as the main functional pathway for inducing inflammation in the skin tissue of rats.

Identification of transcription factors and regulation of ncRNA on modules

We first downloaded the target data from the TRRUST V2 (41) database. Then load ncRNA protein data (score> 0.5) from the RAID 2.0 (42) database and perform a centralized analysis based on this interaction data to determine the regulatory role of these transcription and ncRNA factors in the module. Pivot analysis refers to searching at least two driver interaction modules in the target pair and calculating the importance of the interaction between the driver and the module based on the hypergeometric test. TF and ncRNA with $P< 0.01$ are important pivots of the monitoring module. Finally, through statistical analysis, these pivots are called the main pivots. In addition, Cytoscape (43) is used to display and analyze the network (including connection calculation). Genetic screening is the main molecular regulator that regulates the modular process and is called the intrinsic gene. These endogenous genes may be key detox molecules that can cause burns in mice.

Results

Differentially expressed genes related to inflammation in burned rats

Dysfunction of gene expression often plays an important role in the occurrence of diseases. Therefore, we analyzed the differentially expressed genes based on the expression profiles of four groups of burn diseases at different stages in order to further understand the potential pathogenesis of burn. Combining the four groups,

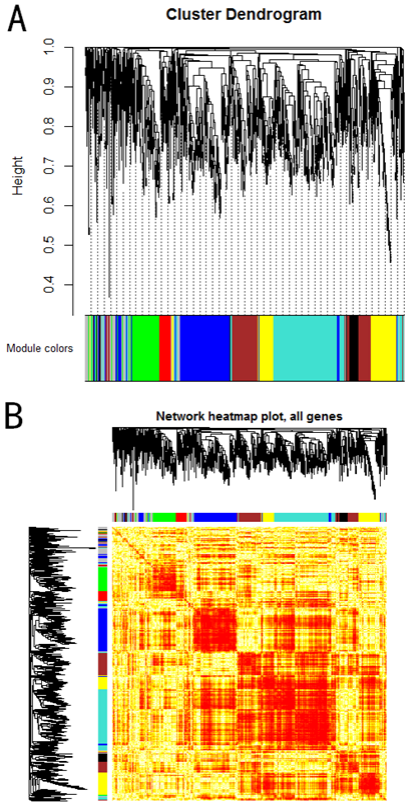

Figure 1. Clustering module of Co-expression relations of burn-related genes in rats. **A:** According to the co-expression relationship of different genes, seven modules are clustered, and one color represents one module. **B:** Thermogram of Modular Gene Expression in Samples. The phenomenon of grouping expression is intuitively presented in burn disease samples.

2679 differentially expressed genes were obtained. These 2679 differentially expressed genes were considered to be the key genes for the imbalance of burn expression in rats.

Co-expression Behavior of Differential Genes in Burn Rats

By combining the differentially expressed genes, we obtained the genes related to the expression disorder of burns in rats, but the regulatory mechanism and the synergistic relationship between them are still unclear. To this end, we continue to study the differential genes of burns in rats, construct the differential gene expression profiles and conduct co-expression analysis. The expression profiles of burn disease samples were analyzed by WGCNA. Seven modules involving 1445 module genes were excavated. These functional modules may be involved in a variety of functions and pathways and may exhibit a variety of monitoring mechanisms that mediate inflammatory-related diseases in burned mice.

Identification of functional dysfunction module in burned rats

The study of the functions and pathways involved in genes is an important tool for identifying and mediating pathogenesis. In order to evaluate the possible manifestations of the genetic diseases of the modules, we analyzed the high yield and path of each module. The results showed that most modules were enriched with rat-related burning functions and paths. We analyzed GO function and KEGG pathway enrichment in 7 functional modules and obtained 9140 functions and 252 KEGG pathway enrichment results. These include 1522 molecular functions (MF), 624 cellular components (CC) and 6994 biological processes (BP) involving genes (Figure 2A, 2B). It is noteworthy that they are significantly involved in the cascade of MAPK signaling pathways, including stress-activated MAPK cascade, negative regulation of stress-activated MAPK cascade and MAPK signaling pathway, which may be the core signaling pathway to induce burns in rats. From the above data, we can find that the MAPK signaling pathway may be closely related to the inflammation of burn skin tissue in rats.

Key ncRNAs and TFs mediating burn dysfunction module in rats

The scientific prediction of ncRNA, which regulates the genes of the dysfunction module, helps us to further discover the regulatory mechanism of burn transcription. For this purpose, a pivotal analysis was performed based on the targeting relationship between ncRNA and genes to predict the ncRNA regulator that disrupted the modules. It was found that 117 ncRNAs had a significant monitoring effect on the modules, including 131 pairs of ncRNA-module interactions (Figure 3).

Statistical analysis of the results showed that miR-17-5p had a strong regulatory effect on the three dysfunction modules. Therefore, ncRNA, identified as the core, was a key disorder molecule in inducing inflammation of burn skin tissue in rats. MiR-122-5p also plays an important role in the dysfunction of a dysfunctional module. In addition, other ncRNAs also have a certain driving effect on the potential dysfunction mechanism

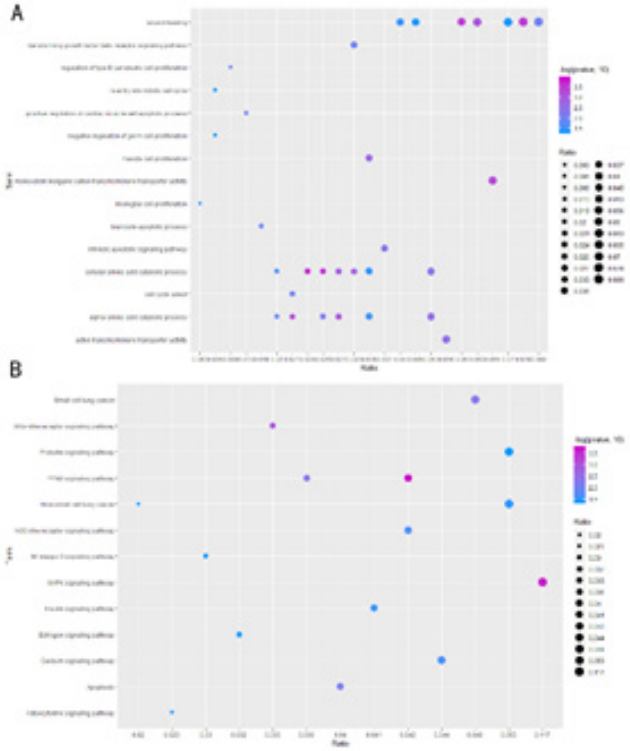

Figure 2. Modular genes involved in the function and pathway detection of rat burn function modules. The results of GO functional enrichment include modular genes. The darker the color, the stronger the density. The larger the circle, the greater the number of GO genes in the modular gene. **B:** Enrichment results of the KEGG pathway related to modular genes. The darker the color, the stronger the density. The larger the circle, the greater the ratio of modular genes to KEGG pathway genes.

Figure 3. The ncRNA regulatory network map of burned rats shows that the green box represents the module and the yellow box represents the ncRNA corresponding to the module.

of burns in rats.

Similarly, transcription factors are equally important in the regulation of gene transcription. Many studies have shown that failure to follow transcription rules can lead to a variety of diseases. Impaired burn function in rats is also closely related to transcription factor dysfunction, and the regulation of transcription factor dysfunction also reflects transcription factor dysfunction. Therefore, we use pivot analysis to predict the module according to the regulation of genes by transcription factors. The results showed that 31 transcription factors had significant transcriptional regulation effects on the potential dysregulation mechanism of burns in rats, involving 36 Pivot-Module interaction pairs (Figure 4). Statistical analysis of these transcription factors showed that both AhR and Foxo1 had a significant regulatory re-

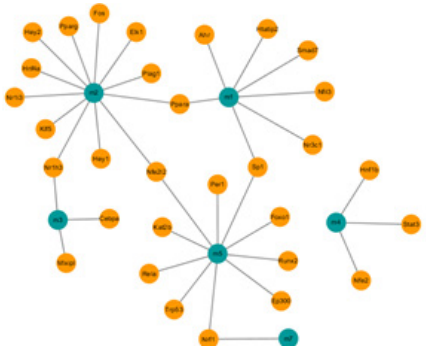

Figure 4. The regulatory network of transcription factors in burns in rats shows that the blue circle represents the module and the orange circle represents the corresponding transcription factors in the module.

lationship with one dysfunction module, and they might be involved in the mechanism of burns in rats. These data suggest that transcription factors may play an important role in the mechanism of burns disorder in rats. These transcription factors, which have significant regulatory effects on multiple dysfunction modules, have been identified as the core transcription factors of burns in rats. In addition, we analyzed the network connectivity based on the dysfunction module, and selected the genes with the highest connectivity in the module, and identified them as introductory genes. There were 10 genes, including Il5, Atp5d and Cox4i1. These intrinsic genes have high connectivity in the network module, which may play a key role in inducing burn-related diseases in rats.

Discussion

Burns are a common type of skin injury. Severe burns are usually accompanied by hypermetabolism, characterized by significant muscle atrophy, which leads to considerable morbidity and mortality (44, 45). Although in recent years, the research on inflammation of burned skin tissue mainly focused on some genes or proteins and related signaling pathways and achieved some results. However, the global regulation of these genes, proteins and signaling pathways in inducing inflammation in burned skin tissue of rats remains unclear. To further explore the core pathways and regulators that induce inflammation in rat burn skin tissue, we established a multi-dimensional integration method based on gene expression data, co-expression analysis, enrichment analysis, transcriptional and post-transcriptional regulatory data and module interaction.

In order to explore the core pathways and regulators of skin inflammation induced by burns in rats, we obtained 1589 differentially expressed genes based on disease-related gene expression data. Among them, Tat plays an important role in inducing inflammation of burn skin tissue in rats. Tat-Rac1 can increase the proliferation and migration of keratinocytes and dermal fibroblasts in vitro, thus playing a key role in wound healing of burned skin (46). Secondly, we integrate the differentially expressed genes and observe their co-expression behavior in the disease samples. From this, we get seven co-expression modules. The genes contained in the modules are considered to have a co-expression phenomenon. Subsequently, enrichment analysis found that genes in seven functional modules mainly participate in the cascade pathway of MAPK signal, including stress-activated MAPK cascade, negative regulation of stress-activated MAPK cascade and MAPK signaling pathway. Therefore, the cascade of the MAPK signaling pathway is considered to be the core signaling pathway to induce inflammation of burn skin tissue in rats. The inflammation signal transduction pathway of MAPK has been found to play an important role in the host response to injury. Local application of p38 MAPK inhibitors is a clinically feasible treatment after burn (47). Subsequently, we identified transcription factors that significantly regulate these seven dysfunction modules, obtained 31 transcription factors and 36 Pivot-Module interaction pairs. Among them, both AhR and Foxo1 have significant regulatory effects on a dysfunction module, which indicates that they play an important role in the process of inducing burns in rats. This is confirmed by Quintana FJ et al (48, 49) that AhR signal transduction is beneficial to solve the inflammation caused by excessive burns, and the overexpression of AhR may also lead to tissue inflammation and autoimmunity. Besides, the Foxo1-mediated gene transcription pathway is a key trigger for protein catabolism in muscle induced by inflammatory reaction (50). Therefore, it can be concluded that AhR and Foxo1 are core transcription factors inducing skin inflammation in burned rats through the cascade of MAPK signals.

In addition, ncRNA has been considered to be an important regulator of disease occurrence and development. We conduct pivot analysis based on the targeting relationship between ncRNA and genes. The predicted results show that 117 ncRNAs have significant regulatory effects on the module. It was found that microRNA-17-5p had significant regulatory effects on three dysfunction modules. It is reported that microRNA-17-5p may inhibit skin inflammation induced by burn injury (51). Mir-122-5p plays a regulatory role in one module, which may promote keratinocyte proliferation and play an important role in the persistent inflammatory response induced by severe burns by down-regulating the expression of SPRY-2 (52). At the same time, we also screened a series of genes with the greatest connectivity as the core molecule of the progress of the regulatory module, that is, the intrinsic gene. In total, there are 10 endogenous genes, which may represent potential disorders that induce inflammation in the burned skin tissue of rats. Among them, Il5 was found to play a major role in local inflammation of burns (53).

A series of regulatory factors predicted in this study have certain regulatory effects on the response process of skin inflammation induced by burns in rats to varying degrees. However, in addition to the above key factors, other unmentioned ncRNA and transcription factors may play a role in the mechanism of inducing imbalance in burned rats, which needs further exploration.

Overall, our study confirms that core transcription factors AhR and Foxo1 are key regulators that can induce inflammation of burn skin tissue in rats through the cascade of MAPK signals. It can not only provide a new way for biologists and pharmacists to study the effect of MAPK signal on burns, but also provide a valuable reference for their follow-up treatment.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
KL designed the study and drafted the manuscript. YF and ZX were responsible for the collection and analysis of the experimental data. CX revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Shanxian Central Hospital, China.

Consent for publication
Not applicable.

Conflict of interest
The authors declare that they have no competing interests.

References

1. Abdullahi A, Amini-Nik S, Jeschke MG. Animal models in burn research. Cell Mol Life Sci 2014; 71: 3241-3255.
2. Vaughn L, Beckel N. Severe burn injury, burn shock, and smoke inhalation injury in small animals. Part 1. burn classification and pathophysiology. J Vet Emerg Crit Care 2012; 22: 179-186.
3. Liang Y, Lin Q, Huang P, Wang Y, Li J, Zhang L, Cao J. Rice Bioactive Peptide Binding with TLR4 To Overcome H2O2-Induced Injury in Human Umbilical Vein Endothelial Cells through NF-κB Signaling. J Agri Food Chem 2018; 66(2): 440-448.
4. Wang L, Lin Q, Yang T, Liang Y, Nie Y, Luo Y, Luo F. Oryzanol modifies high fat diet-induced obesity, liver gene expression profile, and inflammation response in mice. J Agri Food Chem 2017; 65(38): 8374-8385.
5. Lou Y, Shi J, Guo D, Qureshi AK, Song L. Function of PD-L1 in antitumor immunity of glioma cells. Saudi J Boil Sci 2017; 24(4): 803-807.
6. Li H, Tan J, Zhou J, Yuan Z, Zhang J, Peng Y, Wu J, Luo G. Wound management and outcome of 595 electrical burns in a major burn center. J Surg Res 2017; 214: 182-189.
7. Gu XJ, Liu X, Chen YY, Zhao Y, Xu M, Han XJ, Liu QP, Yi JL, Li JM. Involvement of NADPH oxidases in alkali burn-induced corneal injury. Int J Mol Med 2016; 38: 75-82.
8. Yin S. Chemical and Common Burns in Children. Clin Pediatr 2017; 56: 8S-12S.
9. Knowlin LT, Stanford LB, Cairns BA, Charles AG. The effect of preexisting respiratory co-morbidities on burn outcomes. Burns 2017; 43: 366-373.
10. Ellison DL. Burns. Crit Care Nurs Clin North Am 2013; 25: 273-285.
11. Veeravagu A, Yoon BC, Jiang B, Carvalho CM, Rincon F, Maltenfort M, Jallo J, Ratliff JK. National trends in burn and inhalation injury in burn patients: results of analysis of the nationwide inpatient sample database. J Burn Care Res 2015; 36: 258-265.
12. Tanizaki S, Suzuki K. No influence of burn size on ventilator-associated pneumonia in burn patients with inhalation injury. Burns 2012; 38: 1109-1113.
13. Hollywood E, O'Neill T. Assessment and management of scalds and burns in children. Nurs Child Young People 2014; 26: 28-33.
14. Li H, Wang S, Tan J, Zhou J, Wu J, Luo G. Epidemiology of pediatric burns in southwest China from 2011 to 2015. Burns 2017; 43: 1306-1317.
15. Esselman PC. Burn rehabilitation: an overview. Arch Phys Med Rehabil 2007; 88: S3-S6.
16. Zhang J, Li X, Gao Y, Guo G, Xu C, Li G, Liu S, Huang A, Tu G, Peng H, Qiu S, Fan B, Zhu Q, Yu S, Zheng C, Liang S. Effects of puerarin on the inflammatory role of burn-related procedural pain mediated by P2X(7) receptors. Burns 2013; 39: 610-618.
17. Giretzlehner M, Dirnberger J, Owen R, Haller HL, Lumenta DB, Kamolz LP. The determination of total burn surface area: How much difference? Burns 2013; 39: 1107-1113.
18. Lin YH, Lin HH, Shi LP, Yeong EK. The Treatment of Major Burn Injuries. Hu li za Zhi J Nurs 2016; 63: 12-16.
19. Auger C, Samadi O, Jeschke MG. The biochemical alterations underlying post-burn hypermetabolism. Biochim Biophys Acta Mol Basis Dis 2017; 1863: 2633-2644.
20. Guo T, Lin Q, Li X, Nie Y, Wang L, Shi L, Luo F. Octacosanol attenuates inflammation in both RAW264. 7 macrophages and a mouse model of colitis. J Agri Food Chem 2017; 65(18): 3647-3658.
21. Li W, Jia MX, Wang JH, Lu JL, Deng J, Tang JX, Liu C. Association of MMP9-1562C/T and MMP13-77A/G polymorphisms with non-small cell lung cancer in southern Chinese population. Biomol 2019; 9(3): 107-119.
22. Nie Y, Luo F, Wang L, Yang T, Shi L, Li X, Shen J, Xu W, Guo T, Lin Q. Anti-hyperlipidemic effect of rice bran polysaccharide and its potential mechanism in high-fat diet mice. Food Func 2017; 8(11): 4028-4041.
23. Lou Y, Yang J, Wang L, Chen X, Xin X, Liu Y. The clinical efficacy study of treatment to Chiari malformation type I with syringomyelia under the minimally invasive surgery of resection of Submeningeal cerebellar Tonsillar Herniation and reconstruction of Cisterna magna. Saudi J Biol Sci 2019; 26(8): 1927-1931.
24. Lou Y, Guo D, Zhang H, Song L. Effectiveness of mesenchymal stems cells cultured by hanging drop vs. conventional culturing on the repair of hypoxic-ischemic-damaged mouse brains, measured by stemness gene expression. Open Life Sci 2016; 11(1): 519-523.
25. Chen X, Xu Y, Meng L, Chen X, Yuan L, Cai Q, Shi W, Huang G. Non-parametric partial least squares–discriminant analysis model based on sum of ranking difference algorithm for tea grade identification using electronic tongue data identify tea grade using e-tongue data. Sens Actuators B Chem 2020; 127924.
26. Nie Y, Luo F, Lin Q. Dietary nutrition and gut microflora: A promising target for treating diseases. Trends Food Sci Technol 2018; 75: 72-80.
27. Ren Y, Jiao X, Zhang L. Expression level of fibroblast growth factor 5 (FGF5) in the peripheral blood of primary hypertension and its clinical significance. Saudi J Biol Sci 2018; 25(3): 469-473.
28. Young AW, Dewey WS, King BT. Rehabilitation of Burn Injuries: An Update. Phys Med Rehabil Clin N Am 2019; 30: 111-132.
29. Reiband HK, Lundin K, Alsbjorn B, Sorensen AM, Rasmussen LS. Optimization of burn referrals. Burns 2014; 40: 397-401.
30. Fuzaylov G, Murthy S, Dunaev A, Savchyn V, Knittel J, Zabolotina O, Dylewski ML, Driscoll DN. Improving burn care and preventing burns by establishing a burn database in Ukraine. Burns 2014; 40: 1007-1012.
31. Lyu GZ, Xie YJ. Reflection on rescue and treatment of mass burn patients involved in August 2nd Kunshan factory aluminum dust explosion accident. Zhonghua Shao Shang Za Zhi 2018; 34: 326-328.

32. Holmes JH. 4th Critical issues in burn care. J Burn Care Res 29: S180-S187, 2008.
33. Li MY, Mao YG, Guo GH, Liu DW. Application of a hydrosurgery system in debridement of various types of burn wounds. Zhonghua Shao Shang Za Zhi 2016; 32: 574-576.
34. Karyakin NN, Klemenova IA, Luzan AS. The outcomes of lower extremities burn wounds management by using of controlled moist environment. Khirurgiia 2017; 40-43.
35. Kraft R, Herndon DN, Al-Mousawi AM, Williams FN, Finnerty CC, Jeschke MG. Burn size and survival probability in paediatric patients in modern burn care: a prospective observational cohort study. Lancet 2012; 379: 1013-1021.
36. Lo SF. Major Burn Trauma Management and Nursing Care. Hu li za Zhi J Nurs 2015; 62: 82-88.
37. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, L. Robertson C, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-D995.
38. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.
39. Langfelder P, Horvath S: WGCNA. An R package for weighted correlation network analysis. BMC Bioinformatics 2008; 9: 559.
40. Yu G, Wang LG, Han Y, He QY. Cluster Profiler: an R package for comparing biological themes among gene clusters. OMICS 2012; 16: 284-287.
41. Han H, Cho JW, Lee S, Yun A, Kim H, Bae D, Yang S, Kim CY, Lee M, Kim E, Lee S, Kang B, Jeong D, Kim Y, Jeon HN, Jung H, Nam S, Chung M, Kim JH, Lee I.TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. Nucleic Acids Res 2018; 46: D380-D386.
42. Yi Y, Zhao Y, Li C, Zhang L, Huang H, Li Y, Liu L, Hou P, Cui T, Tan P, Hu Y, Zhang T, Huang Y, Li X, Yu J, Wang D. RAID v2.0: an updated resource of RNA-associated interactions across organisms. Nucleic Acids Res 2018; 46: D380-D386.
43. Carlin DE, Demchak B, Pratt D, Sage E, Ideker T. Network propagation in the cytoscape cyberinfrastructure. PLoS Comput Biol 2017; 13: e1005598.
44. Yoshino Y, Ohtsuka M, Kawaguchi M, Sakai K, Hashimoto A, Hayashi M, Madokoro N, Asano Y, Abe M, Ishii T, Isei T, Ito T, Inoue Y, Imafuku S, Irisawa R, Ohtsuka M, Ogawa F, Kadono T, Kawakami T, Kukino R, Kono T, Kodera M, Takahara M, Tanioka M, Nakanishi T, Nakamura Y, Hasegawa M, Fujimoto M, Fujiwara H, Maekawa T, Matsuo K, Yamasaki O, Le Pavoux A, Tachibana T, Ihn H. The wound/burn guidelines - 6: Guidelines for the management of burns. J Dermatol 2016; 43: 989-1010.
45. Ma L, Chu W, Chai J, Shen C, Li D, Wang X. ER stress and subsequent activated calpain play a pivotal role in skeletal muscle wasting after severe burn injury. PloS One 2017;12: e0186128.
46. Fan B, Wang T, Bian L, Jian Z, Wang DD, Li F, Li F, Wu F, Bai T, Zhang G, Muller N, Holwerda B, Han G, Wang XJ. Topical Application of Tat-Rac1 Promotes Cutaneous Wound Healing in Normal and Diabetic Mice. Int J Biol Sci 2018; 14: 1163-1174.
47. Carter D, Warsen A, Mandell K, Cuschieri J, Maier RV, Arbabi S. Delayed topical p38 MAPK inhibition attenuates full-thickness burn wound inflammatory signaling. J Burn Care Res 2014; 35: e83- e92.
48. Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL, Kuchroo VK. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol 2010; 11: 854-861.
49. Quintana FJ, Jin H, Burns EJ, Nadeau M, Yeste A, Kumar D, Rangachari M, Zhu C, Xiao S, Seavitt J, Georgopoulos K, Kuchroo VK. Aiolos promotes TH17 differentiation by directly silencing Il2 expression. Nat Immunol 2012; 13: 770-777.
50. Kashiwagi S, Khan MA, Yasuhara S, Goto T, Kem WR, Tompkins RG, Kaneki M, Martyn JA. Prevention of Burn-Induced Inflammatory Responses and Muscle Wasting by GTS-21, a Specific Agonist for alpha7 Nicotinic Acetylcholine Receptors. Shock 2017; 47: 61-69.
51. Ji ZR, Xue WL, Zhang L. Schisandrin B Attenuates Inflammation in LPS-Induced Sepsis Through miR-17-5p Downregulating TLR4. Inflammation 2019; 42: 731-739.
52. Jiang M, Ma W, Gao Y, Jia K, Zhang Y, Liu H, Sun Q. IL-22-induced miR-122-5p promotes keratinocyte proliferation by targeting Sprouty2. Exp Dermatol 2017; 26: 368-374.
53. Xu H, Xu J, Xu L, Jin S, Turnquist HR, Hoffman R, Loughran P, Billiar TR, Deng M. Interleukin-33 contributes to ILC2 activation and early inflammation-associated lung injury during abdominal sepsis. Immunol Cell Biol 2018; 96: 935-947.