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Comparative gene expression profiling reveals key pathways in septic skeletal muscle

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Abstract. – AIM: Skeletal muscle transcriptome of patients with sepsis was compared with that of controls to elucidate the molecular mechanisms underlying sepsis-induced skeletal muscle dysfunction.

MATERIALS AND METHODS: Gene expression data set GSE13205 was downloaded from Gene Expression Omnibus (GEO), including 13 septic samples and 8 controls. Differentially expressed genes (DEGs) were screened out with t-test. Transcriptional regulatory network was constructed for the DEGs with information from UCSU. In order to identify altered biological functions in sepsis, pathway enrichment analysis was conducted for all the genes in the network with DAVID. Besides, relevant small molecules were retrieved using the Connectivity Map (camp).

RESULTS: A total of 287 DEGs were obtained in sepsis, 149 up-regulated and 138 down-regulated. A transcriptional regulatory network containing 83 nodes and 98 edges was then constructed. Five transcription factors (TFs) and their target genes were acquired. Significantly altered biological pathways included insulin signaling pathway, neurotrophin signaling pathway, fructose and mannose metabolism, circadian rhythm and apoptosis. Besides, a number of relevant molecules were obtained, such as trazodone and thapsigargin.

CONCLUSIONS: Our study provided an insight into the molecular changes sepsis and related skeletal muscle dysfunction. The information could be beneficial in disclosing the pathogenesis and developing effective therapies.

Key Words:
Sepsis, Skeletal muscle dysfunction, Gene expression data, Transcriptional regulatory network, Pathway enrichment analysis, Small molecules.

Abbreviations

GEO = Gene Expression Omnibus; DEGs = Differentially expressed genes; UCSC = University of California Santa Cruz; DAVID = Database for Annotation, Visualization and Integrated Discovery; camp: Connectivity Map; TFs; Transcription factors; SIRS = Systemic inflammatory response syndrome; NFkB = Nuclear factor-kB; AP-1 = Activator protein-1; RMA = Robust Multi-array Average; NCBJ Entrez = National Center for Biotechnology Information – Global Query cross Databases Search System; LIMMA = Linear Models for Microarray Data; KEGG = Kyoto Encyclopedia of Genes and Genomes; Mic = Myelocytomatosis viral oncogene; CBFB = Cote binding factor, beta subunit; FOX01 = Forkhead box 01; NFIL3 = Nuclear factor, interleukin 3; TGIF1 = TGFB-induced factor homeobox 1; PI3K = Phosphoinositide 3-kinase; Akt = Protein kinase B (PKB); IRS1 = insulin receptor substrate1; IRAK1 = interleukin-1 receptor-associated kinase 1; Toll/IL-1 = Toll-interleukin-1 receptor: B cl 2 = B-cell lymphoma2; PPAR-β/δ: Peroxisome-proliferator-activated receptor β/δ; LPS: Lipopolysaccharide.

Introduction

Sepsis is a medical condition featured by a whole-body inflammatory state (called a systemic inflammatory response syndrome or SIRS) caused by severe infection[1]. It leads to millions of deaths globally each year[2] and ranks in the top 10 causes of death[3].

Accelerated proteolysis of muscle is characteristic in patients with sepsis. Researchers have found that ubiquitin-proteasome pathway involves in the muscle proteolysis[4,5]. The gene expression of multiple ubiquitin ligases are up-regulated in skeletal muscle[6]. Williams et al[7] indicate that sepsis stimulates release of myofilaments in skeletal muscle by a calcium-dependent mechanism. Mitochondrial dysfunction also contributes to the muscle impairment as well as organ failure[8]. Besides, Penner et al[9] report that transcription factors nuclear factor-κB (NF-κB) and AP-1 are differentially regulated in skeletal muscle during sepsis.

Considering the complicated pathogenesis of sepsis, microarray technology enables global explorations of the molecular changes. The study by Prucha et al[10] present that microarrays can identify typical gene expression profiles in the blood of

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patients with severe sepsis. Tang et al[11] investigate gene-expression profiles of peripheral blood mononuclear cells in sepsis and find characteristic transcriptional changes that can be used to aid the diagnosis of this disease. Howrylak et al[12] explore the gene signature for acute lung injury in patients with sepsis using microarray technology.

In order to advance the understandings about the molecular mechanisms of sepsis and subsequent muscle dysfunction, transcriptome of skeletal muscle from patients with sepsis was compared with that of controls to identify differentially expressed genes (DEGs), which were further analyzed with bioinformatic tools.

Materials and Methods

Microarray data

Gene expression data set GSE13205[13] was downloaded from Gene Expression Omnibus (GEO)[14]. It contained 13 septic samples and 8 controls. Expression profiles were obtained using GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. Annotation files were collected with raw data.

Screening of DEGs

Raw data were normalized using *R* with Robust Multi-array Average (RMA) method[15] from package Affy. Then probes were mapped to NCBI Entrez. For probes corresponding to a same entrez gene ID, average expression level was calculated as the final number. The probe mapping to more than one gene was removed.

Differential analysis between sepsis and control was performed with using t-test using package LIMMA[16]. p value < 0.05 and $|\log FC| > 1.5$ were set as the cut-offs to filter out differentially expressed genes (DEGs).

Construction of transcriptional regulatory network

Transcriptional regulatory information was acquired from UCSU (http://genome.ucsc.edu)[17-18]. A total of 215 TFs and 214607 target genes were included. Then DEGs were mapped into the whole network and the corresponding network were visualized with Cytoscape[19].

Pathway enrichment analysis

Pathway information came from KEGG Pathway Database[20]. Fisher exact test[21] provided by DAVID[22] was chosen for the pathway enrichment analysis to identify altered biological functions during sepsis. The contingency table for Fisher exact test was shown in Table I. p value was calculated for each term with the following algorithm:

$$p = \frac{\binom{a+b}{a}\binom{c+d}{c}}{\binom{n}{a+c}} = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{a!\,b!\,c!\,d!\,n!}$$

Retrieval of relevant small molecules

Relevant small molecules were retrieved with the Connectivity Map (cmap)[23-24], which is a collection of genome-wide transcriptional expression data from cultured human cells treated with bioactive small molecules. It now contains more than 7056 expression profiles representing 1309 compounds.

The DEGs were divided into up- and down-regulated genes and then mapped to the probes in HG-U133A. The gene-expression changes in sepsis were compared with cmap database and relevant small molecules were acquired according to the enrichment scores.

Results

Differentially expressed genes

According to the criteria (p value < 0.05 and $|\log FC| > 1.5$), a total of 287 DEGs were obtained for sepsis, 149 up-regulated and 138 down-regulated. Details were listed in Supplementary Table I.

Transcriptional regulatory network analysis results

A transcriptional regulatory network containing 83 nodes and 98 edgeswas constructed for the DEGs (Figure 1). Five TFs were included in the network: MYC, CBFB, FOXO1, NFIL3 and TGIF1. The numbers of target genes were 37, 25, 16, 15 and 6, respectively.

Altered biological pathways in sepsis

Pathway enrichment analysis was performed for all the genes in the network. Terms with at least two genes were retained. The top 10 terms were listed in Figure 2. Significantly altered pathways included insulin signaling pathway, neurotrophin signaling pathway, fructose and mannose metabolism, circadian rhythm and apoptosis.

Table I. The contingency table for Fisher exact test.

	DEGs	No DEGs	Total
In Term	M (a)	y-m (b)	y
Not In Term	M-m (c)	Y-M–y+m (d)	Y-y
Total	M	Y-M	Y (n = a+b+c+d)

Y: number of total genes; M: number of DEGs; y: number of genes in a pathway; m: number of DEGs in a pathway.

Relevant small molecules

In order to collect information for treatment of sepsis, relevant small molecules were retrieved using cmap. Top 20 small molecules were listed in Table II. Trazodone (enrichment = –0.93), bezafibrate (enrichment = –0.842) and morantel (enrichment = –0.812) had negative scores, suggesting they could be potential medicines for sepsis. On the contrary, thapsigargin, podophyllotoxin and hexetidine might simulate physical conditions like sepsis or cause the incidence of sepsis. The results provided clues for future drug development.

Discussion

Through a comparative analysis of skeletal muscle transcriptome between septic patients

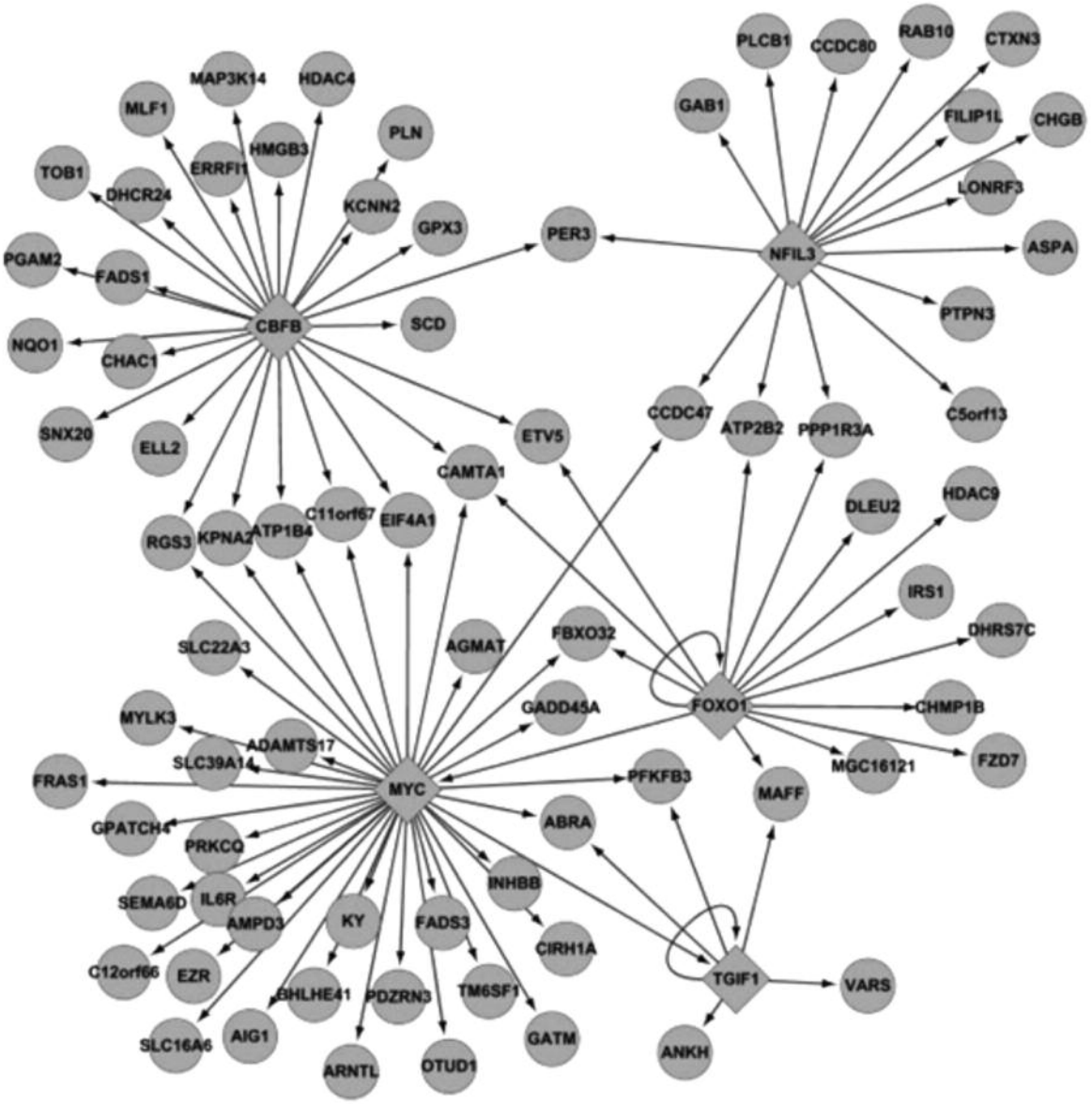

Figure 1. Transcriptional regulatory network for DEGs in the sepsis. Diamonds represent TFs and circles for target genes.

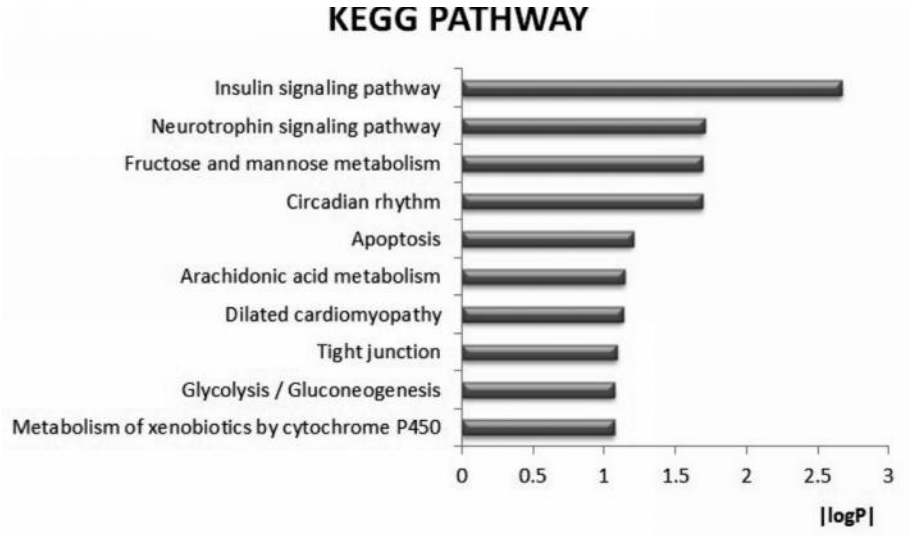

Figure 2. Top 10 pathways enriched in all the genes from the network.

and controls, a range of DEGs were identified for sepsis. To further find out key players in the sepsis-induced muscle dysfunction, transcriptional regulatory network analysis was performed, followed by pathway enrichment analysis, which revealed some interesting and characteristic changes in this disease.

Insulin signaling pathway was the most significantly disturbed pathway. The anabolic effect of insulin in skeletal muscle reflects increased protein synthesis and reduced protein degradation[25]. It has been recognized that insulin resistance and hyperglycemia are very common in septic patients[26]. Wang et al[27] find that insulin resistance causes muscle wasting by mechanisms that involve suppression of PI3K/Akt signaling leading to activation of caspase-3 and the ubiquitin-proteasome proteolytic pathway causing muscle protein degradation. Sepsis poses a great impact on the metabolism of muscle via insulin. Insulin therapy is applied for patients with severe sepsis, but its role remains uncertain[28]. Griesdale et al[29] carry out a meta-analysis and report that intensive insulin therapy significantly increase the risk of hypoglycemia and confer no overall mortality benefit among critically ill patients. Therefore, detailed characterization of the molecular mechanisms holds important clues to modulate the physiological process. Forkhead box O1 (FOXO1) belongs to the forkhead family of transcription factors which are characterized by a distinct forkhead domain. Akt/FOXO signaling participates in both protein loss and the impairment of muscle carbohydrate oxidation during sepsis[30]. Smith et al[31] indicate that sepsis increases the expression and activity of FOXO1 in skeletal muscle by a glucocorticoid-dependent mechanism. Recently published study by Castillero et al[32] reports that PPARβ/δ regulates FOXO1 activation in glucocorticoid- and sepsis-induced muscle wasting and that treatment

Table II. Top 20 small molecules retrieved from cmap.

cmap name	Enrichment score	*p* value
Podophyllotoxin	0.945	0
Monensin	0.922	0
Fludrocortisone	0.754	0
LY-294002	¬–0.379	0
Hexetidine	0.935	2.00E-05
Thapsigargin	0.97	4.00E-05
Tolnaftate	0.857	1.20E-04
15-delta prostaglandin J2	0.538	2.00E-04
Trazodone	–0.93	5.20E-04
Morantel	–0.812	5.60E-04
Atracurium besilate	0.925	8.00E-04
Naringenin	0.844	8.80E-04
Bezafibrate	–0.842	1.13E-03
Heptaminol	0.779	1.16E-03
Chlorhexidine	0.777	1.20E-03
Calcium folinate	0.768	1.50E-03
Pentoxifylline	0.759	1.94E-03
Heliotrine	–0.688	2.20E-03
Procaine	–0.742	2.24E-03
Amitriptyline	0.687	2.42E-03

with a PPARβ/δ inhibitor may ameliorate loss of muscle mass in these conditions. A number of genes were transcriptionally regulated by FOXO1, and some of them were also differentially expressed in sepsis. Insulin receptor substrate 1 (IRS1) is a protein which is phosphorylated by insulin receptor tyrosine kinase. Its downregulation in sepsis might contribute to the insulin resistance. Carvalho-Filho et al[33] report that aspirin attenuates insulin resistance in muscle of diet-induced obese rats by inhibiting inducible nitric oxide synthase production and S-nitrosylation of IRβ/IRS-1 and Akt. Further investigation on the target genes of FOXO1 might bring in new findings.

Neurotrophin signaling pathway was the second pathway affected by sepsis. Neurotrophins are a family of growth factors that are polypeptide in structure and are necessary for the development and maintenance of the vertebrate nervous system. The involvement of this pathway in sepsis is associated with inflammation and apoptosis. Interleukin-1 receptor-associated kinase 1 (IRAK1) participates in the IL1-induced up-regulation of NFκB. Arcaroli et al. report that variant IRAK-1 haplotype is associated with increased NFκB activation and worse outcomes in sepsis[34]. It also mediates LPS-induced myocardial contractile dysfunction. Thomas et al[35] find that IRAK1 deletion disrupts cardiac Toll/IL-1 signaling and protects against contractile dysfunction. B-cell CLL/lymphoma 2 (BCL2) is an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells. Its downregulation may contribute to the death of patient with severe sepsis[36]. Hotchkiss et al[37] report that overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. Therefore, it might be a good target to improve the outcomes of septic patients.

In addition to the above two pathways, fructose and mannose metabolism, apoptosis and arachidonic acid metabolism were also significantly over-represented in DEGs. They might be good directions to investigate the developmental mechanisms of sepsis-induced skeletal muscle dysfunction.

Moreover, relevant small molecules were retrieved from camp. The search is based upon the global match of gene expression profiles and, thus, the results are really suggestive. Negative enrichment score means the small molecule may reverse the effect of sepsis on transcriptome. Oppositely, positive score suggests it may generate a status like sepsis. These results might offer hints to disclose the molecular mechanisms of sepsis. Thapsigargin was of the most positive score. It's a tumor-promoting sesquiterpene lactone and discharges intracellular Ca^{2+} in rat hepatocytes[38]. This was partially in accordance with the calcium disorder in sepsis[39].

Conclusions

Overall, our study offered insights into the molecular mechanisms of sepsis and related skeletal muscle dysfunction. Some DEGs might be targets to modulate the progression of this disease and thus were worthy of further investigations.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

Acknowledgments

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