

Identification of differentially expressed genes associated with burn sepsis using microarray

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Abstract. The aim of the present study was to identify the potential target biomarkers associated with burn sepsis using microarray. GSE1781 was downloaded from Gene Expression Omnibus and included a collective of three biological replicates for each of the three conditions: Sham-Sham, Sham-cecal ligation and puncture (CLP) and Burn-CLP. Subsequently, limma was applied to screen the differentially expressed genes (DEGs). Additionally, functional annotations were predicted by pathway enrichment. Furthermore, the transcription factors were screened according to the transcriptional regulation from patterns to profiles database. Furthermore, the interaction associations of the proteins were obtained from the STRING database and the protein-protein interaction (PPI) network was constructed using Cytoscape. Finally, the gene co-expression analysis was conducted using CoExpress. In total, compared with Sham-Sham, a total of 476 DEGs and 682 DEGs were obtained in Sham-CLP and Burn-CLP, respectively. Additionally, 230 DEGs were screened in Burn-CLP compared with Sham-CLP. *Acadm*, *Ehhadh* and *Angptl4* were significantly enriched in the PPAR signaling pathway. Additionally, *Gsta3*, *Gstm2* and *Gstt1* in Burn-CLP were significantly enriched in glutathione metabolism. In the PPI network, the transcription factor *Ppargcla* interacted with *Angptl4*, while *Acadm* interacted with *Ehhadh*. The gene co-expression analysis showed that *Ehhadh* could be co-expressed with *Aqp8*. In conclusion, *Acadm*, *Ehhadh*, *Aqp8*, *Gsta3*, *Gstm2*, *Gstt1*, *Ppargcla* and *Angptl4* may be potential target genes for the treatment of burn sepsis.

Introduction

Sepsis, which is a systemic inflammatory syndrome, is triggered by severe infections (1). Infection is the most common and serious complication for the patients with a major burn (2). Once the burn wound is infected by the bacteria, bacterium could rapidly proliferate within the damaged tissue, which leads to sepsis and septic shock (3). Previously, it was reported that 50-60% of burn patients with sepsis succumb to the condition (4). Therefore, it is of great urgency to establish the mechanism of burn sepsis.

Previously, a study by Beffa *et al* (4) indicated that the interleukin 6 levels in burn mice were significantly increased by cecal ligation and puncture (CLP). However, the interleukin 6 levels did not decrease in the recovery patients following treatment with statin showing that there should be other mechanisms of sepsis in the burn patients. Additionally, high levels of interleukin 8 are correlated with increased multi-organ failure, sepsis and mortality in post-burn patients (5). Additionally, intestinal regulatory T cell expression has been found to exert immunosuppressive effects on other intestinal T lymphocytes and be closely associated with endotoxin translocation in porcine sepsis following severe burns injuries (6). However, the molecular mechanism of burn sepsis remains unclear.

In 2007, Banta *et al* (7) used moderate burn injury followed by CLP (used for producing sepsis) to construct three groups of rats: Sham Burn-Sham CLP (Sham-Sham), Sham Burn-CLP (Sham-CLP) and Burn-CLP. Subsequently, a microarray expression profile was conducted to screen the differentially expressed genes with the methods of significance analysis of microarrays and false discovery rate of 10% to investigate the contribution of gene expression to metabolic fluxes in hyper-metabolic livers induced by burn injury and CLP in rats and identified that burn injury combined with CLP led to the most significant changes, while CLP alone significantly increased metabolic gene expression; however, it decreased a number of the corresponding metabolic fluxes.

The present study aimed to use the same microarray data to further screen the DEGs between Sham-CLP and Sham-Sham, Burn-CLP and Sham-Sham, as well as Burn-CLP and Sham-CLP with the limma package based on the criteria of $P < 0.05$ and $|\log_2 \text{fold change (FC)}| \geq 2$ and collected the specific genes associated with burn sepsis. Additionally, the

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Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, transcription factor screening, protein-protein interaction (PPI) network construction and co-expression analysis of DEGs were also conducted to illustrate the mechanism of burn sepsis. A previous study proposed that analysis based on differential statistical tests may result in different outcomes (8). Therefore, we hypothesized that certain different results may be obtained from the data of Banta *et al* (7).

Materials and methods

Microarray data. The expression profile of GSE1781 deposited by Banta *et al* (7) was downloaded from Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>), which was based on the platform of the GPL341 [RAE230A] Affymetrix Rat Expression 230A array. GSE1781 included a collection of three biological replicates for each of the three conditions: Sham-Sham, Sham-CLP and Burn-CLP.

Data preprocessing and DEGs screening. The downloaded data were normalized using preprocess Core (9). The probes, which were not mapped to the corresponding gene symbols, were abandoned. Furthermore, the average expression value was used for the genes corresponding to multiple probes. Subsequently, the limma (linear models for microarray data) package in R/Bioconductor was employed to screen the DEGs between Sham-CLP and Sham-Sham, Burn-CLP and Sham-Sham, as well as Burn-CLP and Sham-CLP. $P < 0.05$ and $\log_2FCI \geq 2$ were used as the cut-off criteria for the DEGs.

Pathway enrichment analysis. The Database for Annotation, Visualization and Integrated Discovery is a collection of functional annotation tools for investigators to study the biological meaning behind a large list of genes (10). The KEGG pathway database (<http://www.genome.jp/kegg/pathway.html>), which includes the functions in terms of the network of the interacting molecules, was used to perform the pathway enrichment for the DEGs (11). $P < 0.05$ was the threshold for the pathway enrichment analysis.

Transcription factors screening. The transcriptional regulation from patterns to profiles (TRANSFAC) database (<http://www.gene-regulation.com>) containing the data on transcription factors, their target genes and regulatory binding sites was applied to discover the transcription factors (12). Additionally, different transcription factors between the Sham-CLP and Burn-CLP were further analyzed.

PPI network construction. The interaction associations of the proteins were analyzed using the online tool Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org/>) (13) and the required confidence (combined score) ≥ 0.4 was used as the cut-off criterion. Subsequently, Cytoscape was used to visualize the network (14).

Co-expression analysis of DEGs in Burn-CLP compared with Sham-Sham and Sham-CLP. The union of DEGs in Burn-CLP and Sham-Sham, as well as the DEGs in Burn-CLP and Sham-CLP, was screened. Subsequently, CoExpress (<http://www.bioinformatics.lu/CoExpress/>) was employed

to calculate the correlation coefficient of the DEGs. Pearson correlation coefficient was used to reflect the expression correlation between the DEGs. The Pearson correlation coefficient > 0.9 was taken as the threshold.

Results

DEG analysis. Compared with Sham-Sham, a total of 476 DEGs (including 225 upregulated DEGs, such as *Lcn2* and *Zfhx2*, and 251 downregulated DEGs, such as *Tnnt1* and *Sv2a*) and 682 DEGs (including 324 upregulated DEGs, such as *Arl6ip6* and *Pla2g2a*, and 358 downregulated DEGs, such as *Acadm* and *Ehhadh*) were obtained in Sham-CLP and Burn-CLP, respectively. Additionally, 230 DEGs, including 85 upregulated DEGs, such as *Rbbp9* and *Cla4a*, and 145 downregulated DEGs, such as *Igfals* and *G0s2*, were screened in Burn-CLP compared with Sham-CLP. The 10 most significantly upregulated and downregulated DEGs are listed in Table I. Additionally, the hierarchical cluster analysis is shown in Fig. 1.

KEGG pathway enrichment. Compared with Sham-Sham, the upregulated DEGs, such as *Cxcl14*, *Cxcl16* and *Cxcr4*, in Burn-CLP were significantly enriched in the chemokine-signaling pathway ($P = 0.015$) (Table II). The downregulated DEGs, such as *Acadm* and *Ehhadh*, were significantly enriched in the PPAR signaling pathway ($P = 8.298 \times 10^{-4}$). Additionally, *Gsta3*, *Gstm2* and *Gstt1* in Burn-CLP were significantly enriched in glutathione metabolism ($P = 0.023$) (Table III).

Transcription factor analysis. According to the TRANSFAC database, a total of 18 (including *Ascl2* and *Atf3*) and 19 (including *Ascl2* and *Ppargc1a*) transcription factors were screened among the DEGs in Sham-CLP and Burn-CLP, respectively, compared with Sham-Sham. Additionally, Sham-CLP and Burn-CLP possessed 8 identical transcription factors (Fig. 2A). Their expression levels are exhibited in Fig. 2. Apart from these 8 transcription factors, there were 11 transcription factors (including *Ppargc1a* and *DBP*) in Burn-CLP compared with Sham-Sham (Fig. 2B).

PPI network analysis. The PPI network for the DEGs in the three comparison groups: Sham-Sham versus Sham-CLP, Sham-Sham versus Burn-CLP, Burn-CLP versus Sham-CLP, are shown in Fig. 3C. For the DEGs between Sham-Sham and Sham-CLP, a total of 361 pairs of PPI were obtained from the STRING database. In the PPI network, *Tnf* possessed the highest degree of 25 (Fig. 3A). Additionally, for the DEGs between Sham-Sham and Burn-CLP, a total of 595 pairs of PPI were obtained. In the PPI network, *Esr1* had the highest degree of 31 and *Ppargc1a* could interact with *Angptl4* (Fig. 3B). As for the DEGs between Burn-CLP and Sham-CLP, a total of 110 pairs of PPI were obtained. In the PPI network, *Pik3r1* had the highest degree of 9 (Fig. 3C).

Co-expression analysis. A total of 413 pairs of co-expression associations, including 105 genes, were obtained (Fig. 4). Among these genes, *Cyp3a9* could be co-expressed with 13 genes (such as *Atp1b1* and *Ell2*). In addition, *Ehhadh* could be co-expressed with *Aldh3a2*, *Aqp8* and *Bdhl*.

Table I. Ten most significantly upregulated and downregulated DEGs in Sham-CLP and Burn-CLP compared with Sham-Sham and the DEGs in Burn-CLP compared with Sham-CLP.

Sham-CLP vs. Sham-Sham				Burn-CLP vs. Sham-Sham			Burn-CLP vs. Sham-CLP		
	DEGs	logFC	P-value	DEGs	logFC	P-value	DEGs	logFC	P-value
Upregulated	<i>Lcn2</i>	4.602548	3.83x10 ⁻⁸	<i>Arl6ip6</i>	5.066561	0.008025	<i>Rbbp9</i>	3.296154	0.027772
	<i>Zfhx2</i>	4.02276	0.000262	<i>Pla2g2a</i>	4.675226	5.34x10 ⁻⁷	<i>Clca4</i>	3.295737	1.43x10 ⁻⁵
	<i>Pla2g2a</i>	3.445816	5.64x10 ⁻⁶	<i>Lcn2</i>	4.596656	3.86x10 ⁻⁸	<i>Taf1</i>	2.915533	0.023838
	<i>Plscr2</i>	3.371389	0.037682	<i>Neb</i>	4.304198	0.000744	<i>Tnnt1</i>	2.829013	6.28x10 ⁻⁵
	<i>Neb</i>	3.345386	0.003449	<i>Zfhx2</i>	4.267401	0.000174	<i>Ppargc1a</i>	2.749841	0.036676
	<i>Itgb3bp</i>	3.148196	0.005818	<i>S100a9</i>	3.878773	0.000332	<i>Stard7</i>	2.74522	0.004186
	<i>Nfyb</i>	3.076118	0.006189	<i>Plscr2</i>	3.500379	0.032474	<i>Pitx2</i>	2.567246	0.015435
	<i>Eif4e3</i>	3.026708	0.004705	<i>Ly49s7</i>	3.315606	9.63x10 ⁻⁵	<i>Smim3</i>	2.395974	0.005767
	<i>Mmrn1</i>	3.011887	0.010381	<i>Ddit4</i>	3.230954	0.009239	<i>Aurka</i>	2.347335	0.002993
	<i>Sall2</i>	2.919577	0.03118	<i>Sall2</i>	3.217558	0.020624	<i>Rnf113a1</i>	2.168197	0.011317
Downregulated	<i>Tnnt1</i>	-4.03526	4.48x10 ⁻⁶	<i>G0s2</i>	-5.3812	0.005036	<i>G0s2</i>	-5.50634	0.004437
	<i>Sv2a</i>	-3.9502	0.001074	<i>Igfals</i>	-4.95741	0.001784	<i>Igfals</i>	-4.2644	0.004283
	<i>Pitx2</i>	-3.73666	0.002074	<i>Bdh1</i>	-4.40114	0.009124	<i>Bdh1</i>	-3.23361	0.036533
	<i>Klk1</i>	-3.43142	0.00292	<i>Hsd17b2</i>	-4.07748	0.001695	<i>Tpgs1</i>	-2.91959	0.000561
	<i>Scgb1d2</i>	-3.30923	0.000102	<i>Nim1</i>	-3.73986	0.005966	<i>Tmed1</i>	-2.74594	8.49x10 ⁻⁶
	<i>Rbbp9</i>	-3.25999	0.029075	<i>Car3</i>	-3.69018	0.000853	<i>Rnf213</i>	-2.64719	0.002884
	<i>LOC691984</i>	-3.22479	0.017167	<i>Nrep</i>	-3.53501	0.000151	<i>Slpr3</i>	-2.6437	0.010497
	<i>Kcnj1</i>	-3.19046	0.003682	<i>Lsp1</i>	-3.15146	0.00084	<i>Car3</i>	-2.64229	0.006101
	<i>Gpr85</i>	-3.16711	0.000145	<i>Fabp3</i>	-3.10453	0.000821	<i>Gpr108</i>	-2.62186	0.012436
	<i>Atp1a4</i>	-3.12916	0.015975	<i>Apol9a</i>	-3.09277	0.000172	<i>Obfc1</i>	-2.56692	0.001367

DEGs, differentially expressed genes; CLP, cecal ligation and puncture; FC, fold change.

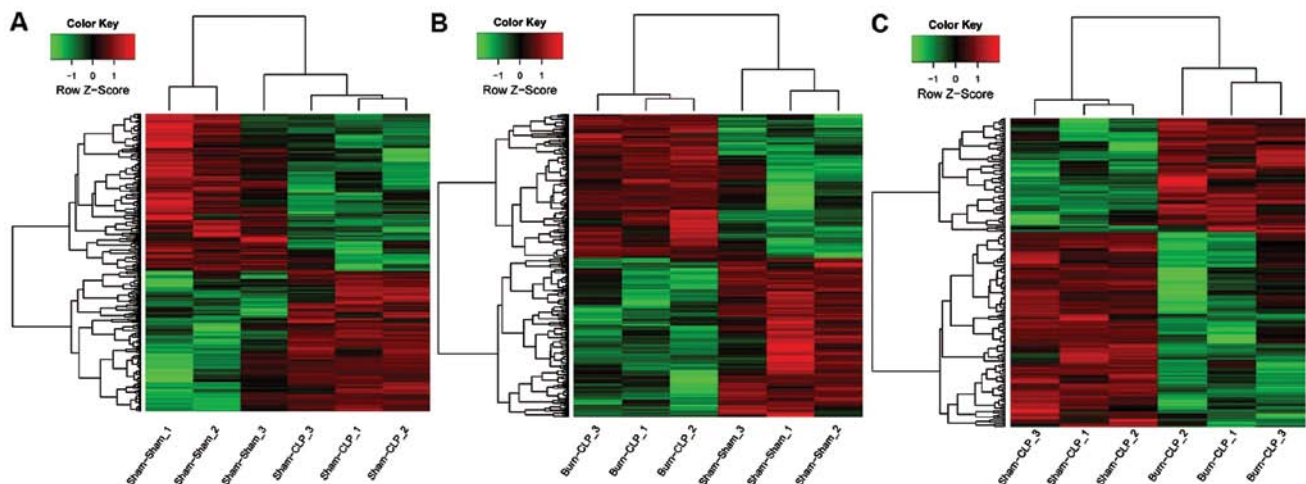


Figure 1. Hierarchical clusters of the DEGs in the three comparison groups. Hierarchical clusters of the DEGs between (A) Sham-Sham and Sham-CLP, (B) Sham-Sham and Burn-CLP and (C) Burn-CLP and Sham-CLP. DEGs, differentially expressed genes.

Discussion

In the present study, 682 DEGs were screened in Burn-CLP compared with Sham-Sham. The downregulated DEGs, such as *Acadm* and *Ehhadh*, were significantly enriched in PPAR signaling pathway. Additionally, in the PPI network, *Acadm* could interact with *Ehhadh*. A former study identified that

the inhibition of PPAR γ could possibly act as protection against T-cell death, which improved the defense mechanisms during systemic inflammation and sepsis (15). Apart from the aforementioned function, PPAR γ could also be involved in the regulation of the mitochondrial dysfunction with tumor necrosis factor α (16). A study by Singer (17) illustrated that mitochondrial dysfunction could give rise to sepsis. As for

Table II. KEGG pathway enrichments for the upregulated DEGs in the Sham-CLP and Burn-CLP compared with Sham-Sham.

Groups	KEGG	P-value	Gene symbol
Sham-CLP vs. Sham-Sham	rno00900: Terpenoid backbone biosynthesis	3.85x10 ⁻⁵	<i>Acat2, Fdps, Hmgcr, Idi1, Mvd</i>
	rno00100: Steroid biosynthesis	8.85x10 ⁻⁵	<i>Cyp51, Ebp, Hsd17b7, Lss, Sqle</i>
	rno04520: Adherens junction	0.004	<i>Acvr1c, Crebbp, Csnk2a1, Ptpn1, Ptpnj, Ssx2ip</i>
Burn-CLP vs. Sham-Sham	rno00830: Retinol metabolism	0.005	<i>Cyp1a1, Cyp26a1, Cyp2b12, Cyp3a9, Dhhrs9, RGD1562200</i>
	rno04060: Cytokine-cytokine receptor interaction	0.012	<i>Amhr2, Ccr1, Csf1r, Cxcl14, Cxcl16, Cxcr4, Il1rap, Lifr, Pf4, Tnfrsf21</i>
	rno04062: Chemokine signaling pathway	0.015	<i>Adcy3, Ccr1, Cxcl14, Cxcl16, Cxcr4, Grk5, Pf4, Rock1, Wasl</i>

DEGs, differentially expressed genes; CLP, cecum ligation and puncture.

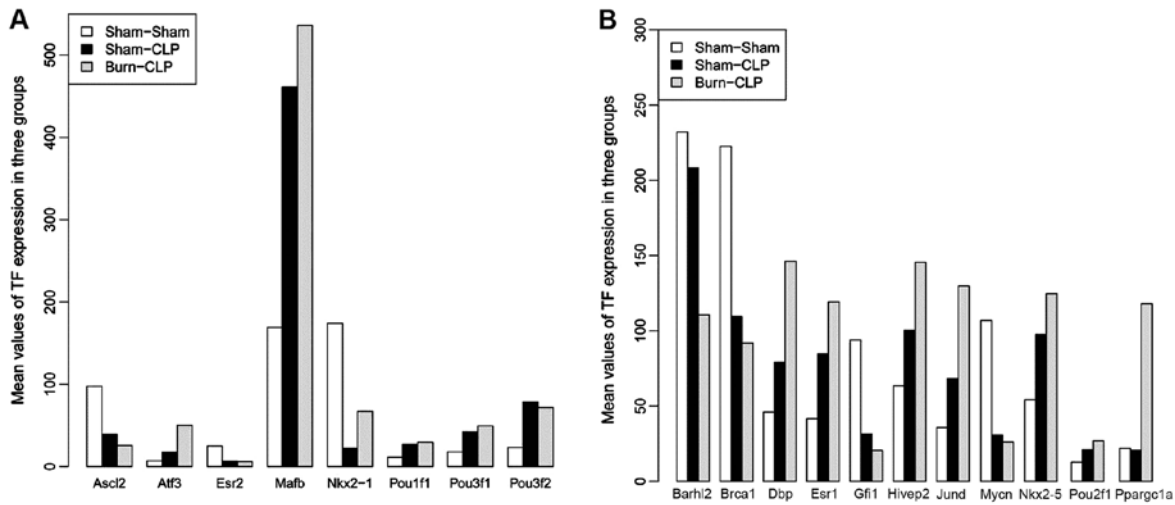


Figure 2. Significantly differentially expressed transcription factors in Sham-CLP and Burn-CLP compared with Sham-Sham. (A) The 8 significantly differentially expressed transcription factors in Sham-CLP and Burn-CLP compared with Sham-Sham. (B) The 11 significantly differentially expressed transcription factors only in Burn-CLP compared with Sham-Sham. CLP, cecum ligation and puncture.

the DEGs enriched in this pathway, *Acadm*, a member of the acyl-CoA dehydrogenase (ACAD) family, has been found to be involved in the metabolism of medium-chain fatty acids (18). In addition, *Ehhadh* has also been proven to be an indispensable element for the production of medium-chain dicarboxylic acids (19). In 2013, Hecker *et al* (20) obtained the conclusion that medium-chain fatty acids could serve as energy for the mitochondrial respiratory capacity and inflammatory conditions in sepsis. Therefore, we hypothesized that the interaction of *Acadm* and *Ehhadh* could be associated with sepsis by modulating the mitochondrial function through the PPAR signaling pathway.

Furthermore, the gene co-expression analysis showed that *Ehhadh* could be co-expressed with *Aqp8*. *Aqp8* is a water channel protein on the inner mitochondrial membrane (21). The upregulation of *Aqp8* has been found to protect the mitochondria from damage in sepsis, which could lead to the loss of energy (22). Additionally, it has been discovered that *Aqp8* was involved in H₂O₂ release and decrease of reactive oxygen

species (ROS) production, which could damage the cells and antioxidant defense system and thus lead to sepsis (23,24). *Ehhadh* has been found to be involved in mitochondrial fatty acid β -oxidation (25). Therefore, we hypothesized that the co-expression of *Ehhadh* and *Aqp8* could be associated with sepsis by regulating the mitochondrial function.

Furthermore, compared with Sham-Sham, the down-regulated DEGs, *Gsta3*, *Gstm2* and *Gstt1*, in Burn-CLP were significantly enriched in glutathione metabolism. The PPI network showed that interactions existed among these three proteins. Previous studies have shown that improved outcomes in animal models of sepsis were obtained by utilizing mitochondrial-targeted antioxidants (26,27). Glutathione could protect the mitochondria from dysfunction by the detoxification of hydrogen peroxide in sepsis (28). *Gsta3*, *Gstm2* and *Gstt1* encode the glutathione S-transferase $\alpha 3$, glutathione S-transferase $\mu 2$ and glutathione S-transferase $\theta 1$, respectively. Glutathione S-transferases are well known for removing endogenous toxic compounds through glutathionylation of

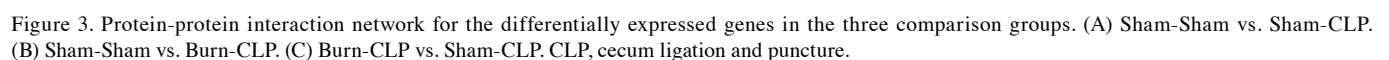


Table III. KEGG pathway enrichments with the downregulated DEGs in Sham-CLP and Burn-CLP compared with Sham-Sham and the downregulated DEGs in Burn-CLP compared with Sham-CLP.

Groups	KEGG	P-value	Gene symbol
Sham-CLP vs. Sham-Sham	rno04080: Neuroactive ligand-receptor interaction	6.575x10 ⁻⁴	<i>Adrb2, Agtr1b, F2rl3, Gabrb1, Galr3, Glra3, Glrb, Htr1a, Lpar2, P2rx5, Pth2r, Tacr3</i>
	rno04260: Cardiac muscle contraction	0.004	<i>Atp1a4, Cacnb1, Cox6a2, Cox8b, Fxyd2, Myl3</i>
	rno05218: Melanoma	0.013	<i>Fgf13, Fgf18, Fgf7, Mapk3, Pdgfa</i>
	rno04660: T cell receptor signaling pathway	0.015	<i>Cd28, Cd8a, Cd8b, Il2, Mapk3, Tnf</i>
	rno04640: Hematopoietic cell lineage	0.020	<i>Cd2, Cd8a, Cd8b, Il3, Tnf</i>
	rno04010: MAPK signaling pathway	0.025	<i>Cacnb1, Fgf13, Fgf18, Fgf7, Hspa1l, Mapk3, Pdgfa, Pla2g1b, Tnf</i>
	rno05330: Allograft rejection	0.034	<i>Cd28, Il12a, Il2, Tnf</i>
	rno04940: Type I diabetes mellitus	0.047	<i>Cd28, Il12a, Il2, Tnf</i>
	rno04514: Cell adhesion molecules (CAMs)	0.048	<i>Cd2, Cd28, Cd8a, Cd8b, Mpz1l, Nrnx3</i>
	rno04060: Cytokine-cytokine receptor interaction	0.048	<i>Agtr1b, Cx3cl1, Il12a, Il2, Il3, Pdgfa, Tnf</i>
Burn-CLP vs. Sham-Sham	rno00650: Butanoate metabolism	6.634x10 ⁻⁴	<i>Aldh3a2, Bdh1, Bdh2, Ehhadh, Hmgcs2, Pdha2</i>
	rno03320: PPAR signaling pathway	8.298x10 ⁻⁴	<i>Acadm, Angptl4, Ehhadh, Fabp3, Fabp7, Fads2, Gk, Hmgcs2</i>
	rno00561: Glycerolipid metabolism	0.003	<i>Aldh3a2, Dak, Gk, Lipc, Mgll, Pnliprp2</i>
	rno00410: β-Alanine metabolism	0.016	<i>Acadm, Aldh3a2, Dpys, Ehhadh</i>
	rno00072: Synthesis and degradation of ketone bodies	0.015	<i>Bdh1, Bdh2, Hmgcs2</i>
	rno00480: Glutathione metabolism	0.023	<i>Gsta3, Gstm2, Gstt1, Idh1, Oplah</i>
Burn-CLP vs. Sham-CLP	rno00072: Synthesis and degradation of ketone bodies	0.004	<i>Acat2, Bdh1, Bdh2</i>
	rno00140: Steroid hormone biosynthesis	0.011	<i>Cyp17a1, Hsd17b1, Hsd17b2, Sult2a2</i>
	rno00650: Butanoate metabolism	0.050	<i>Acat2, Bdh1, Bdh2</i>

DEGs, differentially expressed genes; CLP, cecum ligation and puncture.

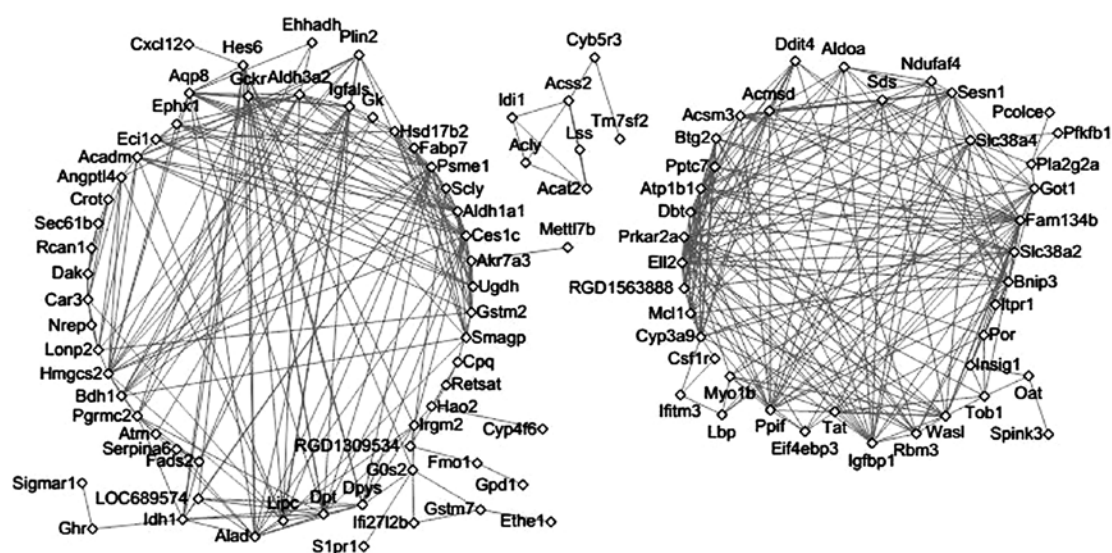


Figure 4. Co-expression network of the differentially expressed genes in Sham-Sham vs. Burn-CLP and Burn-CLP vs. Sham-CLP. CLP, cecum ligation and puncture.

diverse electrophilic substrates and act as antioxidants against ROS (29). Accordingly, we hypothesized that *Gsta3*, *Gstm2* and *Gstt1* may be involved in sepsis through the pathway of glutathione metabolism.

In the present study, *Ppargcla*, which was upregulated in Burn-CLP compared with Sham-Sham, was screened as a transcription factor. However, in Sham-CLP, it was not differentially expressed, suggesting that *Ppargcla* could be associated with burn. AMPK-*Ppargcla* possibly contributes to autophagy activation leading to an antimicrobial response, which is a novel host defense mechanism (30). In the PPI network, *Ppargcla* could interact with *Angptl4*. Additionally, *Angptl4* was significantly enriched in the PPAR signaling pathway. *Angptl4* has been demonstrated to be involved with lipid metabolism, and the disorder of lipid metabolism is a vital issue in septic patients, particularly high-density lipoprotein, which protects against polymicrobe-induced sepsis in mice (31,32). Consequently, we hypothesized that the interaction of *Angptl4* and *Ppargcla* may be associated with sepsis through the PPAR signaling pathway.

In conclusion, *Acadm*, *Ehhadh*, *Aqp8*, *Gsta3*, *Gstm2*, *Gstt1*, *Ppargcla* and *Angptl4* may be potential target genes for the treatment of burn sepsis. However, further studies are required to establish their mechanisms of action in burn sepsis.

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References

1. Taylor FB Jr, Kinasewitz GT and Lupu F: Pathophysiology, staging and therapy of severe sepsis in baboon models. *J Cell Mol Med* 16: 672-682, 2012.
2. Martin GS, Mannino DM, Eaton S and Moss M: The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 348: 1546-1554, 2003.
3. Church D, Elsayed S, Reid O, Winston B and Lindsay R: Burn wound infections. *Clin Microbiol Rev* 19: 403-434, 2006.
4. Beffa DC, Fischman AJ, Fagan SP, Hamrahi VF, Paul KW, Kaneki M, Yu YM, Tompkins RG and Carter EA: Simvastatin treatment improves survival in a murine model of burn sepsis: Role of interleukin 6. *Burns* 37: 222-226, 2011.
5. Kraft R, Herndon DN, Finnerty CC, Cox RA, Song J and Jeschke MG: Predictive value of IL-8 for sepsis and severe infections after burn injury - A clinical study. *Shock* 43: 222-227, 2015.
6. Zu H, Li Q and Huang P: Expression of Treg subsets on intestinal T cell immunity and endotoxin translocation in porcine sepsis after severe burns. *Cell Biochem Biophys* 70: 1699-1704, 2014.
7. Banta S, Vemula M, Yokoyama T, Jayaraman A, Berthiaume F and Yarmush ML: Contribution of gene expression to metabolic fluxes in hypermetabolic livers induced through burn injury and cecal ligation and puncture in rats. *Biotechnol Bioeng* 97: 118-137, 2007.
8. Afsari B, Geman D and Fertig EJ: Learning dysregulated pathways in cancers from differential variability analysis. *Cancer Inform* 13 (Suppl 5): 61-67, 2014.
9. Bolstad B: preprocessCore: A collection of pre-processing functions. R package version 1: 2013.
10. Huang W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4: 44-57, 2009.
11. Kanehisa M and Goto S: KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 28: 27-30, 2000.
12. Matys V, Fricke E, Geffers R, Gössling E, Haubrock M, Hehl R, Hornischer K, Karas D, Kel AE, Kel-Margoulis OV, et al: TRANSFAC: Transcriptional regulation, from patterns to profiles. *Nucleic Acids Res* 31: 374-378, 2003.
13. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C, et al: STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 41: D808-D815, 2013.
14. Saito R, Smoot ME, Ono K, Ruscheinski J, Wang PL, Lotia S, Pico AR, Bader GD and Ideker T: A travel guide to Cytoscape plugins. *Nat Methods* 9: 1069-1076, 2012.
15. Schmidt MV, Paulus P, Kuhn AM, Weigert A, Morbitzer V, Zacharowski K, Kempf VA, Brüne B and von Knethen A: Peroxisome proliferator-activated receptor γ -induced T cell apoptosis reduces survival during polymicrobial sepsis. *Am J Respir Crit Care Med* 184: 64-74, 2011.
16. Chiang MC, Cheng YC, Lin KH and Yen CH: PPAR γ regulates the mitochondrial dysfunction in human neural stem cells with tumor necrosis factor alpha. *Neuroscience* 229: 118-129, 2013.
17. Singer M: The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. *Virulence* 5: 66-72, 2014.
18. Kim JJ and Miura R: Acyl-CoA dehydrogenases and acyl-CoA oxidases. Structural basis for mechanistic similarities and differences. *Eur J Biochem* 271: 483-493, 2004.
19. Houten SM, Denis S, Argmann CA, Jia Y, Ferdinandusse S, Reddy JK and Wanders RJ: Peroxisomal L-bifunctional enzyme (Ehhadh) is essential for the production of medium-chain dicarboxylic acids. *J Lipid Res* 53: 1296-1303, 2012.
20. Hecker M, Sommer N, Voigtmann H, Pak O, Mohr A, Wolf M, Vadasz I, Herold S, Weissmann N, Morty RE, et al: Impact of short- and medium-chain fatty acids on mitochondrial function in severe inflammation. *JPEN J Parenter Enteral Nutr* 38: 587-594, 2013.
21. Calamita G, Ferri D, Gena P, Liquori GE, Cavalier A, Thomas D and Svelto M: The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. *J Biol Chem* 280: 17149-17153, 2005.
22. Wang JQ, Zhang L, Tao XG, Wei L, Liu B, Huang LL and Chen YG: Tetramethylpyrazine upregulates the aquaporin 8 expression of hepatocellular mitochondria in septic rats. *J Surg Res* 185: 286-293, 2013.
23. Schulte J, Struck J, Köhrle J and Müller B: Circulating levels of peroxiredoxin 4 as a novel biomarker of oxidative stress in patients with sepsis. *Shock* 35: 460-465, 2011.
24. Marchisio MJ, Francés DEA, Carnovale CE and Marinelli RA: Mitochondrial aquaporin-8 knockdown in human hepatoma HepG2 cells causes ROS-induced mitochondrial depolarization and loss of viability. *Toxicol Appl Pharmacol* 264: 246-254, 2012.
25. Banasik K, Justesen JM, Hornbak M, Krarup NT, Gjesing AP, Sandholt CH, Jensen TS, Grarup N, Andersson A, Jørgensen T, et al: Bioinformatics-driven identification and examination of candidate genes for non-alcoholic fatty liver disease. *PLoS One* 6: e16542, 2011.
26. Andrade ME, Morina A, Spasić S and Spasojević I: Bench-to-bedside review: sepsis - from the redox point of view. *Crit Care* 15: 230, 2011.
27. Dare AJ, Phillips AR, Hickey AJ, Mittal A, Loveday B, Thompson N and Windsor JA: A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. *Free Radic Biol Med* 47: 1517-1525, 2009.
28. Lowes DA and Galley HF: Mitochondrial protection by the thioredoxin-2 and glutathione systems in an in vitro endothelial model of sepsis. *Biochem J* 436: 123-132, 2011.
29. Ito M, Imai M, Muraki M, Miyado K, Qin J, Kyuwa S, Yoshikawa Y, Hosoi Y, Saito H and Takahashi Y: GSTT1 is upregulated by oxidative stress through p38-MK2 signaling pathway in human granulosa cells: Possible association with mitochondrial activity. *Aging (Albany NY)* 3: 1213-1223, 2011.
30. Yang CS, Kim JJ, Lee HM, Jin HS, Lee SH, Park JH, Kim SJ, Kim JM, Han YM, Lee MS, et al: The AMPK-PPARGC1A pathway is required for antimicrobial host defense through activation of autophagy. *Autophagy* 10: 785-802, 2014.
31. Hato T, Tabata M and Oike Y: The role of angiopoietin-like proteins in angiogenesis and metabolism. *Trends Cardiovasc Med* 18: 6-14, 2008.
32. Guo L, Ai J, Zheng Z, Howatt DA, Daugherty A, Huang B and Li XA: High density lipoprotein protects against polymicrobe-induced sepsis in mice. *J Biol Chem* 288: 17947-17953, 2013.