Screening of Important Factors in the Early Sepsis Stage Based on the Evaluation of ssGSEA Algorithm and ceRNA Regulatory Network

Liou Huang, Chunrong Wu, Dan Xu, Yuhui Cui and Jianguo Tang

Department of Trauma-Emergency and Critical Care Medicine, Shanghai Fifth People’s Hospital, Fudan University, Shanghai, China.

ABSTRACT

BACKGROUND: Sepsis is a dysregulated host response to pathogens. Delay in sepsis diagnosis has become a primary cause of patient death. This study determines some factors to prevent septic shock in its early stage, contributing to the early treatment of sepsis.

METHODS: The sequencing data (RNA- and miRNA-sequencing) of patients with septic shock were obtained from the NCBI GEO database. After re-annotation, we obtained IncRNAs, miRNA, and mRNA information. Then, we evaluated the immune characteristics of the sample based on the ssGSEA algorithm. We used the WGCNA algorithm to obtain genes significantly related to immunity and screen for important related factors by constructing a ceRNA regulatory network.

RESULT: After re-annotation, we obtained 1708 IncRNAs, 129 miRNAs, and 17326 mRNAs. Also, through the ssGSEA algorithm, we obtained 5 important immune cells. Finally, we constructed a ceRNA regulation network associated with SS pathways.

CONCLUSION: We identified 5 immune cells with significant changes in the early stage of septic shock. We also constructed a ceRNA network, which will help us explore the pathogenesis of septic shock.

KEYWORDS: Sepsis, pathogenicity, genes, regulator

Introduction

Sepsis is a dysregulated host response to pathogens. It causes circulatory, cellular, or metabolic circulatory abnormalities and ultimately leads to life-threatening organ dysfunction. Septic shock (SS) is the most severe complication of sepsis and is also the primary cause of mortality in intensive care units (ICUs) worldwide. Early and appropriate regulations are crucial for patient recovery; therefore, it is necessary to explore SS-related factors to help early diagnosis, monitor, and prevent SS development. The pathogenesis of SS is complicated. Cells of immune systems contribute to its occurrence and development. After septic shock, T and B lymphocytes are altered. Therefore, the proportion of circulating and regulatory cells will increase. Additionally, neutrophils play a critical role during septic shock. Early higher neutrophils counts relate to the increase in sepsis severity. Therefore, exploring changes in the type and proportion of immune cells in the early samples of SS will be helpful for rapid diagnosis.

Recently, long non-coding RNAs (IncRNAs) have become a hot spot in disease research. The mutual regulation mode between IncRNA, miRNA, and its downstream target genes is closely related to the occurrence and development of diseases. For example, Manetti et al found at least 77 miRNAs involved in cardiac inflammation and dysfunction during sepsis. Additionally, IncRNA can regulate miRNA (an important factor of post-transcriptional regulation activity) through sponge adsorption. This IncRNA is called competitive endogenous RNA (ceRNA). LncRNA (a ceRNA) can competitively bind to miRNA, thereby regulating the protein level of coding genes and the biological behavior of cells. Therefore, an in-depth understanding of ceRNA-based regulatory mechanisms will help us better understand the pathogenesis of SS and screen important genes.

This research screened out RNAs whose expression levels changed significantly at 24 and 48 hours after SS and constructed a ceRNA regulatory network. Additionally, by gene set enrichment analysis (GSEA), we screened out immune cells with evident differences in different SS periods.

Material and Methods

Data collection

We downloaded the data set, GSE57065, from the NCBI GEO database. There were 82 samples related to SS in GSE57065, and these 82 blood samples were enrolled from 28 ICU patients at the onset of sepsis shock 0, 24, and 48 hours. The sample detection platform was GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. According to the time point, we divided the sample into 2
comparison groups: 24 hours versus 0 hour and 48 hours versus 0 hour. Then, we downloaded the detailed annotation information of the GPL570 Affymetrix Human Genome U133 Plus 2.0 Array platform from the Ensembl genome browser 96 database (http://asia.ensembl.org/index.html), including the probe, gene Symbol, RNA type, and other information. Finally, we re-annotated the expression data in GSE57065 and obtained the lncRNA, miRNA, and mRNA expression levels based on these annotations.

Screening of significantly differentially expressed genes

After re-annotation, we used the limma package of the R software (https://bioconductor.org/packages/release/bioc/html/limma.html) to screen for significantly differentially expressed RNAs (DERs, including lncRNA, miRNA, and mRNA) in the 24 hours versus 0 hour and 48 hours versus 0 hour comparison groups.11 FDR < 0.05 and \(|\log_2 FC| > 0.263\) were considered statistically significant. Heatmaps were generated using pheatmap package (https://cran.r-project.org/web/packages/pheatmap/index.html), and volcano plots were also conducted in R software.12,13 Finally, we retained the overlapping DERs in the 2 comparison groups. These DERs were genes whose expression levels continue to change significantly during the process of 24 to 48 h in the early stage of SS.

Immune-infiltrating cell analysis

Here, we downloaded the immunologic signature gene sets from GSEA database (http://software.broadinstitute.org/gsea/index.jsp). Then we use the ssGSEA algorithm in the GSVA package of the R software (http://www.bioconductor.org/packages/release/bioc/html/GSVA.html)14 to evaluate the type of immune infiltration of the samples in GSE57065. Finally, we compared the proportion of each immune cell in the overlapped pathways and constructed a regulated network associated with SS.

Screening disease progression and immunity-related modules by WGCNA algorithm

For all genes with expression detected in GSE57065, we used the WGCNA R package (https://cran.r-project.org/web/packages/WGCNA/index.html) to screen for significantly stable gene modules that are related to the sample stage and immune cells.15 Screening thresholds: contains at least 100 genes, cutHeight = 0.995.

Then, we mapped the overlapping genes screened in step 2.2 to each WGCNA module. Finally, we calculated the significantly enriched parameter-fold enrichment and enrichment significance \(P\)-value through the hypergeometric algorithm. \[f(k,N,M,n) = \binom{C(k,M)}{C(nk,NM)} / \binom{C(n,N)}{M}\].16

Where, \(N\) represents all genes involved in the analysis of WGCNA; \(M\) represents the number of genes in each module obtained by WGCNA analysis; \(n\) represents the number of DERs obtained in step 2.2; and \(k\) represents the number of DERs in the intersection mapped to the corresponding module.

In this research, we selected \(P < .05\) and fold enrichment \(> 1\) as the thresholds. We took the significantly enriched genes in the target module as the object of further analysis and research.

Construction of the ceRNA network

We used DIANA-LncBase v.2 (http://www.microrna.gr/LncBase) to predict the lncRNA–miRNA interactions based on DELncRNAs obtained from step 2.2.17 StarBase v.2.0 (http://starbase.sysu.edu.cn/)18 was used to search for target genes for the DEMiRNA. Then, we mapped the genes that were significantly enriched in WGCNA modules to the target genes. Additionally, we used these genes to construct interactions. Ultimately, we integrated these interactions and constructed a lncRNA–related ceRNA network with Cytoscape v.3.6.1 based on how lncRNAs can affect the function of miRNAs and act as miRNA sponges to regulate mRNA expression.19

We performed a functional analysis of mRNA in the ceRNA network using DAVID v.6.8 (annotation, visualization, and integrated discovery database, http://david.abcc.ncifcrf.gov/).20,21

Screening disease-related pathway

We used “septic shock” as the keyword to search for the SS-related Kyoto Encyclopedia for Genes and Genomes (KEGG) signal pathways in the Comparative Toxicogenomics Database (CTD) (http://ctd.mdibl.org/).22 Compared with the KEGG signal pathways obtained from step 2.5, we kept the overlapped pathways and constructed a regulated network associated with SS.

All data-related scripts were provided in Supplemental Material 1.

Result

Screening of significantly differentially expressed genes

After re-annotation, we got 1708 lncRNAs, 129 miRNAs, and 17326 mRNAs. Then, 874 and 1546 DERs were selected by Limma in the 24 hours versus 0 hour and 48 hours versus 0 hour comparison groups, respectively. The volcano map (Figure 1 left) and heatmap (Figure 1 right) of the lncRNAs, miRNAs, and mRNAs showed that these DERs could separate different samples based on time. By comparing the DERs screened in the 2 comparison groups, 644 overlapped DERs were retained, including 26 lncRNAs, 7 miRNAs, and 611 mRNAs. These DERs were RNAs whose expression levels were significantly different in the 24 and 48 hours samples.

Immune-infiltrating cell analysis

Here, we downloaded the immunologic signature gene sets from GSEA database (http://software.broadinstitute.org/gsea/index.jsp). Then we use the ssGSEA algorithm in the GSVA package of the R software (http://www.bioconductor.org/packages/release/bioc/html/GSVA.html)14 to evaluate the type of immune infiltration of the samples in GSE57065. Finally, we compared the proportion of each immune cell in the overlapped pathways and constructed a regulated network associated with SS.

Screening disease progression and immunity-related modules by WGCNA algorithm

For all genes with expression detected in GSE57065, we used the WGCNA R package (https://cran.r-project.org/web/packages/WGCNA/index.html) to screen for significantly stable gene modules that are related to the sample stage and immune cells.15 Screening thresholds: contains at least 100 genes, cutHeight = 0.995.

Then, we mapped the overlapping genes screened in step 2.2 to each WGCNA module. Finally, we calculated the significantly enriched parameter-fold enrichment and enrichment significance \(P\)-value through the hypergeometric algorithm. \[f(k,N,M,n) = \binom{C(k,M)}{C(nk,NM)} / \binom{C(n,N)}{M}\].16

Where, \(N\) represents all genes involved in the analysis of WGCNA; \(M\) represents the number of genes in each module obtained by WGCNA analysis; \(n\) represents the number of DERs obtained in step 2.2; and \(k\) represents the number of DERs in the intersection mapped to the corresponding module.

In this research, we selected \(P < .05\) and fold enrichment \(> 1\) as the thresholds. We took the significantly enriched genes in the target module as the object of further analysis and research.

Construction of the ceRNA network

We used DIANA-LncBase v.2 (http://www.microrna.gr/LncBase) to predict the lncRNA–miRNA interactions based on DELncRNAs obtained from step 2.2.17 StarBase v.2.0 (http://starbase.sysu.edu.cn/)18 was used to search for target genes for the DEMiRNA. Then, we mapped the genes that were significantly enriched in WGCNA modules to the target genes. Additionally, we used these genes to construct interactions. Ultimately, we integrated these interactions and constructed a lncRNA–related ceRNA network with Cytoscape v.3.6.1 based on how lncRNAs can affect the function of miRNAs and act as miRNA sponges to regulate mRNA expression.19

We performed a functional analysis of mRNA in the ceRNA network using DAVID v.6.8 (annotation, visualization, and integrated discovery database, http://david.abcc.ncifcrf.gov/).20,21

Screening disease-related pathway

We used “septic shock” as the keyword to search for the SS-related Kyoto Encyclopedia for Genes and Genomes (KEGG) signal pathways in the Comparative Toxicogenomics Database (CTD) (http://ctd.mdibl.org/).22 Compared with the KEGG signal pathways obtained from step 2.5, we kept the overlapped pathways and constructed a regulated network associated with SS.

All data-related scripts were provided in Supplemental Material 1.

Result

Screening of significantly differentially expressed genes

After re-annotation, we got 1708 lncRNAs, 129 miRNAs, and 17326 mRNAs. Then, 874 and 1546 DERs were selected by Limma in the 24 hours versus 0 hour and 48 hours versus 0 hour comparison groups, respectively. The volcano map (Figure 1 left) and heatmap (Figure 1 right) of the lncRNAs, miRNAs, and mRNAs showed that these DERs could separate different samples based on time. By comparing the DERs screened in the 2 comparison groups, 644 overlapped DERs were retained, including 26 lncRNAs, 7 miRNAs, and 611 mRNAs. These DERs were RNAs whose expression levels were significantly different in the 24 and 48 hours samples.
Figure 1. DEGs identified in 24 h versus 0 h (A) and 48 h versus 0 h (B). Left: Volcano map. Red dots: upregulated genes; Blue dots: downregulated genes. The horizontal red dotted line indicates false discovery rate (FDR) = 0.05, and the 2 vertical red dotted lines indicate |log₂ FC| (fold change) = 0.263. Right: Heatmap.

Figure 2. Distribution diagram of immune cell types with significant differences between the 0, 24, and 48 h time points. *P < .05. **P < .01. ***P < .005.
Immune-infiltrating cell analysis

Based on the gene expression data, using the ssGSEA algorithm, we obtained 28 immune cell ratios. First, we compared the differences in the proportion of various immune cells at 0, 24, and 48 h. Then, we kept the immune cell types that were significantly different at 24 h or 48 h time points compared with the time at 0 h, and finally got 5 immune cells with significant differences (Figure 2). The 5 immune cells were activated CD4 T cell, T follicular helper cell, Regulatory T cell, activated B cell, and neutrophils.

Module detection

First, we assume that the gene network is subject to scale-free distribution. Then, we selected a soft threshold (power) to make the constructed network a scale-free network. In this research, 9 was set as the soft threshold to meet the selected criteria of power value (Figure 3A). Based on the standard of a dynamic cut tree, 100 genes were set as the least gene number of each gene networks and 0.995 as the cut height, respectively. Finally, 13 modules were screened out.

Based on results of step 3.2, the correlation between modules, immune-infiltrating cells, and time was calculated, and modules related to different immune-infiltrating cells and time were screened (Figure 4).

Based on the hypergeometric enrichment algorithm described in the method, we mapped 611 overlapped DERs to WGCNA modules. Therefore, 277 DERs are located in WGCNA modules (As shown in Table 1). Furthermore, they were significantly enriched in black and magenta modules, containing 73 and 43 genes, respectively.
Table 1. Modules.

| ID     | COLOR  | MODULE SIZE | # OVERLAPPED DEGS | ENRICHMENT INFORMATION          | ENRICHMENT FOLD [95%CI] | P Hippo |
|--------|--------|-------------|-------------------|---------------------------------|-------------------------|---------|
| Module 1 | Black  | 205         | 73                | 6.943 [5.104-9.377]             | 2.20E-16                |
| Module 2 | Blue   | 414         | 12                | 0.566 [0.286-1.015]             | 5.75E-02                |
| Module 3 | Brown  | 341         | 14                | 0.801 [0.428-1.386]             | 5.23E-01                |
| Module 4 | Green  | 223         | 6                 | 0.525 [0.189-1.176]             | 1.53E-01                |
| Module 5 | Greenyellow | 118    | 1                 | 0.165 [0.00414-0.947]           | 4.61E-02                |
| Module 6 | Gray   | 2388        | 90                | 0.735 [0.570-0.941]             | 1.25E-02                |
| Module 7 | Magenta | 169        | 43                | 4.496 [3.390-7.140]             | 1.32E-14                |
| Module 8 | Pink   | 176         | 9                 | 0.998 [0.444-1.966]             | 9.99E-01                |
| Module 9 | Purple | 161         | 2                 | 0.242 [0.0289-0.898]            | 2.43E-02                |
| Module 10 | Red   | 217         | 1                 | 0.0899 [0.00226-0.510]          | 4.80E-04                |
| Module 11 | Tan   | 113         | 1                 | 0.173 [0.00432-0.990]           | 4.41E-02                |
| Module 12 | Turquoise | 560    | 25                | 0.871 [0.549-1.327]             | 6.12E-01                |
| Module 13 | Yellow | 320         | –                 | –                               | –                       |

The modules in bold were the modules with significantly enriched DERs.

Figure 4. Identification of modules associated with immune cells and time.
Figure 5. ceRNA network. Squares, triangles, and circles represent DElncRNA, DEmiRNA, and DEMRNA, which are significantly enriched in modules.
Table 2. KEGG signaling pathway with significant mRNA correlation in the ceRNA regulatory network.

| TERM                        | COUNT | P-VALUE |
|-----------------------------|-------|---------|
| hsa00410: beta-Alanine metabolism | 4     | 4.13E-05 |
| hsa00360: phenylalanine metabolism | 2     | 7.86E-03 |
| hsa00340: histidine metabolism | 2     | 1.01E-02 |
| *hsa01100: metabolic pathways | 10    | 1.16E-02 |
| hsa00350: tyrosine metabolism | 2     | 1.55E-02 |
| hsa00260: glycine, serine, and threonine metabolism | 2     | 1.71E-02 |
| hsa00071: fatty acid degradation | 2     | 1.83E-02 |
| hsa00280: valine, leucine, and isoleucine degradation | 2     | 2.03E-02 |
| *hsa04060: cytokine-cytokine receptor interaction | 3     | 3.26E-02 |

Construction of the ceRNA network

Prediction of lncRNA–miRNA–mRNA interactions

First, we used the experimental module DIANA-LncBase v.2.27 (http://www.microrna.gr/LncBase) to predict the lncRNA–miRNA interactions based on the overlapped DELncRNAs. Then, T starBase v.2.0 (http://starbase.sysu.edu.cn/) was used to predict the interactions between DEMiRNAs and DEMRNAs. Next, we mapped DERNAs enriched in black and magenta modules to regulate target genes and predict miRNA–mRNA interactions. Finally, we constructed a lncRNA-related ceRNA network using Cytoscape v.3.6.1 (Figure 5). It shows which lncRNAs can affect the function of miRNAs and how they regulate mRNA expression.

Gene function analysis. We used DAVID v.6.8 for KEGG signaling pathway enrichment annotation analysis on mRNAs in the ceRNA regulatory network, and 1 of 9 KEGG signal pathways was obtained (Table 2).

Screening disease-related pathway. We used “septic shock” as a keyword in the CTD database and searched 75 KEGG signal pathways directly related to SS. Compared with the pathways obtained before, Metabolic and cytokine–cytokine receptor interaction signaling pathways were screened. Then, we separately extracted the parts of the ceRNA regulatory network directly related to these 2 disease pathways (Figure 6). In these 2 pathways, has-miR-4668, has-miR-6847, has-miR-601, and has-miR-6281 contribute to SS pathogenesis. GABPB1-AS1 can regulate most of the miRNAs in the ceRNA network.

Conclusions

Septic shock is a clinical emergency and needs rapid diagnosis; however, the diagnosis of shock is complex and more difficult for severe infection. This review found some bio-markers that help rapid diagnosis of SS. The ratio of activated CD4 T cells, T follicular helper cells, regulatory T cells, activated B cells, and neutrophils will change significantly after septic shock. This provides us with new ideas for the rapid diagnosis of SS.
Authors’ Contributions
Jianguo Tang and Liou Huang were responsible for the conception and design of the research, and drafting the manuscript. Chunrong Wu and Dan Xu performed the data acquisition. Liou Huang and Yuhui Cui performed the data analysis and interpretation. All authors have read and approved the manuscript.

ORCID iD
Jianguo Tang https://orcid.org/0000-0003-1263-7327

Supplemental material
Supplemental material for this article is available online.

REFERENCES
1. Esposito S, De Simone G, Boccia G, De Caro F, Pagliano P. Sepsis and septic shock: New definitions, new diagnostic and therapeutic approaches. J Glob Anti-microb Resist. 2017;10:204-212.
2. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet. 2005;365:63-78.
3. Gustave CA, Gosses M, Demaert J, et al. Septic shock shapes B cell response toward an exhausted-like/immunoregulatory profile in patients. J Immunol. 2018;200:2418-2425.
4. Rimmelé T, Payen D, Cantaluppi V, et al. Immune cell phenotype and function in sepsis. Shock. 2016;45:282-291.
5. Park I, Kim M, Choe K, et al. Neutrophils disturb pulmonary microcirculation in sepsis-induced acute lung injury. Eur Respir J. 2019;53:1800786.
6. Manetti AC, Maiese A, Paolo MD, et al. MicroRNAs and sepsis-induced cardiac dysfunction: a systematic review. Int J Mol Sci. 2020;22:321.
7. Wang J, Liu X, Tu H, et al. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. Nucleic Acids Res. 2010;38:5366-5383.
8. Liu XH, Sun M, Nie FQ, et al. Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. Mol Cancer. 2014;13:92.
9. Zhou X, Gao Q, Wang J, Zhang X, Liu K, Duan Z. Lnc-RNA-RoR acts as a "spoon" against mediation of the differentiation of endometrial cancer stem cells by microRNA-145. Gynecol Oncol. 2014;133:333-339.
10. Ritchie ME, Phipson B, Wu D, et al. LImma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47.
11. Wang L, Cao C, Ma Q, et al. RNA-seq analyses of multiple meristems of soybean: novel and alternative transcripts, evolutionary and functional implications. BMC Plant Biol. 2014;14:169.
12. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA. 1998;95:14863-14868.
13. Ye L, Zhang T, Kang Z, et al. Tumor-infiltrating immune cells act as a marker for prognosis in colorectal cancer. Front Immunol. 2019;10:2368.
14. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559.
15. Cao J, Zhang S. A Bayesian extension of the hypergeometric test for functional enrichment analysis. Biometrics. 2014;70:84-94.
16. Paraskevopoulou MD, Vlachos IS, Karagkouni D, et al. DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts. Nucleic Acids Res. 2016;44:D231-D238.
17. Liu B, Tan X, Liang J, et al. A reduction in reactive oxygen species contributes to dihydroartemisinin-induced apoptosis in human hepatocellular carcinoma cells. Sci Rep. 2014;4:7041.
18. Shuffler P, Markiel A, Ozier O, et al. Cytopro: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-2504.
19. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4:44-57.
20. Huang W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37:1-13.
22. Davis AP, Grondin CJ, Johnson RJ, et al. Comparative Toxicogenomics Database (CTD): update 2021. *Nucleic Acids Res*. 2021;49:D1138-d1143.

23. Huet O, Chin-Dusting JP. Septic shock: desperately seeking treatment. *Clin Sci*. 2014;126:31-39.

24. Heffernan DS, Monaghan SF, Thakkar RK, Machan JT, Cioffi WG, Ayala A. Failure to normalize lymphopenia following trauma is associated with increased mortality, independent of the leukocytosis pattern. *Crit Care*. 2012;16:R12.

25. Hoser GA, Skirecki T, Złotorowicz M, Ziębińska-Borkowska U, Kawiak J. Absolute counts of peripheral blood leukocyte subpopulations in intraabdominal sepsis and pneumonia-derived sepsis: a pilot study. *Folia Histochem Cytobiol*. 2012;50:420-426.

26. Mitchell K, Barreyro L, Todorova TI, et al. IL1RAP potentiates multiple oncogenic signaling pathways in AML. *J Exp Med*. 2018;215:1709-1727.

27. Warda W, Larosa F, Neto Da, Rocha M, et al. CML hematopoietic stem cells expressing IL1RAP can be targeted by chimeric antigen receptor-engineered T cells. *Cancer Res*. 2019;79:663-675.

28. Shen J, Wang C, Ying J, Xu T, McAlinden A, O’Keefe RJ. Inhibition of 4-aminobutyrate aminotransferase protects against injury-induced osteoarthritis in mice. *JCI Insight*. 2019;4:e128568.

29. Chen X, Cao Q, Liao R, et al. Loss of ABAT-mediated GABAergic system promotes basal-like breast cancer progression by activating Cal(2+)-NFAT1 axis. *Theranostics*. 2019;9:34-47.

30. Nakakido M, Tamura K, Chung S, et al. Phosphatidylinositol glycan anchor biosynthesis, class X containing complex promotes cancer cell proliferation through suppression of EHD2 and ZIC1, putative tumor suppressors. *Int J Oncol*. 2016;49:868-876.

31. Hua YY, Zhang Y, Gong WW, et al. Dihydromyricetin improves endothelial dysfunction in diabetic mice via oxidative stress inhibition in a SIRT3-dependent manner. *Int J Mol Sci*. 2020;21:6699.

32. Li Q, Fang Y, Zhu P, et al. Burkholderia pseudomallei survival in lung epithelial cells benefits from miRNA-mediated suppression of ATG10. *Autophagy*. 2015;11:1293-1307.