Article

Comparative Analysis of Whole Transcriptome Profiles in Septic Cardiomyopathy: Insights from CLP- and LPS-Induced Mouse Models

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Abstract: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, with septic cardiomyopathy being a common and severe complication. Despite its significant clinical impact, the molecular mechanisms underlying sepsis-induced cardiomyopathy (SICM) remain incompletely understood. In this study, we performed a comparative analysis of whole transcriptome profiles in two widely used mouse models of septic cardiomyopathy, the cecal ligation and puncture (CLP) model and the lipopolysaccharide (LPS) model. Our aim was to identify key genes and pathways involved in the development of septic cardiomyopathy and to evaluate the similarities and differences between the two models. Our findings suggested that 1) both methods can induce septic heart dysfunction within 24 hours; 2) distinct whole transcriptome expression profiles are revealed; 3) potentially different pathways are involved in causing heart failure in sepsis. The comprehensive comparison provides valuable insights into the molecular basis of septic cardiomyopathy and contributes to the ongoing search for effective treatment strategies, triggered by different factors for SICM.

Keywords: Sepsis-induced cardiomyopathy; Gene sequencing; Whole transcriptome profiles; Septic animal models; Cecal ligation and puncture; Lipopolysaccharide

1. Introduction

Sepsis continues to be a major global health challenge, accounting for a significant portion of morbidity and mortality worldwide. [1, 2]. Septic cardiomyopathy is a frequent and severe sepsis complication, contributing to the increased risk of death in affected patients. Despite ongoing research efforts, the molecular mechanisms underlying septic cardiomyopathy are not yet fully understood, hindering the development of targeted therapies. Different methods of sepsis induction, such as Cecal Ligation and Puncture (CLP) and Lipopolysaccharide (LPS) administration, are widely used in experimental models to study the pathophysiology of septic cardiomyopathy [3-5]. While these models share similarities in the manifestation of sepsis, they may also have unique molecular signatures that can impact the generalizability of the findings. For example, mortality in LPS induced sepsis occur rapidly due to intense inflammatory response on cardiovascular system, whereas delayed mortality is observed in CLP model of sepsis [6]. Both models displayed similarities and differences in gene expression patterns depending on the severity of the insult and the animal model used [7]. Understanding the gene expression profiles

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associated with different methods of sepsis induction is essential for elucidating the molecular mechanisms of SICM and identifying potential therapeutic targets.

Systemic inflammation followed by bacterial infection may lead to severe form of sepsis and can cause cardiac dysfunction. Experimental data have demonstrated the link between inflammation, sepsis, and cardiac dysfunction [8, 9]. Analysis of echocardiographic data sowed reduced cardiac contractility, cardiac index, and ejection fraction (EF) and diastolic dysfunction are associated with sepsis [10, 11]. Clinical data indicated that septic patients with diastolic dysfunction have higher mortality than those diagnosed with sepsis but without diastolic dysfunction [12, 13]. The underlying molecular markers responsible for cardiac dysfunction during sepsis are not well documented. Although sepsis-induced cardiac dysfunction has been extensively studied, the underlying mechanisms are not yet fully understood. Several potential mechanisms have been proposed, including: inflammatory cytokine production[14], endothelial dysfunction [15], oxidative stress due to increased reactive oxygen species[16], insufficient ATP production leading to mitochondrial dysfunction [17], impaired calcium ion channels [18], and myocardial stunning [11].

In this study, we conducted a comparative analysis of the whole transcriptome expression profiles in mouse models of septic hearts induced by either CLP or LPS. Our results il-lustrate distinct gene expression patterns and pathways associated with each method of sepsis induction. Such as G protein-coupled receptor binding, seem highly upregulated only by LPS, while significant downregulation of HIF-1 signaling pathway and PI3K-Akt signaling pathway are only observed in CLP-induced septic hearts. Importantly, we ob-served that several crucial aspects, such as regulation of inflammatory response, regulation of reactive oxygen species (ROS) metabolic process, regulation of endothelial cell chemotaxis, leukocyte migration, and apoptotic processes, as well as some other molecular pathways, are commonly dysregulated in septic cardiomyopathy, regardless of the induction method. By emphasizing these shared or distinct molecular characteristics, we underscore the importance of considering the method of sepsis induction in the study of septic cardiomyopathy. Our comparative analysis of CLP and LPS-induced mouse models contributes to the ongoing effort to decipher the molecular basis of SICM and identify promising therapeutic targets for future interventions.

2. Materials and Methods

2.1. Animal Models and Experimental Design:

Adult male C57BL/6 mice, aged 8-10 weeks, were randomly assigned to one of three groups: CLP-induced sepsis, LPS-induced sepsis, or a control group. Since no differences in cardiac function were observed between PBS injection and sham CLP operation, we reduced the number of animals used for control by employing only sham-operated mice as controls for both septic animal models. Both male and female mice were used in the study; however, only male mice were included in the gene sequencing analysis to minimize potential confounding effects related to sex-specific differences in gene expression, hormonal regulation, and immune responses. All animal procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee.

2.2. Cecal Ligation and Puncture (CLP) procedure induced sepsis.

CLP induced sepsis was performed as previously described[19],. Briefly, mice in the CLP group were anesthetized using 1% isoflurane and underwent a midline laparotomy. Under aseptic conditions, the cecum was gently exteriorized, and the area below the ileocecal valve was ligated to preserve blood supply and valve function. The ligated cecum was then punctured twice using a 20-gauge needle, ensuring minimal tissue damage. A small amount of fecal material was carefully extruded from the puncture sites to confirm bacterial leakage into the peritoneal cavity. Subsequently, the cecum was repositioned into the abdominal cavity. The abdominal incision was closed in two layers using sterile

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sutures, ensuring proper wound healing. Postoperatively, the mice received subcutaneous fluid resuscitation to maintain hydration and promote recovery. The warmed 0.9% saline solution, with a volume of 1 ml, was administered subcutaneously into the flank area. For the control group, mice were sham-operated. The mice were closely monitored for any signs of distress, infection, or complications following the injection.

2.3. Lipopolysaccharide (LPS) administration induced sepsis.

Mice in the LPS group were subjected to an intraperitoneal injection of lipopolysaccharide (LPS) to induce sepsis as previously described [20]. A predetermined dose of LPS (5 mg/kg body weight) was dissolved in sterile saline and administered using a sterile syringe and needle. The injection site was first cleaned with an antiseptic solution to minimize the risk of infection. The needle was carefully inserted into the lower abdominal quadrant, and the LPS solution was slowly injected into the peritoneal cavity. Following the injection, the needle was gently withdrawn, and the injection site was monitored for any signs of leakage or adverse reactions.

2.4. Echocardiographic Image Acquisition

Echocardiographic assessment was performed 24 hours after the CLP or LPS procedure in all experimental groups, including the control group. Echocardiographic imaging was conducted using a VisualSonics Vevo 2100 system equipped with an MS400 linear array transducer on mice anesthetized with 1% isoflurane. Mice were imaged at baseline (sham operated) and 24 hours post-CLP or LPS, following a previously described protocol [21, 22]. To assess cardiac output, the recordings of at least 10 independent cardiac cycles for each experiment were analyzed. Cardiac output, the volume of blood pumped by the heart per minute, was then calculated based on these echocardiographic measurements, taking into consideration the heart rate, stroke volume, and the dimensions of the left ventricle. This method provides a comprehensive evaluation of the heart's capacity to meet the body's circulatory needs under septic conditions.

2.5. Sample collection and RNA extraction

24 hours after either LPS IP injection or CLP operation, mice were euthanized, and their hearts were rapidly excised. Heart tissues were snap-frozen in liquid nitrogen and stored at -80°C until further processing. Total RNA was isolated from 30 ug heart tissue using RNeasy Fibrous Tissue Mini Kit (74704, Qiagen, MD) following the manufacturer's protocol. RNA integrity was confirmed using a Cytation3 microplate reader (BioTek, VT). The RNA profiling was conducted using Illimina NovaSeq 6000 sequencer of the University of Chicago Genomics Facility (Chicago, IL). Libraries were prepared using Illumina TruSeq Small RNA Sample Preparation Kit (RS-930-1012, Illumina, CA).

2.6 RNA-Seq Analysis

Transcriptome profiles of CLP and LPS inducted septic heart were analysis using RNA-seq technology. The raw sequencing data quality was pre-processed with FastQC (version 0.11.9)[23] to ensure high sequencing quality for all sequencing reads. We then used STAR2.7.9 [24] to perform reference alignments to the mouse reference genome based on GENCODE M27 (https://www.gencodegenes.org/). All transcriptome genes were assembled via featureCounts [25] based on the same version of corresponding gtf file from GENCODE. The assembled transcriptome count table was normalized via TMM from edegR [26, 27] and differential expressed genes were conducted in edgeR with the statistical additive model using the control mouse samples without any sepsis inductions as the base lines for comparisons.

Differentially expressed genes were selected at the statistical significancy level (FDR $\leq$ 0.05 and FC $\geq \pm 1.5$). Venn-diagram was created via ggVennDiagram package in R

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(https://cran.r-project.org/). The low expressed genes were detected with expression in at least 2 samples with expression values count per million (cpm>1). The scaled expression values (x-scores) were calculated based on the logarithm normalized values with base 2, and used for heatmap visualizations via R package ComplexHeatmap [28, 29].

Functional enrichment analysis was conducted via clusterProfiler [30] on the top 200 identified DEGs based on fold change ratio from CLP and LPS sepsis inductions. We further selected specific import enriched functions and pathways at an FDR corrected p-value of 0.05 to visualize the regulated functions differences of these 2 different sepsis inductions methods. Gene regulation networks exploration was performed via ReactomeFIViz[[31] in cytoscape v3.9.1 (http://www.cytoscape.org/) on the identified DEGs with respect to LPS and CLP septic inductions, where we included all background linker genes from 2021 ReactomeFIViz database. We then classified genes based on the total number of genes interactions from the network, and defined genes with the highest number of interactions as network essential genes. Mmp3 associated genes networks with respect to 2 different septic induction mechanisms were further selected and visualized via cytoscape v3.9.1.

3. Results

3.1. CLP and LPS-induced septic animal models developed cardiac dysfunction.

Both CLP and LPS-induced septic animal models exhibited cardiac dysfunction within 24 hours, as evidenced by multiple functional and structural alterations (Fig.2C-E). We observed that LPS (5mg/kg) and 40% CLP led to high mortality rates, with 75% and 50% mortality after 72 hours, respectively (Fig. 2A, B). Interestingly, LPS-induced sepsis (5mg/kg) demonstrated slightly lower mortality when compared to 40% CLP. Cardiac dysfunction was apparent as early as 6 hours (unpublished paper) post-sepsis induction in both models, suggesting a rapid onset of functional impairment. At 24 hours post-induction, both animal models displayed a marked reduction in cardiac output, providing a comprehensive evaluation of the heart's capacity to meet the body's circulatory needs under septic conditions (Fig. 2C-E).

3.2. Transcriptome Changes in Mouse Hearts Following Sepsis Induction

There is clear transcriptome differences among those samples with and without sepsis inductions as shown in Fig. 3A, At FDR corrected p-value of 0.05 and fold change (FC) of 1.5, 439 genes were detected upregulated in CLP induction mouse hearts, and 893 genes upregulated with LPS inductions whereas 497 genes downregulated with CLP induction, and 793 genes downregulated with LPS inductions. (Fig. 3B, C). Even though the sepsis induction mechanisms are different, we still can see 513 genes commonly regulated in the heart by both sepsis induction methods (Fig. 3D). Importantly, we found significantly higher expression of certain Matrix Metalloproteinases (MMPs), known to regulate cardiac remodeling, in LPS-induced sepsis compared to CLP-induced sepsis (Figure 3E). Especially, Mmp3 were upregulated in both septic induction models (Fig. 3E), similarly for several other Mmp family genes including Mmp2, Mmp8, Mmp9, Mmp14, Mmp17 were upregulated in both models of sepsis, whereas Mmp11, Mmp15, Mmp19, Mmp17 do not fully upregulated in CLP septic induction in comparison with LPS septic induction (Fig. 3E).

Functional enrichment analysis revealed that the upregulated genes in CLP induced septic hearts were primarily involved in positive regulation of cell migration, inflammatory response, immune effector process and cellular response to IL-17 (Fig. 4A). Additionally, the downregulated genes were responsible for extracellular matrix organization, integrin cell surface interactions and antigen processing and presentation of exogenous peptide antigen via MHC class-II (Fig. 4B). Transcriptional regulatory networks analysis indicates that the upregulated genes in CLP induced septic hearts were regulated mainly by NFκB1, Sp1, Jun and Egr1 (Fig. 4C). Induction of sepsis through LPS administration

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was found to result in a higher number of upregulated genes. Notably, the top 10 upregulated genes were Saa3, Cxcl13, Acod1, Fpr1, Lcn2, Ms4a4c, Serpina3m, Marco, Ms4a8a and Chil, which are involved in various biological processes and are expressed in different cells within the mouse heart. The upregulation of these genes suggests that they play a role in the body's response to sepsis induced by LPS administration. Upregulated genes in LPS induced sepsis were primarily involved in innate immune response, inflammatory response and positive regulation of response to external stimulus (Fig. 5A), whereas downregulated genes were involved in phagosome, peptide-ligand binding receptors and developmental biology (Fig. 5B). Transcriptional network analysis showed NFκB, Jun and Irf1 mainly regulate expression of genes in LPS-induced sepsis (Fig. 5C). These findings revealed that CLP- and LPS-induced septic hearts exhibit distinct transcriptional changes, reflecting the different molecular mechanisms and biological processes involved in the body's response to sepsis.

3.3. Functional mechanisms comparison between CLP versus LPS induced sepsis.

The comparative functional enrichment analysis of regulated genes in both septic hearts revealed a shared involvement primarily in mediating inflammatory response, regulation of reactive oxygen species metabolic processes, leukocyte apoptotic processes, and positive regulation of the toll-like receptor signaling pathway (Fig. 6A). Moreover, we observed that specific biological functions and pathways were downregulated differently in septic hearts, depending on the induction method. For instance, CLP-induced septic hearts exhibited downregulation of the PI3K-Akt signaling pathway, calcium signaling, and HIF-1 pathways, while LPS-induced septic hearts displayed downregulation of polysaccharide metabolic processes, glycogen metabolic processes, and glucan metabolic processes.

Functional enrichment of DEGs between CLP and LPS induced septic hearts revealed that numerous genes associated with mitochondrial function were downregulated, such as Slc25a33, Tsfm, Mrps22, Mrpl45, Taco1, Sdhaf4, and Sco1 (Fig. 7). Mitochondria play a vital role in energy production and various other cellular processes, with their dysfunction potentially leading to cellular stress and impaired heart function [32, 33]. Furthermore, certain apoptotic processes can be regulated exclusively by a specific sepsis induction method (Fig. 7). For example, the neutrophil apoptotic process can potentially be regulated by the CLP induction method, whereas LPS influences apoptotic processes in endothelial cells, neurons, and myeloid cells. Interestingly, we observed that endothelial cells respond differently to the two sepsis induction methods: CLP-induced sepsis positively regulates endothelial cell migration, while LPS-induced sepsis negatively regulates endothelial cell proliferation.

3.4. Gene regulation networks comparisons between CLP and LPS sepsis inductions.

Our analysis revealed that both JUN and EP300 play crucial roles in the two sepsis induction models (Fig. 8A, B). In particular, JUN can directly activate sepsis-related genes, altering biological mechanisms associated with essential sepsis functional genes (Fig. 8A, B). Notably, Mmp3, a gene involved in the regulation of cardiac remodeling, can be directly regulated by JUN for both sepsis induction methods (Fig. 8C, D). These findings highlight the significance of JUN and EP300 as central regulators in the gene regulation networks of CLP and LPS-induced septic hearts, potentially influencing key molecular pathways and functional outcomes in sepsis-induced cardiomyopathy. Further investigation into the specific roles and downstream targets of these transcription factors may provide valuable insights into the molecular mechanisms underlying septic cardiomyopathy and reveal novel therapeutic targets.

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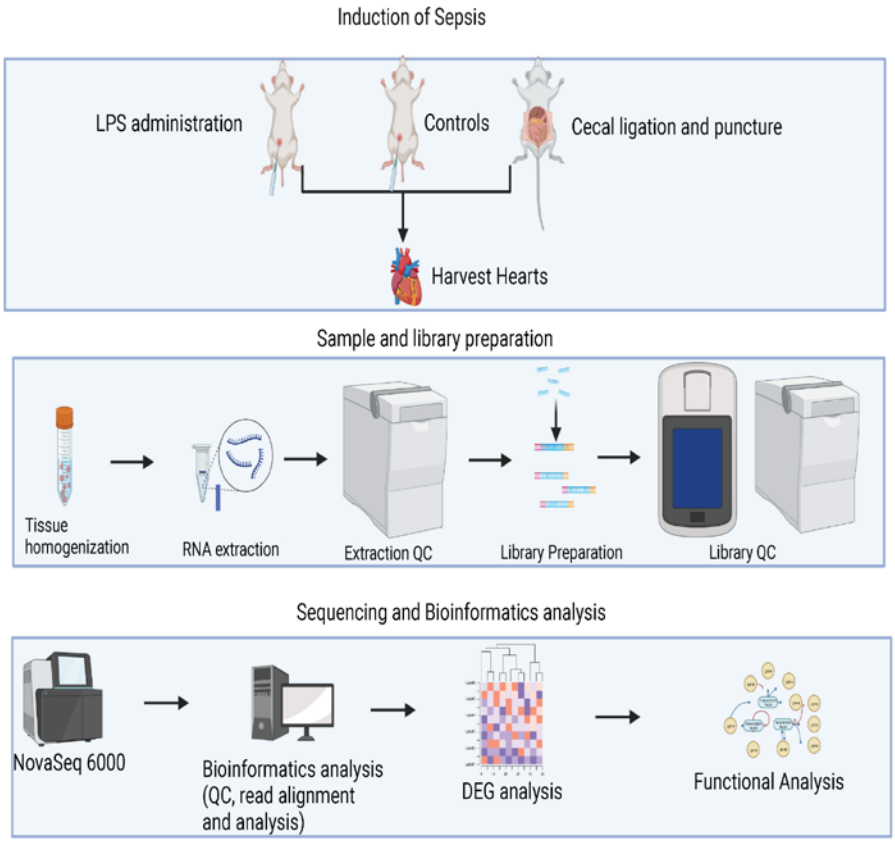

Figure 1. Schematic diagram of RNA-sequencing experimental design. 24 hours after either LPS IP injection or CLP operation, mice were euthanized, and their hearts were rapidly excised. Sample and library preparation was followed by Illumina NovaSeq 6000 sequencing and bioinformatics analysis of DEGs. The figure was created by BioRender.com.

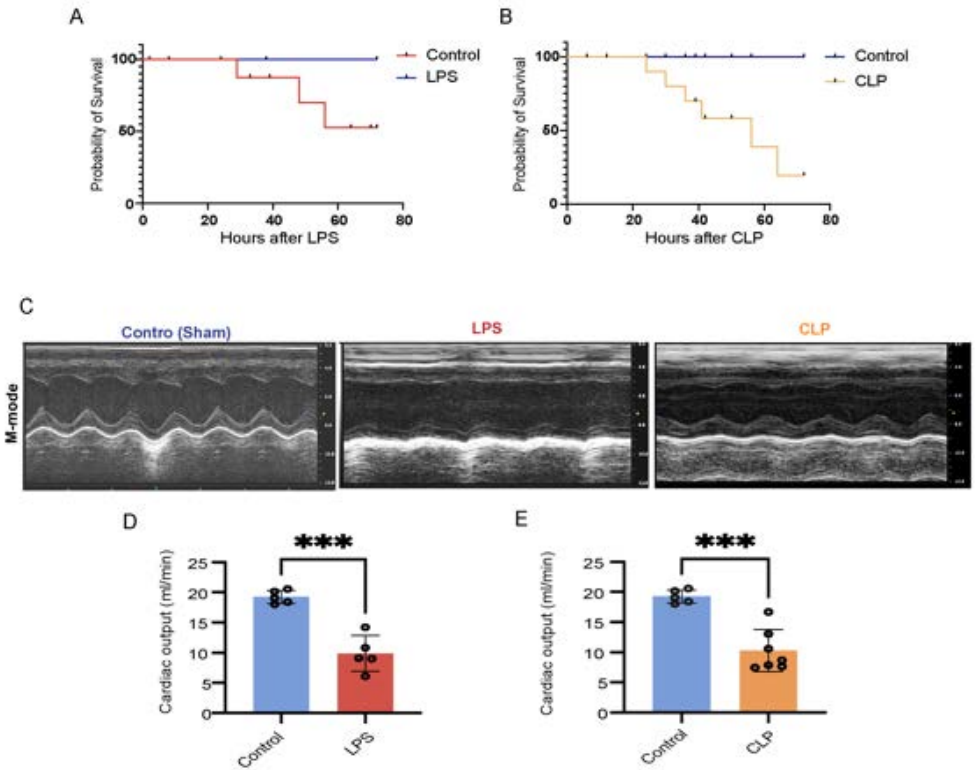

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Figure 2. Assessment of Cardiac Output and Mortality in Septic Mice. Mice weighing 20-25g were subjected to either 40% cecal ligation and puncture (CLP, n=15) or lipopolysaccharide (LPS, 5mg/kg, n=16) injection. Mortality in mice was observed over a duration of 5 days, and cardiac output was measured by echocardiography 24 hours post-sepsis induction. A) LPS-induced mortality (P<0.001). B) CLP-induced mortality (P<0.001). C) Cardiac output in LPS-induced sepsis. D) Cardiac output in CLP-induced sepsis. Data are represented as Mean ± SD, n=5, ***P<0.001.

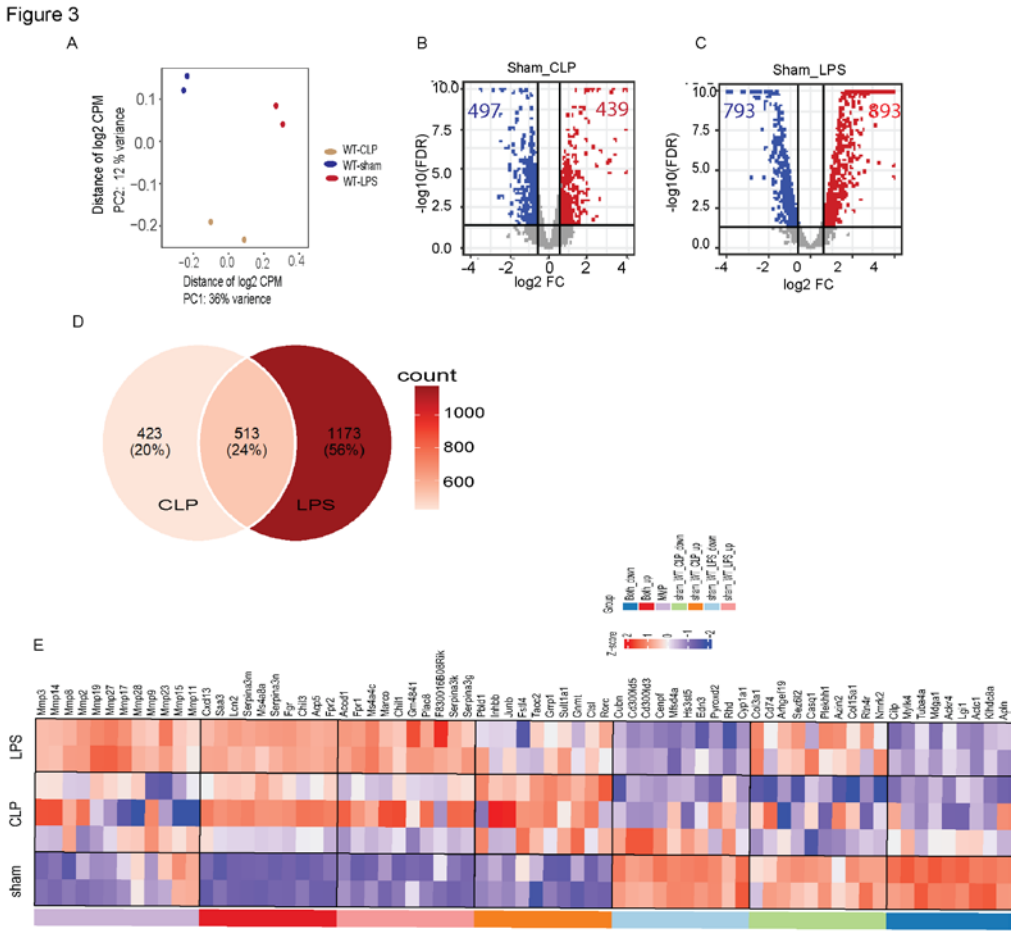

Figure 3. Differential Gene Expression and Functional Enrichment Analysis in CLP versus LPS-Induced Sepsis. (A) Principal Component Analysis (PCA) plot of six samples, including duplicates for each experimental condition. Labels are as follows: Sham denotes Control Sham-operated samples; CLP indicates sepsis induction with Cecal Ligation and Puncture; LPS refers to sepsis induction with Lipopolysaccharide. (B-C) Volcano plot of Differential Gene Expression (DEG) analysis, with the horizontal line indicating a False Discovery Rate (FDR) corrected p-value threshold of 0.05, and two vertical lines indicating cvFold Change (FC) at 0.05. (D) Venn diagram illustrating the comparative analysis of identified DEGs from both CLP and LPS sepsis induction. (E) Heatmap of selected highly expressed genes in relation to different sepsis induction methods in the heart.

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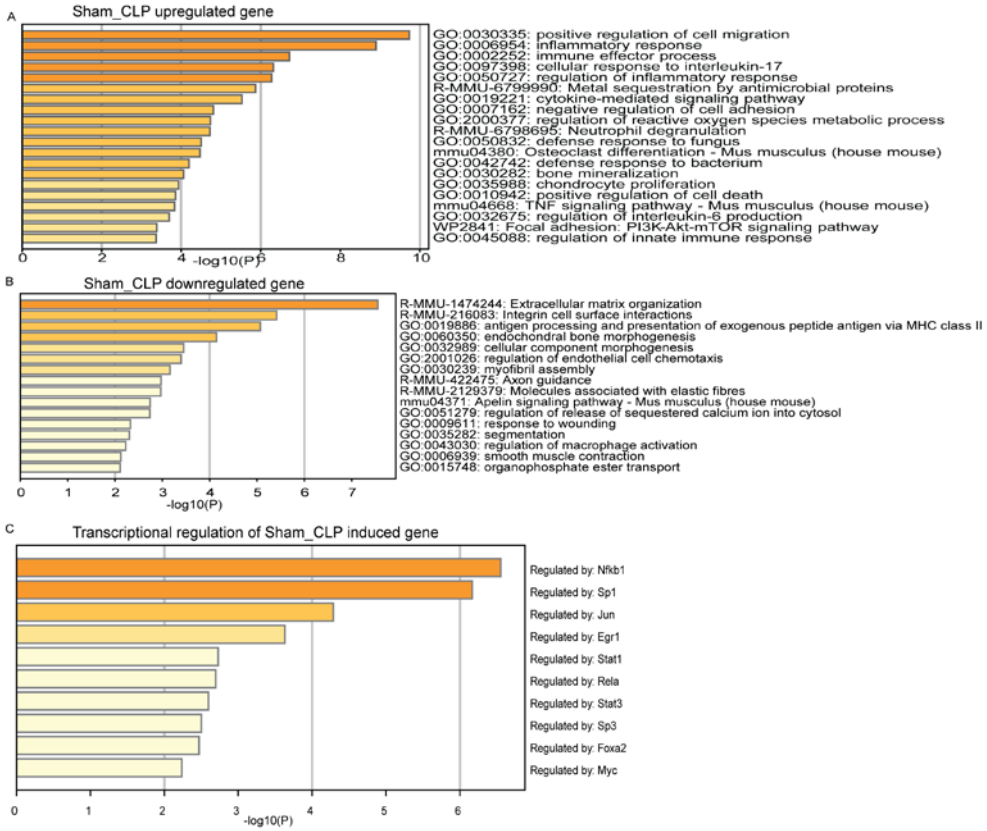

Figure 4: Enrichment Analysis of Genes in CLP-Induced Septic Hearts. (A) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of selected up-regulated genes. (B) GO/KEGG enrichment of selected down-regulated genes. (C) GO-Transcriptional Regulatory Regions in Human Untranslated Sequences (TRRUST) enrichment of transcriptional regulators in CLP-induced samples, compared to their respective sham controls. Analyses were performed using Metascape.com.

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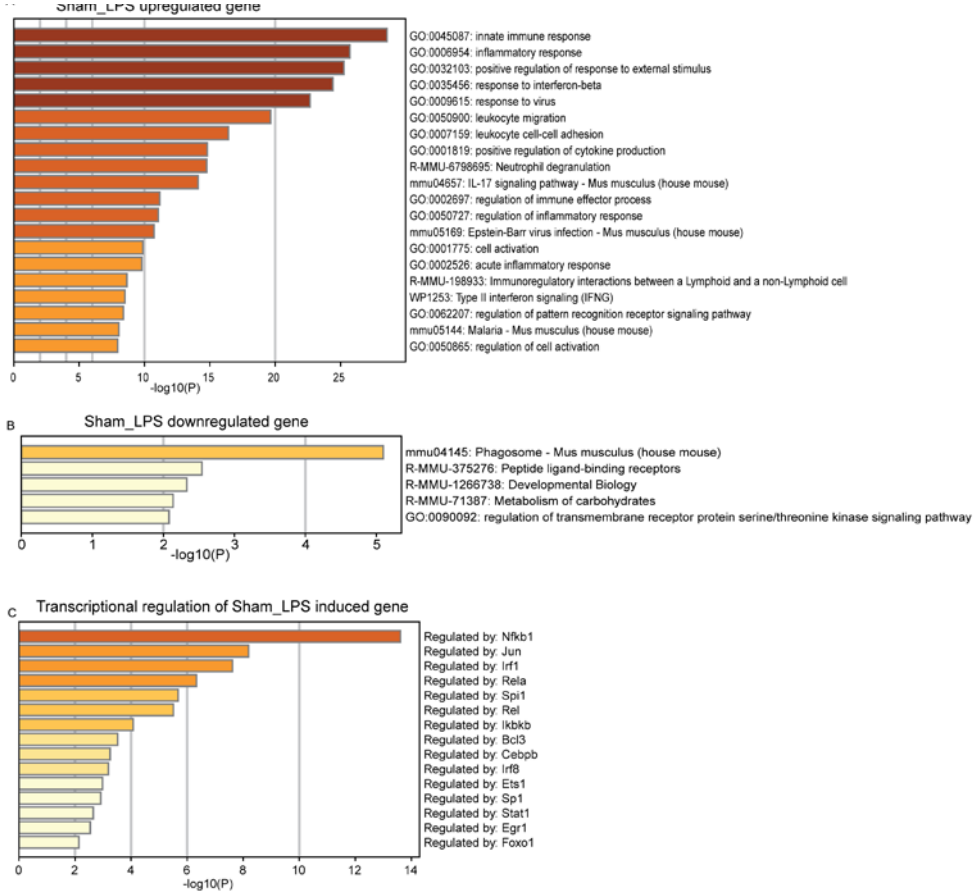

Figure 5: Enrichment Analysis of Genes in LPS-Induced Septic Hearts. (A) GO/KEGG enrichment of selected up-regulated genes. (B) GO/KEGG enrichment of selected down-regulated genes. (C) GO-TRRUST enrichment of transcriptional regulators in LPS-induced samples, compared to their respective sham controls. Analyses were performed using Metascape.com.

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Figure 6

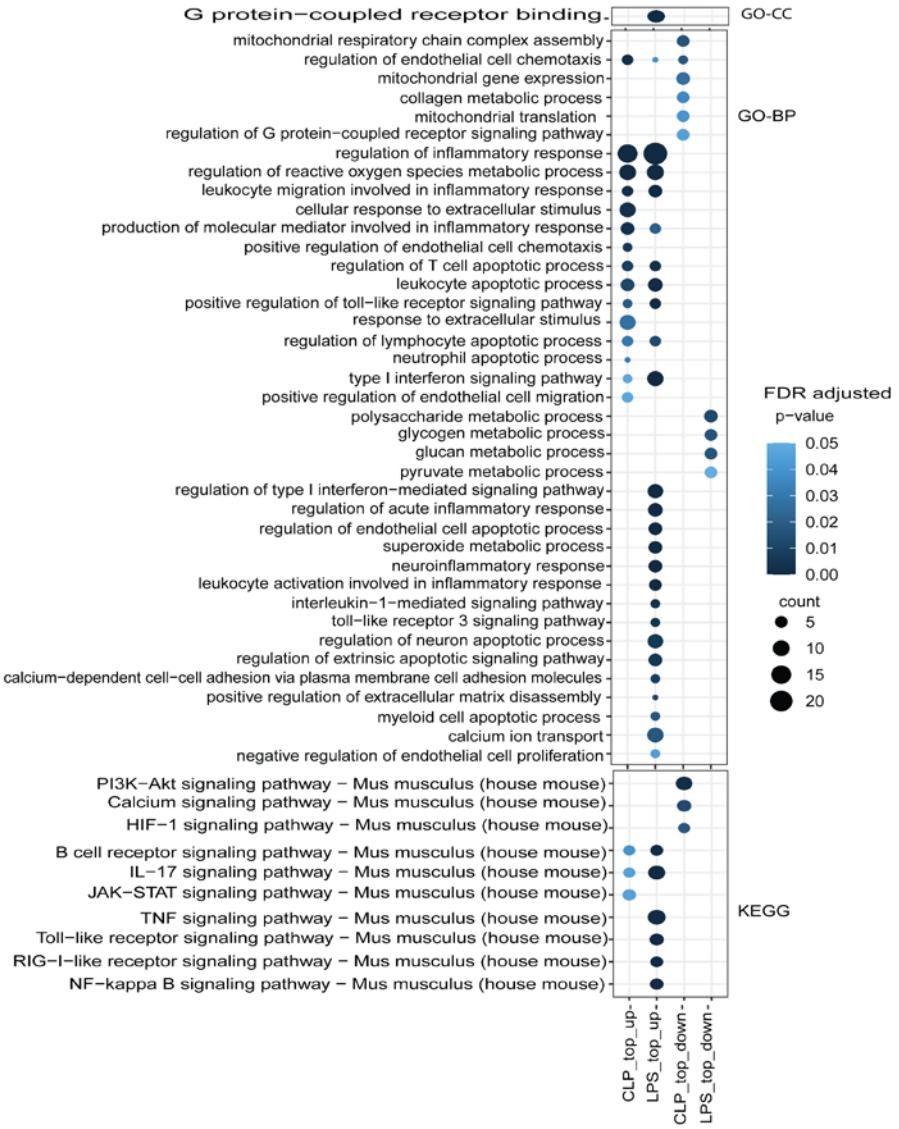

Figure 6: Functional Enrichment Analysis of Differential Gene Expressions (DEGs). Highlighted are selected potentially regulated functions associated with the two sepsis induction methods, using Gene Ontology (GO) cellular components, biological processes, and KEGG databases.

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Figure 7

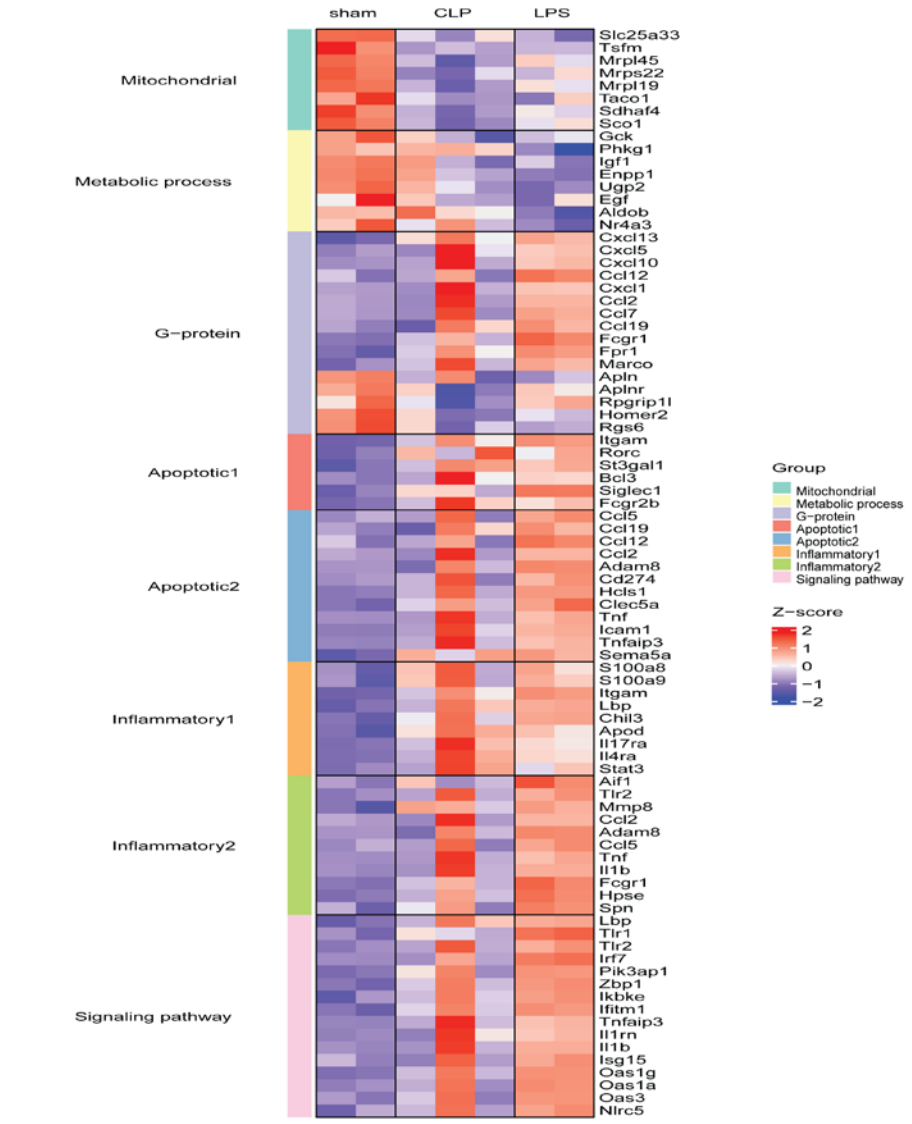

Figure 7. Heatmap of Selected Enriched Functional Genes. This figure represents the differential expression levels of genes.

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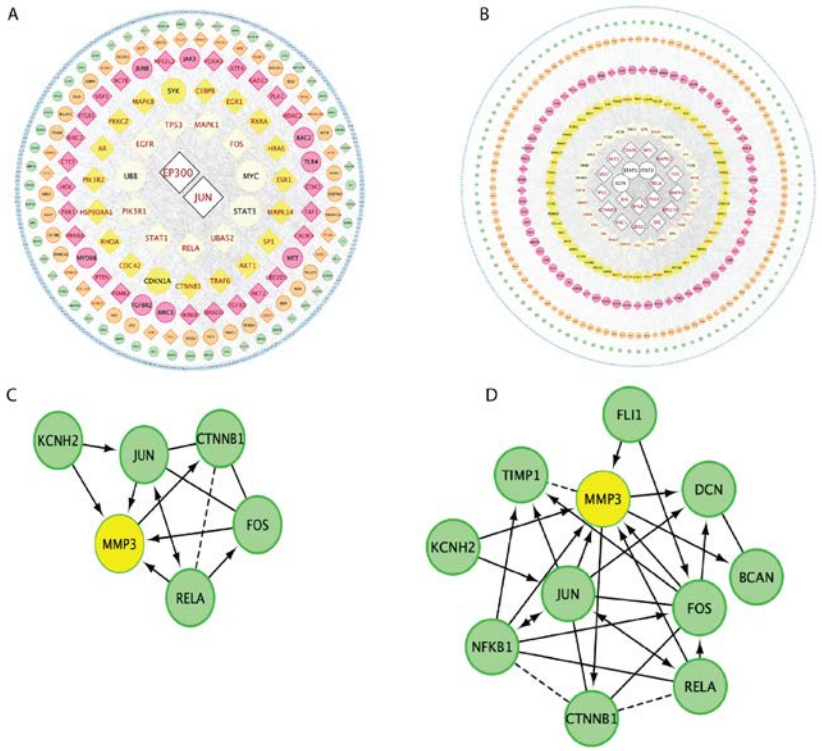

Figure 8: Network Analysis of Regulated Genes. (A) Network of regulated genes in CLP-induced sepsis, with central, essential genes representing those with more than 20 interactions. (B) Network of regulated genes in LPS-induced sepsis, with central, essential genes representing those with more than 20 interactions. (C) Interaction network of genes involving MMP3 in CLP-induced sepsis. (D) Interaction network of genes involving MMP3 in LPS-induced sepsis.

4. Discussion

Septic shock is a severe medical condition that arises due to a systemic inflammatory response to infection, resulting in a range of life-threatening symptoms such as hypotension, ischemia, multiple organ failure, and even death [8].To understand the underlying molecular pathways involved in the pathogenesis of sepsis, numerous in vivo and in vitro models have been developed to explore candidate therapeutic agents aiming to mimic sepsis in human and its treatment [34-42]. Although gene expression patterns during sepsis have been studied in different organs, the common set of genes induced by both methods, particularly in the heart, remains unclear.

In this study, we employed RNA sequencing to analyze changes in transcriptional profiles and molecular pathways in heart tissue samples from mice induced with sepsis through lipopolysaccharide (LPS) or cecal ligation and puncture (CLP). Our results demonstrate that both LPS and CLP are effective methods in inducing septic cardiomyopathy and mortality. Compared to their respective controls, both models of sepsis lead to the activation or inhibition of different signaling pathways, resulting in altered

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transcription of genes. Our study specifically highlights the differential expression of Matrix Metalloproteinases (MMPs) such as Mmp2-3, Mmp8-9, Mmp14, Mmp17, Mmp19, Mmp28-29, with significantly higher expression in LPS-induced sepsis compared to CLP-induced sepsis. MMPs are known to regulate cardiac remodeling, and elevated MMP expression is strongly associated with left ventricular dysfunction in heart failure patients [43, 44]. Mmp9 is a commonly induced gene among the MMPs in both models of sepsis, and previous studies have reported its upregulation in the liver of CLP-induced septic rats [45].

In this study, we first compared gene expression data from heart tissue samples of mice induced with sepsis through LPS or CLP with their respective control groups to identify differential gene expression. Our findings revealed that both models of sepsis induce different sets of inflammatory genes. Specifically, LPS-induced sepsis activates acute inflammation via Toll-like receptor (TLR) pathways, while CLP-induced sepsis activates inflammation via STAT3 and cytokine expression. In addition to inflammatory genes, we also identified differential expression of several unique genes responsible for metabolism and mitochondrial function in both models of sepsis.

Next, we compared each group of differentially expressed genes to identify common genes regulated by each model of sepsis. We found that among the 2109 differentially expressed genes, 24% were commonly regulated by both models of sepsis. Using bioinformatics analysis, we identified major differentially expressed genes and distinct gene clusters that regulate inflammation, mitochondrial respiration, oxidative stress, endothelial permeability, extracellular matrix, and apoptosis. Previous studies have shown that LPS administration increases the expression of inflammatory genes by activating NFκB and promotes cardiac tissue rearrangement [46], while RNA-sequencing of CLP-induced septic heart revealed increased expression of Cxcr2, Cxcl1, Uchl1, Ace2, Itgb6, Lrg1, Hmgb2, Itgam, and Icam-1 genes [47].]. In our study, we observed upregulation of TNF, Toll-like receptor, and NFκB signaling pathways in LPS-induced septic heart tissues, whereas CLP-induced sepsis upregulates JAK-STAT, IL-17, and B-cell receptor signaling pathways.

Although genome-wide association studies (GWAS) and high-throughput OMICs technologies have suggested several common biomarkers of sepsis, including tumor necrosis factor α (TNFα), interleukin (IL)-1, IL-6, IL-10, and macrophage migration in-hibitory factor [40-42, 48, 49] the exact molecular markers associated with septic cardi-omyopathy are not clearly known. Despite significant progress in medicine, the man-agement of sepsis remains largely limited to anti-infective measures, fluid resuscitation, maintenance of multi-organ function, and other comprehensive therapies [50, 51]. Identification of commonly expressed genes in cardiac tissue across diverse models of sepsis may provide new insights into the pathogenesis of this condition and the development of novel therapies aimed at ameliorating septic cardiomyopathy.

In conclusion, our study highlights the importance of identifying commonly expressed genes in cardiac tissue across two different models of sepsis to gain new insights into the pathogenesis of septic cardiomyopathy and the development of novel therapies. Our study identified MMPs as potential targets for therapeutic intervention of septic cardiomyopathy.

Author Contributions: Writing - Original Draft: KU and YL; Bioinformatic Analysis: YL, QL, and KU; Model Induction: SA and RW; Experiment Performance: KU, KP, and TN; Writing - Review and Editing: RW, QS, and WS; Supervision: RW. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal research in this study was conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of the University of Chicago. (Animal protocol number 72392, approved on November 15, 2021).

Data Availability Statement: Data will be made available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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