American Journal of BioMedicine
AJBM 2014;2(4): 288–299
Research Article doi: 10.18081/2333-5106/014-04/288-299

Expression of ATF3 in mouse protects the liver against sepsis via inhibiting HMGB expression

Ya Yun, Xing Zheng, Lijuan Chen, Yong Su, Chin-Fen Kim, Mao Zhao[1*]

Abstract

Lipopolysaccharides are components of Gram-negative enterobacteria that cause septic shock in mammals and triggers innate immunity mainly via TLR4 signaling. HMGB1 play a critical role in regulating innate immunity-induced sepsis. ATF3 is a negative regulator of TLR4 signaling and the mechanism of HMGB1 induced liver injury after sepsis are incompletely understood. In this study, we investigated the protective effects of ATF3 after LPS injection. Adult (4-6 months) C57/BL6 mice and ATF3 knockout mice were treated with a low dose of LPS (0.5 mg/kg, iv) for 6, 12 hrs. Liver enzymes and cytokines (TNF-α, IL-1β and IL-6) are assessed. The neutrophil and mononuclear cells in the liver tissue were examined using immunofluorescent staining. We found that serum HMGB1 levels were 8-fold higher in C57/BL6 mice with sepsis than ATF3 knockout with greater densities of neutrophils and mononuclear cells in the liver tissue, and higher levels of TNF-α, IL-1β and IL-6 in the circulation and liver tissue as well as associated with an increase in the mortality rate. In conclusion, upregulation of ATF3 contributes to the reduced release of HMGB1, and increased the survival rate of mice after LPS treated. Therefore, suppressing LPS-induced inflammation with ATF3 induction may be an important strategy for sepsis therapy.

Keywords: ATF3, Endotoxemia, Proinflammatory cytokine, HMGB1

[*]Corresponding author email: Maoz@yahoo.com
[1]Huaihe Hospital of Henan University, Henan University, China.
Received 11 June 2013; accepted November 23, 2014, Published December 15, 2014
Copyright © 2014 MZ
This is article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

Introduction

The word sepsis originated from the Greek word "σηψις" (pronounced as "sipsis") that is originally used in Greek language for describing deterioration of the animal or vegetable organic matter due to the presence of microbes [1].

Severe sepsis and septic shock remain a thorny issue in public health care because of their high mortality rate, which has been reported to be between 30% and 50% [2]. Sepsis can evolve to multiple organ dysfunction syndrome (MODS), whose severity accounts for a high mortality rate. During sepsis, liver dysfunction is one of the MODS components and usually is associated with a poor prognosis but its precise incidence remains unclear. Whereas the liver plays a pivotal role in regulating a wide range of key metabolic, homeostatic, and host-defense

Research Article doi: 10.18081/2333-5106/014-04/288-299

activities, liver dysfunction is commonly viewed only as a consequence of shock and initial tissue hypoperfusion. In fact, the injured liver may be considered one of the main actors in the genesis and amplification of multiple organ failure. However, the lack of reliable diagnostic tools does not allow detection of early liver dysfunction [2]. This concise overview aims to describe the epidemiology and prognostic value of liver dysfunction during sepsis, then to review the pathophysiological aspects and clinical features of liver dysfunction, and finally to propose a main therapeutic axis and perspectives on specific treatment.

High mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP) [2], serves as a transcription factor in the nucleus and also as a proinflammatory cytokine when released into the extracellular fluids [2]. HMGB1 is involved in the activation of innate immune mechanisms in the context of viral and bacterial infections [3]. Clinical reports reveal that its levels are increased significantly in critically ill patients with sepsis [4]. Protective effects of activated protein C in severe sepsis may partially be mediated through the inhibition of HMGB1 signaling [16]. Receptor for advanced glycation end products (RAGE) and certain TLRs serve as the major signaling receptors for HMGB1 at the cell surface and inside the cell. Further, HMGB1 interacts specifically with TLR4/MD2 and activates macrophages and monocytes to release proinflammatory cytokines such as TNFα [4].

The role of ATF3 and HMGB1 in immune regulation and their connections to inflammatory diseases have been reported, and they both function in TLR-related pathways. However, it is unclear whether ATF3 plays a role in the HMGB1 regulation of LPS-induced endotoxemia. The purpose of this study is to characterize the relation between ATF3 and HMGB1 [5]. We found that ATF3 protects against LPS-induced endotoxemia in mice through reducing HMGB1 expression [6].

Sepsis, a major cause of morbidity and mortality worldwide [8], occurs in 18 to 42% of patients with Gram-negative bacterial infection [7]. Lipopolysaccharides (LPS) are endotoxins derived from the outer membranes of Gram-negative bacteria and are main triggers of innate immunity and acute inflammation that are vital for antimicrobial defense reactions [8]. LPS binds to Toll-like receptor 4 (TLR4) to activate a crucial proinflammatory transcription factor NF-κB that elicits expressions of several proinflammatory cytokines and chemokines such as TNF-α, IL-6 [9], and MCP-1 [10].

Analyses of endotoxin-stimulated macrophages revealed that ATF3 is a rapidly induced transcription factor that represses IL-6 transcription [11]. ATF3, a member of the ATF/cyclic adenosine monophosphate (cAMP) responsive element-binding protein (ATF/CREB) family of transcription factors, is a stress-inducible transcription factor. The ATF3 promoter comprises several TLR-responsive elements such as AP-1 and NF-κB sites; hence, the early phase of ATF3 induction is a foremost response to TLR signaling [12]. In addition, ATF3 induction during sepsis augments susceptibility to secondary infections [13]. ATF3 has been implicated as a strong target for prophylactic or therapeutic agent in endotoxic shock or bacterial sepsis [14].

Research Article doi: 10.18081/2333-5106/014-04/288-299

Methods and Materials

Animal

The ATF3-KO mice were kindly provided by Henan University, China. The ATF3-KO mice allele was backcrossed into C57BL/6J (B6) mice for at least seven generations before LPS-induced endotoxemic experiments. Male mice (8 to 10 weeks old and weighing 25–35 g) maintained under standard conditions at Tzu Chi University's Animal Center were used. All experimental procedures were approved by the Animal Care and Use Committee of Henan University, China.

Procedures

LPS serotype 0127:B8 (Sigma-Aldrich Chemical, St. Louis, USA) was dissolved in sterile physiological saline immediately before use. Mice were divided randomly into 4 groups, administered LPS (5 or 50 mg/kg, ip), and sacrificed at 0, 3, 6, and 24 hrs after LPS challenge under anesthesia (pentobarbital, 50 mg/kg, ip), and the lung tissues were removed for subsequent experiments. Supernatants of blood samples also were collected and frozen at −80°C for subsequent assays. In another study, mice were injected intraperitoneally with recombinant AAV (adeno-associated virus)-PGK (phosphoglycerate kinase) or AAV-ATF3 vectors (2×10^8 viruses). Two weeks later, mice were treated with LPS (5 mg/kg, ip) for 24 hrs, and the liver were removed for further studies.

Culture of RAW 264.7 Macrophage Cell Line

RAW 264.7 cells were obtained from the Henan University, China. Cells were cultured in a high-glucose DMEM medium containing 10% heat-inactivated fetal bovine serum (FBS, Hyclone Laboratories, Logan, USA), antibiotics (100 U/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 μg/mL amphotericin, Biological Industries, Beit Haemek, Israel) and maintained at 37°C in a humidified incubator containing 5% CO_2. Cells were cotransfected with plasmids using lipofectamine 2000 (Invitrogen, Carlsbad, USA). Three days after transfection, cells were treated with LPS (200 ng/mL) for 24 hrs.

Plasma TNF-*α* and IL-6 Measurement

Plasma TNF-*α*, IL-6, and HMGB1 concentrations were measured by enzyme-linked immunosorbent assay (TNF-*α* and IL-6: from Enzo Life Science, USA; HMGB1: from Uscn Life Science, Houston, USA) according to manufacturer's instructions.

IL-6 and TNF-*α* mRNA Measurement

IL-6 and TNF-*α* mRNA were measured by RT-PCR using a Super Script kit (Invitrogen) according to manufacturer's instructions. Total RNA was extracted from the lung tissue using trizol (Invitrogen) according to our previous report [15]. mRNA samples were quantified by spectrophotometer, and equal amounts of mRNA were reversely transcribed into first-strand cDNA. PCR amplifications were performed in triplicate using mixture of Master SYBR Green supermix (Roche, Switzerland). cDNA and specific primers for TNF-α and IL-6. The real-time PCR was performed for 45 cycles of 95°C for 15 s and 60°C for one minute using an ABI Prism 7300 (Life Technologies, USA).

Western Blotting

Protein concentrations were quantified by bicinchoninic acid (BCA) protein assay reagent (Pierce Chemicals, Rockford, USA). Standard techniques for SDS polyacrylamide gel electrophoresis and immunoblotting were followed. The antibodies used were mouse iNOS monoclonal IgG (1 : 500, BD Pharmagen, California, USA), mouse HMGB1 monoclonal IgG (1 : 1000, Abcam), rabbit ATF3 polyclonal IgG (1 : 1000, Santa Cruz Biotechnology), rabbit NF-κB monoclonal antibodies (1 : 500, Santa Cruz Biotechnology), mouse antiactin antibody (1 : 4000, Chemicon, IL, USA), and rabbit histone H2A polyclonal antibody (1 : 2000, Cell Signaling Technology, Beverly, CA, USA). The immunoreactivities were visualized and scanned into a computer. Individual bands were analyzed by Image J software (National Institute of Mental Health, NIH, Bethesda, USA).

Statistical analysis

Results were expressed as mean values7SEM and represent the mean of five independent experiments. ANOVA followed by Dunnett test or Bonferroni test was used to analyze differences among experimental groups. Statistical significance was considered at $P<0.05$.

Results

Survival Rate of Mice

Sepsis and septic shock are thorny problems, which cause high mortality from severe inflammatory response resulting in multiple organs dysfunction [17]. To confirm the pathological involvement of ATF3 in LPS-induced endotoxemia, WT and ATF3-KO mice were administered a lethal dose of LPS (50 mg/kg, ip), and the survival rate was examined. Figure 1 shows that less than 20% of the LPS-treated ATF3-KO mice survived for 24 hrs. In contrast, greater than 80% of WT mice survived after 24 hrs. These observations suggested that enhanced expression of ATF3 may protect mice from LPS-induced shock.

American Journal of BioMedicine
AJBM 2014;2(4): 288–299
Research Article doi: 10.18081/2333-5106/014-04/288-299

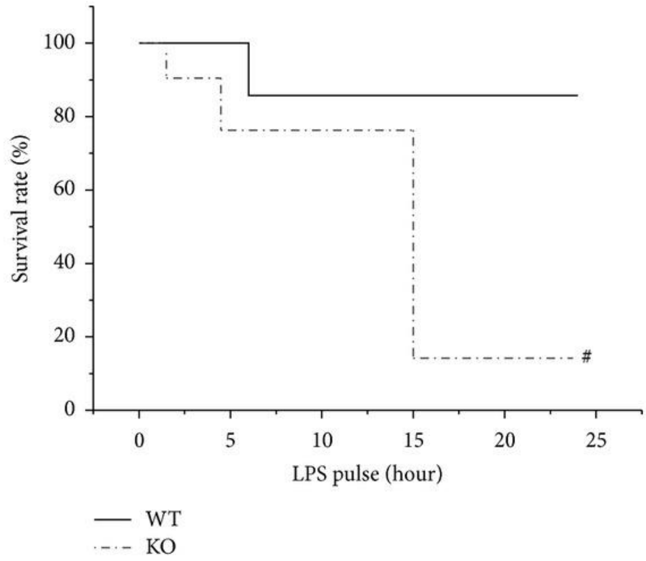

Figure 1.

The Kaplan-Meyer survival rate of WT (wild type) and KO (ATF3 knockout) mice at 0, 3, 6, and 24 hrs following administration of a lethal dosage of LPS (50 mg/kg, ip). Data represent three independent experiments (21–24 animals used). #, $P < 0.05$, WT versus KO mice.

ATF3 Knockout Increased MCP-1 Expression

It has been shown that the secretion of chemokine, MCP-1, by LPS-activated endothelial cells contributes substantially to the pathogenesis of sepsis [18]. However, the mechanism involved in LPS-induced MCP-1 production in the lung is not well understood. We examined the expression of MCP-1 in the lungs of WT and ATF3$^{-/-}$ mice under LPS treatment. As shown in Figure 3, ATF3$^{-/-}$ mice showed marked elevated level of MCP-1 compared with WT controls (Figure 2(a)), and MCP-1 predominately was expressed within the cytoplasmic region of MCP-1-positive cells (Figure 2(a) and insets). Furthermore, we compared the liver injury score in mice after LPS injection, and histological analysis of the lliver tissues from LPS-treated mice showed that ATF3 knockout further increased the accumulation of activated alveolar macrophages (Figure 3(a)) and the thickness of intra-alveolar septa (Figure 3(b)). Similarly, ATF3$^{-/-}$ mice revealed augmented liver injury score caused by LPS (Figure 3(c)). Together, these results indicate that ATF3 can reduce MCP-1 expression and decrease liver injury after sepsis occurrence in mice.

American Journal of BioMedicine
AJBM 2014;2(4): 288–299
Research Article doi: 10.18081/2333-5106/014-04/288-299

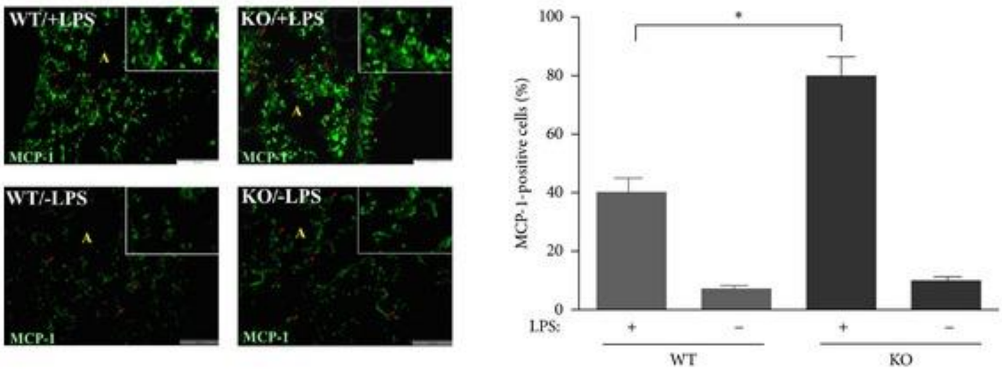

Figure 2.

ATF3 knockout increased LPS-induced MCP-1 level in the liver tissues of mice. Localization of MCP-1 induced by LPS in the liver tissues of WT and ATF3-KO mice detected by immunofluorescence. A: hepatic. Red arrows show MCP-1 staining of hepatic septa with few neutrophils. Insets show MCP-1 expression in the cytoplasm. Scale bar = 50 μm. MCP-1-positive cells were calculated from 4 fields with a total number of 200 cells counted. Data represent mean ± SEM ($n = 4$). $*$, $P < 0.01$.

Patients with Sepsis Syndrome Had Elevated Serum HMGB1 Levels

Both ATF3 and MCP-1 have been reported to be novel biomarkers for detecting inflammation-induced nephropathology [19]. Since ATF3 modulates the secretion and release of HMGB1, which is a down-stream proinflammatory mediator and may more closely reflect the status of sepsis, we, therefore, examined its level in sepsis patients. In a preliminary human study, serum samples were collected from ten inpatients whose diagnosis indicated sepsis, ten inpatients without infection, and four healthy volunteers. The serum levels of HMGB1 of inpatients suffering from sepsis were significantly higher when compared to the control groups (Figure 3). These results indicate that elevated serum HMGB1 level reflects the severity of sepsis syndrome.

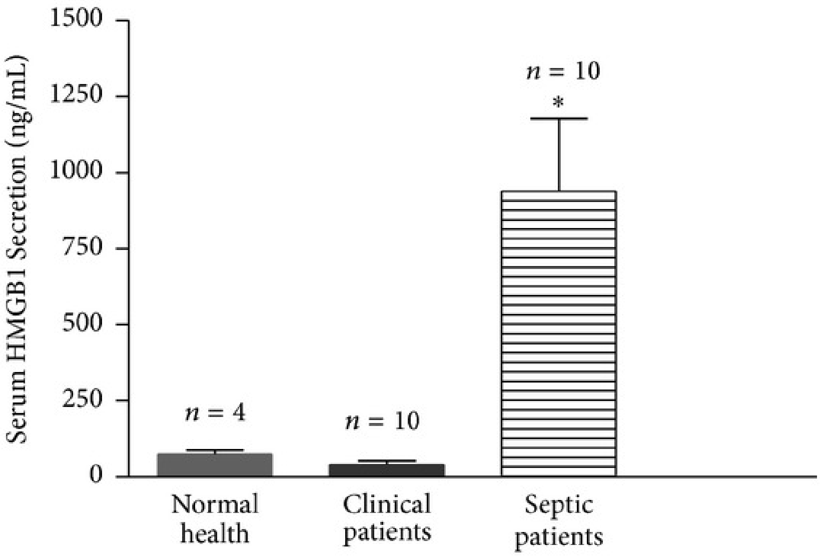

Figure 3.

Serum HMGB1 levels in septic patients. Serum HMGB1 was measured by an ELISA kit in the healthy volunteers and inpatients with or without sepsis. ∗, $P < 0.05$.

We further used the loss of function assay to inhibit ATF3 function in *in vitro* system. Cultured macrophages (RAW264.7) were transfected with ATF3 siRNA and control vector plasmid followed by LPS treatment. As shown in Figure 3, LPS-induced ATF3 gene expression was blocked as indicated by Western blotting (Figure 3(a)). HMGB1 release and secretion (Figure 3(b)), and NO (Figure 3(c)) induced by LPS were markedly enhanced when ATF3 functions were silenced by ATF3 siRNA compared with control cells.

Research Article doi: 10.18081/2333-5106/014-04/288-299

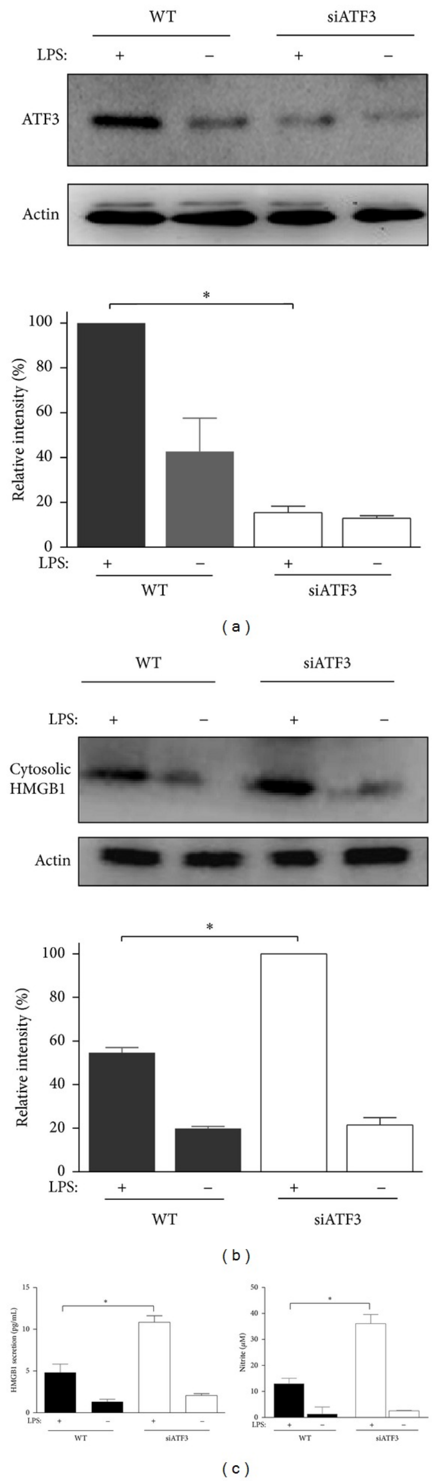

Figure 3.

ATF3 siRNA enhanced LPS-induced elevation of ATF3 and cytosolic HMGB1 expression and medium HMGB1 and nitrite concentrations in macrophage culture. (a) Representative Western blots of ATF3 expression in RAW264.7 cells treated with LPS (200 ng/mL) and ATF3 siRNA. The intensity of ATF3 was normalized to that of actin, which was served as the internal standard, and the percentage of control in the presence of LPS (200 ng/mL) was set as 100%. Data represent mean ± SEM ($n = 3$). ∗, $P < 0.01$. (b) Western blots of cytosolic HMGB1 expression.

Research Article doi: 10.18081/2333-5106/014-04/288-299

Discussion

No specific therapeutics for liver sepsis dysfunction/failure are currently available. Nevertheless, a set of recommendations could be given from the perspective of liver dysfunction/failure [19]. Thus, sepsis liver management relies first on early goal-directed resuscitation as recommended (that is, early antibiotic therapy and infection source control, fluid resuscitation, and vasopressor support to restore perfusion in the liver and other organs as well as support for the associated organ failure) [20].

Indeed, an appropriate hemodynamic restoration permits the restoration of liver perfusion and is an essential step in avoiding liver dysfunction. Interestingly, experimental data suggest that catecholamines could have an effect in the inflammatory response and participate in hepatic dysfunction [21]. Corticosteroid use in septic shock is still debated, but with respect to the liver, experimental data suggest that they may have an immunomodulation effect on sepsis-induced cholestasis through the induction of hepatobiliary transporters and restoration of bile transport [22].

Moreover, in the CORTICUS (Corticosteroid Therapy of Septic Shock) study, hydrocortisonetreated patients demonstrated a faster improvement in liver failure (SOFA hepatic score of 3 or 4) during the first week ($P < 0.0001$) [22]. However, at present, the use of corticosteroids cannot be recommended for the treatment of sepsis-induced hepatic dysfunction.

Although ATF3 expression is believed to play an important role in response to various stresses [23], little is known about its function in endotoxemia. In this study, we demonstrated that ATF3 exhibited a protective effect against LPS- induced endotoxemia in mice.

ATF3 expression not only decreased LPS-induced elevation inflammatory mediators, such as IL-6, TNF-α, NO, iNOS, MCP-1, and HMGB1, in the lung tissues and blood, but also increased the survival rate in endotoxemic mice. The upstream regulator of these inflammatory mediators is TLR4-NF-κB pathway [24], and ATF3 negatively regulates this pathway. We showed that *atf3* deficiency in mice could augment NF-κB presentation, and treatment of macrophages with ATF3 siRNA enhanced the nuclear translocation of NF-κB p65.

We also found elevated expression of serum HMGB 1 in septic patients in a preliminary study (Figure 6). Although the relationship between HMGB1 and ATF3 is not completely understood, we showed that in ATF3 KO mice, the serum HMGB1 level was elevated; however, administering AAV-ATF3 vector could reverse the HMGB1 level. These data indicate that ATF3 exhibits a protective effect against endotoxemia through an anti-inflammatory mechanism involving HNGB1 molecule. Further, HMGB1 could be a useful marker for sepsis [25].

The neutralization of inflammatory mediators with endotoxin-specific antibodies [26], tumor necrosis factor (TNF) inhibitors [27], or interleukin receptor antagonists [3] does not improve the overall survival of people with sepsis. In spite of the current advances in antibiotic therapy and intensive care, sepsis is still the most common reason of death in intensive care units [28]. In this study, we demonstrated that ATF3 is involved in most steps of innate immune response after pathogen invasion; ATF3 affects neutrophils recruitment, macrophage activation, proinflammatory cytokines production, and HMGB1-TLR4 circle. ATF3 presents in the early stage of endotoxemia to prevent macrophage activation and inhibit NO, IL-6, and TNF-*α* release that eventually will trigger an uncontrolled systemic inflammatory response that may lead to sepsis. ATF3 can block the processes of innate immune response induced by invading pathogens in the early stage but also regulate the late-acting mediators of sepsis, such as HMGB1. Therefore, suppressing LPS-induced inflammation with ATF3 induction will be an important target in pharmacologic treatment of sepsis.

Conclusion

Sepsis-induced liver dysfunction is a frequent event and is strongly associated with mortality. During the past few decades, its pathophysiology, including hypoxic and cholestasis aspects, has been better understood. However, the tools to diagnose liver dysfunction earlier and more accurately remain limited. At this time, the treatment of liver dysfunction is included only in the general therapeutic steps on sepsis syndrome management. An earlier and better identification of patients with liver dysfunction is warranted and may be the way to evaluate new therapeutic strategies and further improve the prognosis of sepsis.

Competing interests

The authors declare that they have no competing interests.

References

1. Muller-Decker K, Manegold G, Butz H, et al. Inhibition of cell proliferation by bacterial lipopolysaccharides in TLR4-positive epithelial cells: independence of nitric oxide and cytokine release. J Invest Dermatol. 2005;124:553–561. [PubMed]
2. Tobias PS, Soldau K, Gegner JA, Mintz D, Ulevitch RJ. Lipopolysaccharide binding protein-mediated complexation of lipopolysaccharide with soluble CD14. J Biol Chem. 1995; 270:10482–10488. [PubMed]
3. Hood DA, Takahashi M, Connor MK., Freyssenet D. Assembly of the cellular powerhouse: current issues in muscle mitochondrial biogenesis. Exercise and Sport Sciences Reviews 2000; 28(2): 68–73, 2000. View at Scopus
4. Abraham WT, Gilbert EM, Lowes BD, et al. Coordinate changes in Myosin heavy chain isoform gene expression are selectively associated with alterations in dilated cardiomyopathy phenotype. Molecular Medicine Cambridge, Mass. 2002; 8:750–760. [PubMed]
5. Molkentin JD, Kalvakolanu DV, Markham BE. Transcription factor GATA-4 regulates cardiac muscle-specific expression of the alpha-myosin heavy-chain gene. Mol. Cell. Biol 1994;14: 4947–4957. [PubMed]
6. Piantadosi CA, Suliman HB. Mitochondrial transcription factor A induction by redox activation of nuclear respiratory factor 1. J. Biol. Chem 2006; 281: 24–333. [PubMed]
7. Laurie K. Russella, Brian N. Fincka, Daniel P. Kelly. Mouse models of mitochondrial dysfunction and heart failure. Journal of Molecular and Cellular Cardiology 2005; 38(1): 81-91. View at Publisher

8. Crouser ED. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. Mitochondrion. 2004; 4: 729–741. [PubMed]
9. Huttemann M, Lee I, Samavati L, Yu H, Doan JW: Regulation of mitochondrial oxidative phosphorylation through cell signaling**.** Biochim Biophys Acta 2007; 1773:1701-1720. [PubMed]
10. Zapelini PH, Rezin GT, Cardoso MR, Ritter C, Klamt F, Moreira JC, et al.: Antioxidant treatment reverses mitochondrial dysfunction in a sepsis animal model. Mitochondrion 2008; 8: 211-218. [PubMed]
11. Yousif NG. Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK–PI3K/Akt pathway. Cell biology international 2014; 38 (1): 5-91. [PubMed]
12. Hanada T, Yoshimura A. Regulation of cytokine signaling and inflammation. Cytokine Growth Factor Rev 2002; 13: 413-421. [PubMed]
13. Orrenius S, Gogvadze A, Zhivotovsky B: Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol 2007; 47:143-183. [PubMed]
14. Echtay KS, Murphy MP, Smith RA.J. Talbot DA, Brand MD. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. J. Biol. Chem 2002; 77: 47129–47135. Abstract/FREE Full Text
15. Lin TK, Liou CW. Chen SD. et al., Mitochondrial dysfunction and biogenesis in the pathogenesis of Parkinson's disease. Chang Gung Medical Journal 2009; 32(6); 589–599. View at Scopus
16. Hock MB, Kralli A. Transcriptional control of mitochondrial biogenesis and function. Annual Review of Physiology 2009; 71: 177–203. View at Publisher · View at Google Scholar · View at Scopus
17. Lagouge M, Argmann C, Gerhart-Hines Z. et al., Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell 2006; 127(6): 1109–1122. View at Publisher · View at Google Scholar · View at Scopus
18. Suliman HB, Carraway MS, Ali AS, Reynolds CM, Welty-Wolf KE, Piantadosi CA. The CO/HO system reverses inhibition of mitochondrial biogenesis and prevents murine doxorubicin cardiomyopathy. Journal of Clinical Investigation 2007; 117(12): 3730–3741. View at Publisher
19. Nakai A, Yamaguchi O, Takeda T, et al. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. Nat Med 2007; 13: 619–624. CrossRefMedline
20. Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 2004; 6: 463–477. CrossRefMedline
21. Shimomura H, Terasaki F, Hayashi T, Kitaura Y, Isomura T, Suma H. Autophagic degeneration as a possible mechanism of myocardial cell death in dilated cardiomyopathy. Jpn Circ J. 2001; 65: 965–968. CrossRefMedline
22. Valentim L, Laurence KM, Townsend PA, et al. Urocortin inhibits Beclin1-mediated autophagic cell death in cardiac myocytes exposed to ischaemia/reperfusion injury. J Mol Cell Cardiol 2006; 40: 846–852.CrossRefMedline
23. Austin EW, Yousif NG, Ao L, Cleveland JC, Fullerton DA, Meng X. Ghrelin reduces myocardial injury following global ischemia and reperfusion via suppression of myocardial inflammatory response. AJBM 2013; 1(2): 38-48. View at Publisher
24. Klionsky DJ, Abeliovich H, Agostinis P, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy 2008; 4: 151–175. Medline
25. Brocheriou V, Hagege AA, Oubenaissa A, Cardiac functional improvement by a human Bcl-2 transgene in a mouse model of ischemia/reperfusion injury. J Gene Med 2000; 2: 326–333. CrossRefMedline
26. Munusamy S, MacMillan-Crow LA. Mitochondrial superoxide plays a crucial role in the development of mitochondrial dysfunctionduring high glucose exposure in rat renal proximal tubular cells Free Radic. Biol. Med 2009; 46:1149–1157 Article View Record in Scopus
27. Ventura-Clapier R, Garnier A, Veksler V. Transcriptional control of mitochondrial biogenesis: the central role of PGC-1alpha Cardiovasc. Res 2008; 79: 208–217 | Full Text via CrossRef
28. Yousif NG, Al-amran FG. Novel Toll-like receptor-4 deficiency attenuates trastuzumab (Herceptin) induced cardiac injury in mice. BMC cardiovascular disorders 2011;11(1): 62. [PubMed]

Research Article doi: 10.18081/2333-5106/014-04/288-299

American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net