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Mitochondrial protein UCP1 mediates liver injury induced by LPS through EKR signaling pathway

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Abstract. – OBJECTIVE: Mitochondria are abundant in liver. The roles of mitochondrial protein in liver injury and related signaling pathways are still unclear. UCP1 is a novel mitochondrial transmembrane protein. Its expression pattern and function in liver still needs further investigation.

MATERIALS AND MATERIALS: A mouse model of liver injury was established by the treatment of LPS. UCP1 expression in the liver tissue was detected by Western blot and qRT-PCR. ERK signaling activity was tested by enzymatic activity kit. ATP production was evaluated by flow cytometry. Cell apoptosis was determined by Western blot and flow cytometry. ERK signaling pathway inhibitor, U0126, was used t treat mice. Liver tissue from sepsis patien Va collected from the surgery.

RESULTS: Our data showed that the lev UCP1 was upregulated, ERK si was tivated, ATP production was duce and c apoptosis was enhanced in e w or i jury model caused by LP U te on significantly suppresse CP1 ession, inhibited ERK signalin hway, e ed ATP production, and re in iver cell apoptosis in mice liver inju model. increased, ERK signaling acti d, and cell tosis elevated in the liver ti e of sis patients.

CONCLU S CP1 plays a role in the liver tissue of m iver ry model and sepsis patie ough dulation of mitochondri TP oducti nd cell apoptosis by ERK si ing y.

Key Wo
Mitoch ial protein UCP1, ERK signaling pathway, LPS, Liver injury.

Introd on

LPS induced liv jury, wh gs to a kind of liver inflamm y re se sy ome. It is characterized by ig ce, h h case fatality rate, high cost increa in ence[1,2]. Liver injury caused S can le tissue hypoperfusion, septi hock, an dysfunction, and death throug ultiple me isms[3-5]. Therefore, effective de tion is the pre se and foundation of the treatent of liver injury induced by LPS[6,7]. According to current teria, the treatment to the mice with s nmation became less effective[8]. At present, it is urgently needed to explore the biomarker h good specificity and sensitivity for early diagosis, treatment, and prognosis of LPS-induced liver injury. Previous studies showed that biomarkers for LPS-induced liver injury mainly included the indicator of tissue perfusion, organ function, inflammatory cytokines, and liver tissue dynamics. However, the limitation was that all of these indicators were lack of specificity and high sensitivity[9,10]. Therefore, a better molecular biomarker is needed for the diagnosis of liver injury caused by LPS in clinic[11]. LPS-induced liver injury involves a variety of mechanisms, including the structure and function changes of mitochondria associated with inflammatory mediators[12,13]. Myocardial mitochondria are the important target of liver cell during liver injury. Following the development of biogenetics and the concept of cellular hypoxia, it was speculated that the mitochondrial energy metabolism had certain relationship with its own dynamics changes[14,15]. Mitochondrial protein UCP played an important role

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in mitochondrial function[16], which may be closely related to liver injury[17,18]. In this study, we intend to explore the potential relationship between UCP1 levels in liver cells and liver injury induced by LPS. As there is still lack of evidence about the relationship between UCP1 and LPS induced liver injury[19], we selected patients with liver injury from ICU and tested UCP1 level in the liver based on the data concerning mouse model of liver injury, in order to evaluate the value of UCP1 as molecular biomarker in liver injury induced by LPS and provide theoretical basis for the early diagnosis and prognosis of liver injury.

Materials and Methods

Experimental Model

Mouse liver injury model was established using LPS according to previous report[10]. Female C57BL/6 mouse at 6-8 weeks old was intraperitoneal injected by LPS at 25 μg/kg. The mouse injected by normal saline was treated as control. The experiment was approved by the Ethics Committee in our hospital conforming to anima[illegible] fare[10]. The liver tissue collected from th[illegible]sis patients received surgery was used as expe[illegible]tal group, while the liver tissue obtained fro[illegible]ver operation due to traffic accident was treat[illegible] as control. The experiment was ap[illegible]d by the Ethics Committee in Qingdao M[illegible] Hospital. All the subjects had signed [illegible]me[illegible]nsent.

Liver Tissue Preparation

The liver tissue was ex[illegible]cted [illegible] mouse or operation. The tissue [illegible]lysed an[illegible]d by normal saline and ace[illegible] for four time[illegible]en, the homogenate was [illegible]d at 150 g and stored at -20°C.

Caspase-3 Ac[illegible]y Detection

ERK signal[illegible] activi[illegible] was detected by kinase activity kit. [illegible] casp[illegible] activity was tested by microplate[illegible]20[illegible]otal c[illegible] × 10^5 liver cells were adde[illegible]ate t[illegible]ease the protein. Then, the mixture[illegible]ed with caspase-3 chrom[illegible] substa[illegible] room temperature avoi[illegible]r 20 min. At last, cells were tested on [illegible]ropla[illegible]492 nm[17].

At[illegible]tion

Mito[illegible] ATP production in liver tissue was detecte[illegible]ow cytometry. The liver tissue was added with[illegible]ysis to prepare the suspension. Next, the mixture was added[illegible]n fluid and tested on microplate[illegible]er at [illegible]n to calculate the ATP concent[illegible]on accordi[illegible]the standard curve.

Western Blot

The protein was [illegible]acted from[illegible] tissue and quantified. N[illegible]the pr[illegible]in was se[illegible]rated by SDS-PAGE and [illegible]lyze[illegible] Imag[illegible] software to obtain the gra[illegible].

Real-time[illegible]R

Total R[illegible] was extracte[illegible]m the liver tissue using [illegible]itrogen, Carlsbad, CA, USA) and r[illegible]e [illegible]d for Real-time PCR[21]. Total RNA was re[illegible]scribed to cDNA using p[illegible]t 42°C for 60 m[illegible]he PCR reaction system [illegible]ained 1 μl cDNA, 2 μl primers, 4 μl dNTP, 2 [illegible]oading buffer and 1 μl Taq enzyme. The PCR [illegible]ction was com[illegible]ed of 94°C for 5 min, followed [illegible]0 cycles of [illegible]C for 30 s and 55°C for 30 s. The [illegible] were as follows.

[illegible]TTAGAGGGTGGGGTGGATTGT-3' and 5'- CCACCTAAATCAACCTCCAACCA-3'; [illegible]in, 5'-TTATTAGAGGGTGGGGCGGATCGC-3' [illegible]ACCTAAATCGACCTCCGACCG-3'.

0126 Pretreatment

ERK signaling pathway inhibitor U0126 was [illegible]sed to treat mouse through intraperitoneal [illegible]jection at 10 μg/kg. Next, the mouse was intraperitoneal injected by LPS at 25 μg/kg. The mouse injected by normal saline was treated as control.

Statistical Analysis

All data analyses were performed on SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The measurement data were depicted as mean ± standard deviation. $p<0.05$ was considered as statistical significance.

Results

UCP1 Expression in Liver Cells from Mouse Liver Injury Model

As shown in Figure 1A, qRT-PCR results demonstrated that UCP1 mRNA significantly increased in liver cells from liver injury model compared with that in normal control ($p=0.0017$). As shown in Figure 1B and C, Western blot results revealed that the level of UCP1 protein obviously elevated in liver cells from liver injury model compared with that in normal control ($p=0.0027$).

ERK Signaling Activated, Mitochondrial ATP Production Reduced, and Cell Apoptosis Enhanced in Liver Injury Mouse

To further discuss the molecular mechanism of UCP1 in LPS induced liver injury model, we tested mitochondrial ATP production and cell apoptosis. As shown in Figure 2, our data exhibited that ERK signaling pathway was activated, mitochondrial ATP production was reduced, and cell apoptosis was enhanced in mice with liver injury.

The Influence of ERK Signaling Pathway Inhibitor U0126 Pretreatment

Next, to investigate the specific mechanism of UCP1 in liver injury induced by LPS, we detected ATP level and cell apoptosis in mice model pretreated by U0126. As shown in Figure 3, U0126 intervention significantly suppressed UCP1 expression, inhibited ERK signaling pathway, enhanced ATP production, and restrained liver cell apoptosis in mice, suggesting a key role of U0126 on ERK signaling pathway.

UCP1 Expression and Cell Apoptosis the Liver Tissue from Sepsis Patient

To study UCP1 expression and cell apopt sepsis patients, we collected the liver tissue f sepsis patients who received surgery. As shown Figure 4, the level of UCP1 was inc d, along with the increasing activation of naling pathway and elevation of cell a osis the liver tissue of sepsis patients.

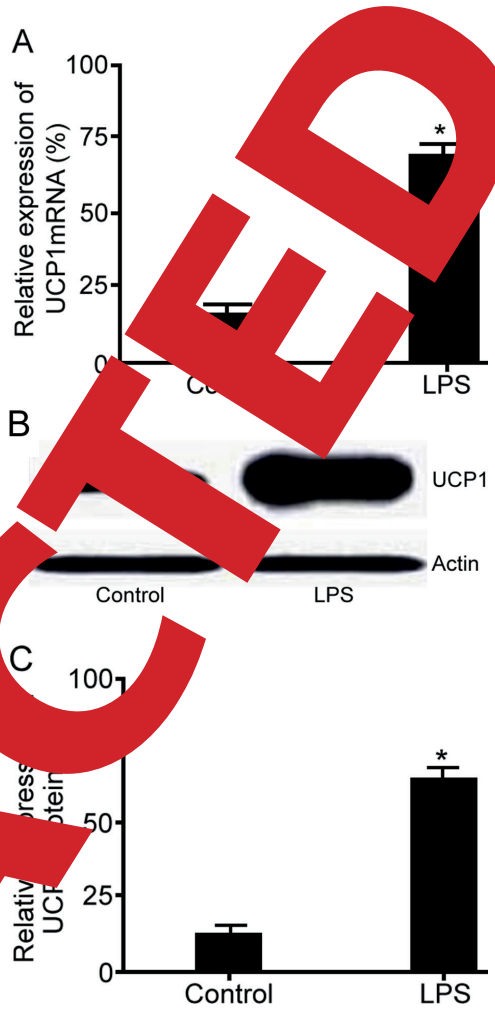

Figure 1. UCP1 expression in liver cells from mouse liver injury model. ***(A)*** qRT-PCR detection. ***(B)*** Western blot detection. ***(C)*** Western blot analysis. *$p<0.05$, compared with control.

Discus n

LPS seriously threat mammal's life the cause of liver inflam mong which, the function of mitochondrial uncoupling protein (UCP1) in liver injury induced by LPS remains to be further investigated. Early diagnosis and treat-

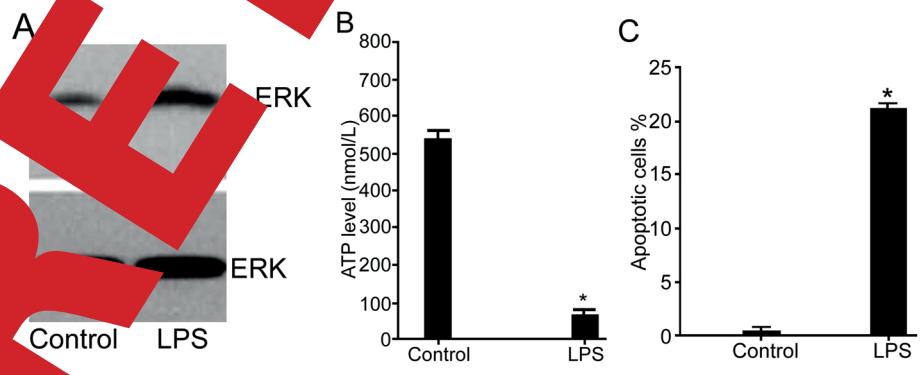

Figure 2. ER ng activated, mitochondrial ATP production reduced, and cell apoptosis enhanced in liver injury mouse. ***(A)*** Western blot detection. ***(B)*** ATP production. ***(C)*** Cell apoptosis detection.

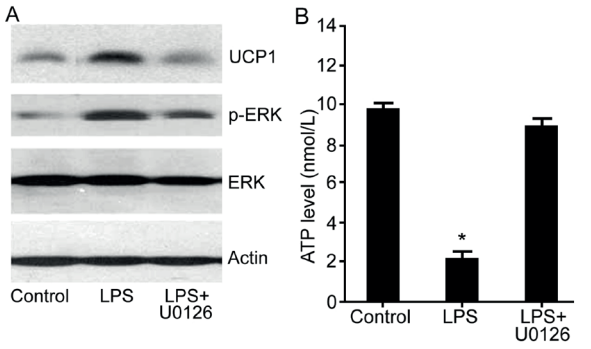

Figure 3. The influence of ERK signaling pathway inhibitor U0126 pretreat *(A)* Weste tion. *(B)* ATP production. *(C)* Cell apoptosis detection. *$p<0.05$, compared with control.

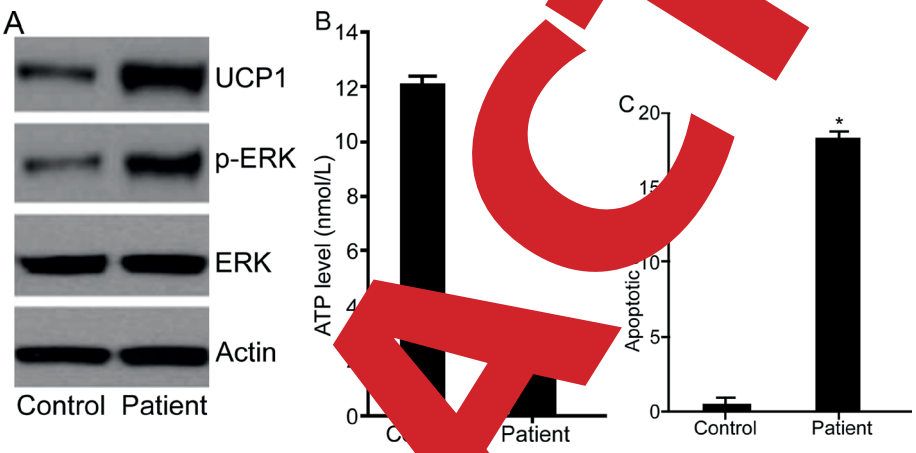

Figure 4. UCP1 expression and cell apo in the liver from sepsis patients. *(A)* Western blot detection. *(B)* ATP production. *(C)* Cell apoptosis detection ompared w ntrol.

ment were of prime importa jury in duced by LPS[22,23], but cu t m biomarker provided an inconve t and u ry solution to evaluate thi ease[24]. Therefo this study aimed to explo ibility of UCP1 as the biomarker in ea diag prognosis of liver injury induced by LPS. I ed liver injury is a co ated inflammat response process induc by in ction. A variety of inflammatory kines eract with each other to induce inte m respo during the process of liver us le g to mouse death[1,2]. Under this c pro-inflammatory cytok aling p s were activated to lead age in orga s and tissues of mamm d an matory strategy should b ted p flammatory diseases[25-27]. The application of reasonable detection method detection and prognosis is of great signi In our study on the molecular mechanism of UCP1 in liver injury, we found that UCP1 was upregulated, ERK signaling was activated, ATP production was reduced, and cell apoptosis enhanced in mouse model with liver injury induced by LPS. Also, with the treatment of U0126, the expression of UCP1 was significantly suppressed, ERK signaling pathway was inhibited, ATP production was increased, and liver cell apoptosis was decreased in mice. It suggested that UCP1 played its role in liver injury through the regulation of ERK signaling pathway, ATP production and cell apoptosis. Endotoxin injection in mouse showed the production of reactive oxygen species (ROS), sustained inflammatory activation and over expression of immune cytokines[14]. Moreover, levels of inflammatory cytokines were upregulated when ROS was activated[15,16]. Thus, the role of inflammatory cytokines in regulating reactive oxygen species and the influence of molecular target still needs further exploration. There are also deficiencies in this study. Firstly, the sample scale was small and larger scale was needed to

investigate the possibility of UCP1 as a practical biomarker in liver injury induced by LPS. Secondly, since most liver injury patients received chemotherapy and other treatments[28], the impact of treatment on UCP1 level is still to be further elucidated. Lastly, UCP1 as the target in animal liver injury model requires to be evaluated.

Conclusions

UCP1 exerted an essential effect on mice with liver injury and sepsis patients by regulating mitochondrial ATP production, cell apoptosis and ERK signaling pathway.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest.

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