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# HMGB1/TLR4 promotes apoptosis and reduces autophagy of hippocampal neurons in diabetes combined with OSA

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ABSTRACT

*Aims:* Obstructive sleep apnea (OSA) combined with type 2 diabetes (T2DM) may lead to cognitive dysfunction. We previously reported that cognitive impairment is exacerbated in KKAy mice exposed to intermittent hypoxia (IH), during which the DNA binding protein HMGB1 mediates hippocampal neuronal apoptosis by maintaining microglia-associated neuroinflammation, but the underlying mechanism remains largely unknown.

*Materials and methods:* We performed immunofluorescence, Western blotting, and immunohistochemistry experiments in mouse hippocampal tissues and HT22 cells. KKAy type 2 diabetes model mice and normal C57BL/6J mice were exposed to IH or intermittent normoxia. HT22 cells were cultured in high glucose medium and exposed to IH or intermittent normoxia. We transfected HMGB1 siRNA into HT22 cells and then treated them with high glucose combined with intermittent hypoxia.

*Key findings:* In conclusion, IH aggravated apoptosis and autophagy defects in T2DM mice, and increased the protein expression of HMGB1 and TLR4. This was also confirmed in HG + IH-treated hippocampal HT22 cells. HMGB1 siRNA can significantly reduce the protein expression of HMGB1 and TLR4, reverse neuronal apoptosis and enhance autophagy.

*Significance:* We believe that HMGB1 is a key factor in the regulation of hippocampal neuronal apoptosis and autophagy defects in T2DM combined with OSA. Targeting HMGB1/TLR4 signaling as a novel approach may delay or prevent the increased apoptosis and decreased autophagy induced by T2DM combined with OSA, and may ultimately improve cognitive dysfunction.

## 1. Introduction

The incidence of type 2 diabetes (T2DM) is increasing globally and has become a large burden on public health. T2DM has many comorbidities; one of the most common comorbidities is obstructive sleep apnea (OSA), which is mainly caused by recurrent partial or complete upper airway obstruction [1,2]. It is characterized by intermittent hypoxia (IH) during sleep, which leads to the loss of hippocampal neurons and cognitive dysfunction [3,4]. Studies have shown that chronic IH induces neuronal apoptosis in the hippocampal CA1 region, and apoptotic neuronal death contributes to IH-induced cognitive dysfunction [5]. It has been reported that OSA affects the dentate gyrus (DG) and further affects children's cognitive function [6]. Meanwhile, researchers have confirmed the occurrence of neuronal apoptosis in the hippocampal CA1 region of diabetes [7]. In addition, T2DM significantly reduces neurogenesis and cell proliferation in the mouse DG [8]. Hippocampal autophagy in diabetic GK rats with hyperglycemia is inhibited, and exposure to high glucose directly inhibits autophagy in mouse primary hippocampal neurons [37]. Apoptosis is one of the main causes of hippocampal neuronal damage [10]. We previously reported that hippocampal neuronal apoptosis is exacerbated in KKAy T2DM model mice exposed to IH. However, the relationship between autophagy and apoptosis in patients with OSA and T2DM has not been elucidated.

High mobility group protein box 1 (HMGB1) is a nuclear nonhistone DNA-binding protein that is mainly present in the nucleus and cytoplasm of cells and can also be actively secreted or passively released into the extracellular space [11]. As a damage-associated molecular pattern (DAMP), extracellular HMGB1 binds to a variety of cell surface receptors, including advanced glycation end products (RAGEs) and

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Toll-like receptor (TLR)-4; TLR4 is a major receptor for endogenous extracellular HMGB1 [12]. HMGB1 and TLR4 form a ligand-receptor pair that is involved in diseases characterized by cell death and injury, including diabetes [13] and Alzheimer's disease [11]. Studies have shown that HG can induce TLR4 overexpression and that HMGB1 is an endogenous ligand for HG-induced TLR4 signaling [14]. Extracellular HMGB1 stimulates glutamate release, which mediates neuronal cytotoxicity [12]. Overstimulated neurons can actively release HMGB1, and cell apoptosis can also lead to elevated levels of HMGB1 [12,15]. Experiments have shown that neurons release HMGB1 during seizures and that this release is accompanied by an increased expression of TLR4 [15]. A previous study found that the TLR4 signaling pathway inhibits autophagy [16]. We hypothesized that extracellular HMGB1 may combine with TLR4 to aggravate neuronal damage.

Our previous studies showed that HG + IH can induce oxidative stress and apoptosis in HT22 neurons and that the hippocampus of KKAy mice exposed to IH are severely damaged and that the Morris water maze (MWM) test showed a deterioration of cognition [17]. In the present study, we tested whether HG + IH exposure regulates apoptosis and autophagy by regulating the HMGB1/TLR4 pathway in the hippocampus of IH-exposed KKAy mice and HG + IH-treated HT22 cells. We look forward to finding new ways to inhibit apoptosis and promote protective autophagy to develop new therapeutic strategies for neuroprotection in patients with OSA complicated with T2DM.

## 2. Materials and methods

### 2.1. Animals and protocol for IH

Male wild-type C57BL/6J mice (C57, body weight, 26–30 g, 12 weeks old) and KKAy mice (KKAy, body weight, 35–38 g, 12 weeks old, Beijing HFK Biotechnology Co., Ltd.) were supplied with water and food in an air-conditioned room. They were then randomly assigned to 4 experimental groups as follows: the control group (C57, n = 8); the group of C57 mice exposed to IH (C57 + IH, n = 8); the KKAy mice group (KKAy, n = 8); and the group of KKAy mice exposed to IH (KKAy + IH, n = 8). We developed a gas delivery program in a computer language to deliver a specific gas into the exposure chamber to simulate IH like that of OSA [18]. The C57 group and the KKAy group were exposed to intermittent normoxic air for 8 h/d for 4 weeks, and the $O_2$ concentration in the exposed chamber was maintained at 21%. The C57 + IH group and the KKAy + IH group were exposed to IH for 30 cycles per hour for 8 h/d for 4 weeks. For each cycle, the $O_2$ concentration decreased from 21% to 5% over 30 s and then rebounded to 21% and was maintained 90 s. All experimental mice were anesthetized by intraperitoneal injection of 10% chloral hydrate and then sacrificed by cervical dislocation. The animal research program was approved by the Animal Ethics and Use Committee of Tianjin Medical University.

### 2.2. Cell culture and protocol for IH

HT22 cells (Beijing Chuanglian Biotech Institute, Beijing, China) were grown in Dulbecco's modified Eagles medium (DMEM, Life Technologies) with 10% fetal bovine serum (FBS, Life Technologies) and supplemented with penicillin (100 U/ml) and streptomycin (100 g/ml) (SV30010, Thermo, USA) and incubated at 37 °C in a humidified 5% $CO_2$ incubator. Then, the HT22 cells were seeded into 6-well plates and 24-well plates and assigned to the NC group, IH group, HG group and HG + IH group. The HT22 cells in the HG group and HG + IH group were treated with 50 mM glucose for 16 h, and the cells in the NC group and the HG group were subjected to intermittent normal oxygen exposure to maintain a 21% $O_2$ concentration for 8 h. The cells in the IH group and HG + IH group were exposed to IH in which 1.5% $O_2$ was for 30 s [19]and 21% $O_2$ was maintained for 90 s for 8 h [20].

### 2.3. Western blotting

To extract proteins from the frozen samples of cells and brain tissues, the cells and tissues were homogenized at 4 °C, the supernatant was collected and diluted with loading buffer, and the proteins were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to the little pole polyvinylidene fluoride (PVDF) membranes by the semidry transfer method. The membranes were blocked for 2 h with 5% nonfat milk. The membranes were incubated overnight at 4 °C with primary antibodies against LC3 (microtubule-associated protein 1 light chain 3) (Proteintech, cat# 14600-1-AP), Beclin 1 (Proteintech, cat# 11306-1-AP), p62 (Proteintech, cat# 18420-1-AP), cleaved caspase-3 (Proteintech, cat# 19677-1-AP), Bcl2 (Proteintech, cat# 12789-1-AP), Bax (Proteintech, cat# 50599-2-Ig), HMGB1 (Abcam, cat# ab18256), TLR4 (Proteintech, cat# 19811-1-AP) and GAPDH (Proteintech, cat# 10494-1-AP). A goat anti rabbit secondary antibody was diluted at 1:3000 and incubated for 1 h at room temperature. The membranes were then exposed to ECL solution, and the results were analyzed by Image J software and normalized to GAPDH.

### 2.4. Immunofluorescent labeling

Hippocampal frozen sections and HT22 hippocampal cells were fixed. After washing in PBS for 15 min at room temperature, the samples were permeabilized, blocked and then incubated overnight at 4 °C with the following primary antibodies (diluted with 1% BSA (Sigma-Aldrich)): anti-LC3 (Proteintech, cat# 14600-1-AP), anti-p62 (Proteintech, cat# 18420-1-AP), anti-Beclin 1 (Proteintech, cat# 11306-1-AP), and anti-cleaved caspase-3 (Proteintech, cat# 19677-1-AP). The samples were incubated with FITC labeled anti-rabbit or anti-mouse secondary antibodies diluted with 1% BSA for 1 h at 37 °C. The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, Beyotime, cat# C1002) for 2 min. The sections were coverslipped, and images were taken under a fluorescence microscope and analyzed with Image J.

### 2.5. Immunohistochemistry (IHC)

Paraffin sections of the hippocampus were dewaxed with xylene, hydrated with ethanol, and rinsed 3 times with PBS. Then, they were boiled for 20 min in 0.01 M citrate buffer. Antigen retrieval was carried out with 0.03% $H_2O_2$ at room temperature, and then the sections were washed three times with PBS, and blocked with 1% BSA for 30 min at room temperature. After rinsing, the slides were incubated with Ki67 (Bioworld, cat# BS90769), TLR4 (Proteintech, cat# 19811-1-AP), and cleaved caspase-3 (Proteintech, cat# 19677-1-AP) primary antibodies diluted in 1% BSA overnight at 4 °C. The next day, the sections were washed three times with PBS and then incubated with anti-rabbit secondary antibody diluted 1:5000 in 1% BSA for 1 h at 37 °C, and then washed in PBS. Diaminobenzidine (DAB) staining was performed for 2 min until light brown staining appeared, hematoxylin was used for counterstaining for 1 min, and the sections were finally dehydrated and fixed and covered for observation.

### 2.6. HMGB1 small interfering RNA (siRNA) transfection

HT22 cells were cultured in 6-well plates to 70% confluence. Lipofectamine 2000 (Thermo, cat# 11668027) was uniformly mixed with HMGB1 siRNA and NC siRNA (GenePharma Co., Ltd., Shanghai, China) in Opti-MEM according to the instructions and added to a 6-well plate for transfection for 6 h. Then, the original medium was replaced with HG medium, the cells that had been transfected were exposed to IH for 8 h, and finally, the cell protein was collected immediately.

### 2.7. Statistical analysis

The measurement data were calculated by SPSS 21.0 software (IBM Corp., Armonk, NY, USA). Graphs were created with GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All data between groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. All data are expressed as the mean ± S.E.M. $P < 0.05$ represents a significant difference. All independent experiments were performed at least three times.

## 3. Results

### 3.1. Decreased autophagy and increased apoptosis of hippocampal neurons in T2DM mice exposed to IH

Immunofluorescence was used to analyze the frozen hippocampal tissue sections from each group of mice. We found that the expression levels of LC3 and Beclin 1 in the hippocampus of IH-exposed C57 mice and KKAy mice were lower than those in the C57 group, while the expression of p62 increased correspondingly. There were no obvious differences between the C57 + IH group and the KKAy group. In addition, the KKAy + IH group showed a more significant decrease in LC3 and Beclin 1 expression and a significant increase in p62 (Fig. 1A). We also found that the expression of cleaved caspase-3 increased in the C57 + IH and KKAy group and especially in the KKAy + IH group (Fig. 1D). The expression of cleaved caspase-3 was significantly increased in the C57 + IH group and the KKAy group compared with the C57 group, that is most increased in the KKAy + IH group than the other group as determined by immunohistochemistry. While the expression of Ki67 was significantly more decreased in the KKAy + IH group than the C57 + IH group and the KKAy group, there are statistical differences (Fig. 1F and H).

Therefore, we speculated that there was a decrease in autophagy and an increase in apoptosis of hippocampal neurons of in KK-Ay mice and IH-exposed C57 mice and that this phenomenon was aggravated in the KKAy + IH group compared with the other groups. We determined Western blotting and found that LC3-I was converted to LC3-II (through the lipidation of LC3, the autophagosome-related form), and the protein expression of Beclin 1 was decreased in the C57 + IH group and the KKAy group, especially the KKAy + IH group. The expression of p62 (an LC3-binding protein that is degraded by autophagy and is a characteristic autophagic substrate) increased, and cleaved caspase-3 and Bax/Bcl2 expression was increased in the C57 + IH group and KKAy group compared with the C57 group. The KKAy + IH group showed the most obvious change (Fig. 1B and E). Statistical analysis showed that the C57 + IH group and the KKAy group were statistically different from the C57 group, and there was no difference between the C57 + IH group and the KKAy group. The KKAy + IH group was statistically different compared with the other three groups (Fig. 1C and G). We found that T2DM mice exhibited significantly inhibited autophagy and promoted hippocampal neuronal apoptosis after IH exposure. These results indicated that hippocampal damage was most severe when T2DM mice were exposed to IH.

### 3.2. Decreased autophagy and increased apoptosis of HT22 cells exposed to HG + IH

We first observed by immunofluorescence that, compared with those in the normal group, the expression levels of LC3、Beclin 1 were decreased and the expression level of p62 was increased in the IH group and the HG group, the expression in the HG + IH group was the most obvious than that of IH group and HG group (Fig. 2A). For cleaved caspase-3, the expression in the IH group and the HG group was higher than that in the normal group, and the most obvious increase was in the HG + IH group (Fig. 2D). We did not observe a significant difference between the HG group and the IH group. We found that exposure to IH or HG weakened autophagy and enhanced apoptosis in hippocampal neurons. To further demonstrate this phenomenon, we measured the protein expression of LC3, p62, Beclin 1, cleaved caspase-3, Bax/Bcl2 by Western blotting (Fig. 2 B and E). The expression of LC3 and Beclin 1 in HG + IH group was significantly lower than that in IH group and HG group. The expression of p62 and cleaved caspase-3 and Bax/Bcl2 was significantly higher than that in IH group and HG group (Fig. 2 B and E). Statistical difference showed between the IH group、the HG group and the normal group. There was a statistical difference between the HG + IH group and the other group. There was no difference between the IH group and the HG group (Fig. 2C and F). Therefore, we observed from the cell point of view that autophagy was impaired and apoptosis was increased in the IH group and the HG group and especially in the HG + IH group. We concluded that hippocampal neurons treated with HG + IH exhibited the lowest level of autophagy and the highest level of apoptosis, representing the most severe damage.

### 3.3. Autophagy is suppressed, and apoptosis is promoted via the HMGB1/TLR4 signaling pathway

The protein expression of HMGB1 and TLR4 was significantly increased in the C57 + IH group and the KKAy group compared with the normal group, and there were statistically significant differences. The most significant increase was observed in the KKAy + IH group. And there were statistically significant differences between the KKAy + IH group and the C57 + IH group、the KKAy group. There was no significant difference between the C57 + IH group and the KKAy group (Fig. 3 A and B). We also found that the expression of TLR4 in the C57 + IH group and the KKAy group was significantly higher than that in the normal group by immunohistochemistry. The expression of TLR4 in the KKAy + IH group was significantly higher than that in other groups, and the above comparisons were statistically significant (Fig. 3 E and F). In addition, we verified in the cell experiments that compared with the normal group, the protein expression of HMGB1 and TLR4 was significantly increased in the IH group and the HG group, and the expression of HMGB1 and TLR4 in the HG + IH group was significantly increased compared with the other three groups. These were statistically different (Fig. 3C and D). We therefore found that the HMGB1-TLR4 signaling pathway may be involved in the reduction of autophagy and the increase of apoptosis of hippocampal and HT22 cells.

### 3.4. HMGB1 siRNA reverses apoptosis and enhances autophagy in HT22 cells

To further determine whether HMGB1 is involved in apoptosis and autophagy in HT22 cells, we transfected HMGB1 siRNA into HT22 cells and found that, compared to that in the HG + IH + NC-si group, the expression of HMGB1 and TLR4 was decreased in the HG + IH + HMGB1-si group. The statistical analysis showed significance (Fig. 4 A and B). We also found that the protein expression levels of LC3 and Beclin1 were significantly increased, p62 expression was decreased, and the expression levels of the cleaved caspase-3 and Bax/Bcl2 proteins were decreased in the HG + IH + HMGB1-si group. Statistical analysis showed that the above changes were significant (Fig. 4C, D, E and F). It is not difficult to see that HT22 cells transfected with HMGB1 siRNA exhibited increased autophagy and decreased apoptosis after HG combined with IH treatment. In conclusion, HG + IH treatment inhibited autophagy and promoted apoptosis in HT22 cells through the HMGB1- TLR4 signaling pathway.

## 4. Discussion

The global incidence of T2DM is high [22], and it has numerous comorbidities [23]. Studying the pathogenesis of T2DM and its comorbidities is critical. OSA is one of the most common comorbidities in T2DM patients [24]. The prevalence of OSA in adults with T2DM is

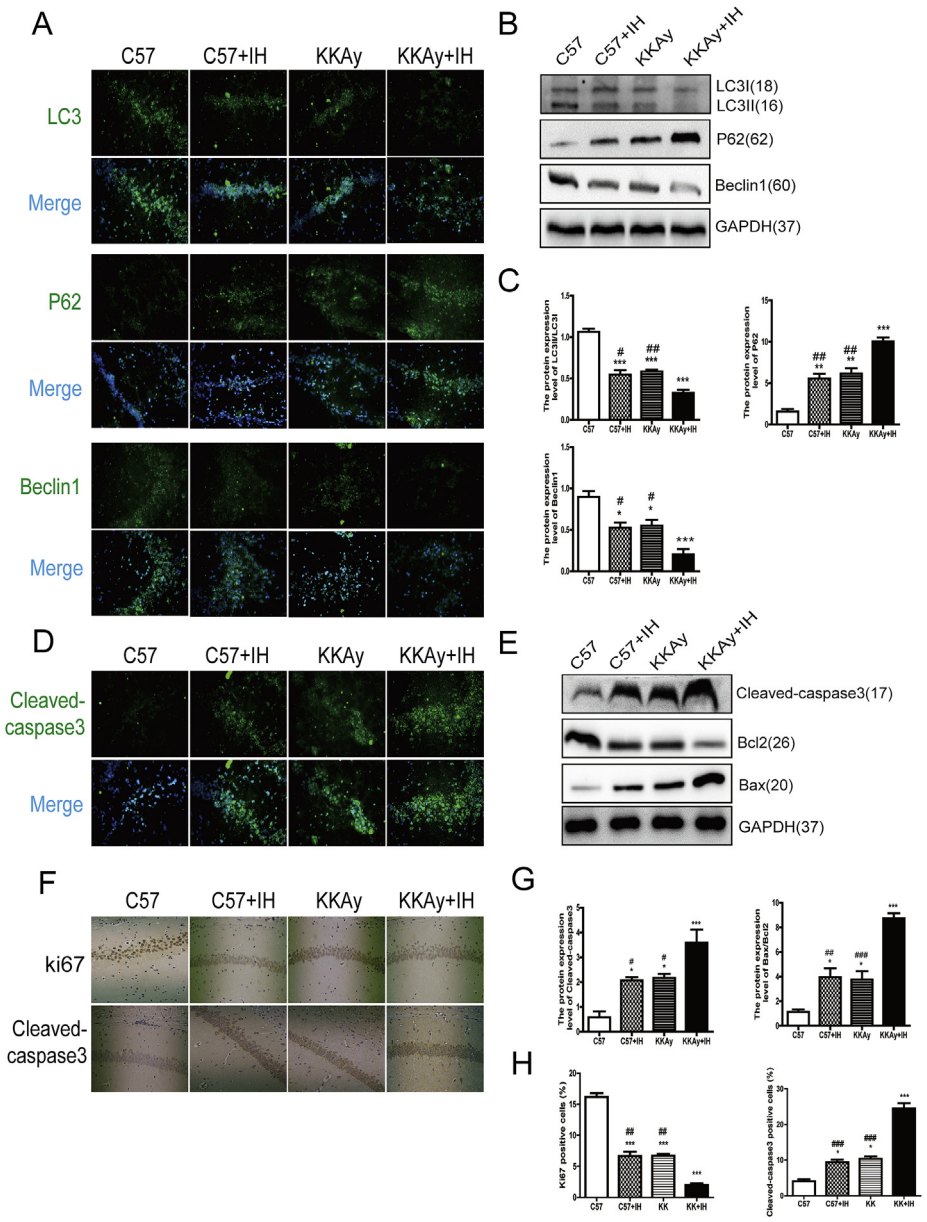


**Fig. 1. KKAy mice exposed to IH exhibit aggravated hippocampal neuronal apoptosis and impaired autophagy.** (A and B) Neuronal apoptosis and autophagy were observed by immunofluorescence in frozen sections of mouse hippocampal tissue. The green and blue fluorescence in the cells are markers of cytoplasmic and nuclear fusion. The detected indicators include LC3, p62, Beclin 1, cleaved caspase-3. (B and C) LC3, p62, Beclin 1 and GAPDH protein levels were determined by Western blotting. (E and G) The expression of cleaved caspase-3, Bcl2, Bax and GAPDH was analyzed by Western blotting. (F and H) Immunohistochemistry was used to observe the expression of Ki67 and cleaved caspase3. All results are presented as the mean ± S.E.M (n = 8 for each group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus the C57 group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus the KKAy + IH group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reported to be approximately 40–97%, which is significantly higher than the prevalence in the general population, and about approximately 23% of patients with T2DM have OSA [25,26]. Intermittent hypoxemia, as the main pathological feature of OSA, is associated with the development of T2DM [24]. There was a recent report that the association between type 2 diabetes and OSA is bidirectional [27]. The underlying mechanisms of association between OSA and diabetes may include intermittent hypoxia and increased oxidative stress [28].Chronic intermittent hypoxia has a combined effect of increasing oxidative stress and activating inflammatory pathways, which promotes beta cell apoptosis and lead to insulin resistance and pancreatic beta cell dysfunction [29,30]. Chronic hyperglycemia can also promote the development of OSA by increasing oxidative stress [31,32]. We found that oxidative stress can activate apoptosis [33]. Interestingly, autophagy has also been found to be impaired in numerous comorbidities [34] of T2DM. To some extent, neurodegenerative diseases caused by T2DM and OSA are closely related to autophagy impairment [35]. In addition, rats exposed to IH exhibit identified learning disorders and increased apoptosis in the CA1 region of the hippocampus [36]. There is also evidence that both T2DM animal models and T2DM patients exhibit cognitive changes in the central nervous system, such as learning and memory impairments [38,39]. Cognitive deficits caused by T2DM are accompanied by the upregulation of neuronal apoptosis [40]. In addition, stress can inhibit DG neurogenesis in the hippocampus [41]. Hypoxia may alter hippocampal function by inactivating the G protein in the DG [42]. The hippocampal DG region is indeed negatively affected by neuroinflammation caused by the development of metabolic syndrome [43]. The DG is a neurogenic region associated with memory and learning processes, and changes to the DG area of T2DM mice manifest as cognitive impairment [44]. Interestingly, CA1 has been found to be the most vulnerable region of the hippocampus to hypoxic injury [45]. Whereas neurons in the DG are more resistant to ischemia and hypoxia, the lower blade of the DG is able to almost completely resist depolarization in response to hypoxia, aglycemia, or the combination of both insults [46]. A study showed that the autophagy-apoptosis relationship varies based on the brain region [47]. The hippocampus is one of the most vulnerable brain regions for various neurobiological injuries [48]. In this experiment, we ignored the different areas of the hippocampus and studied the hippocampus as a whole.

Our previous studies found that IH and T2DM have synergistic

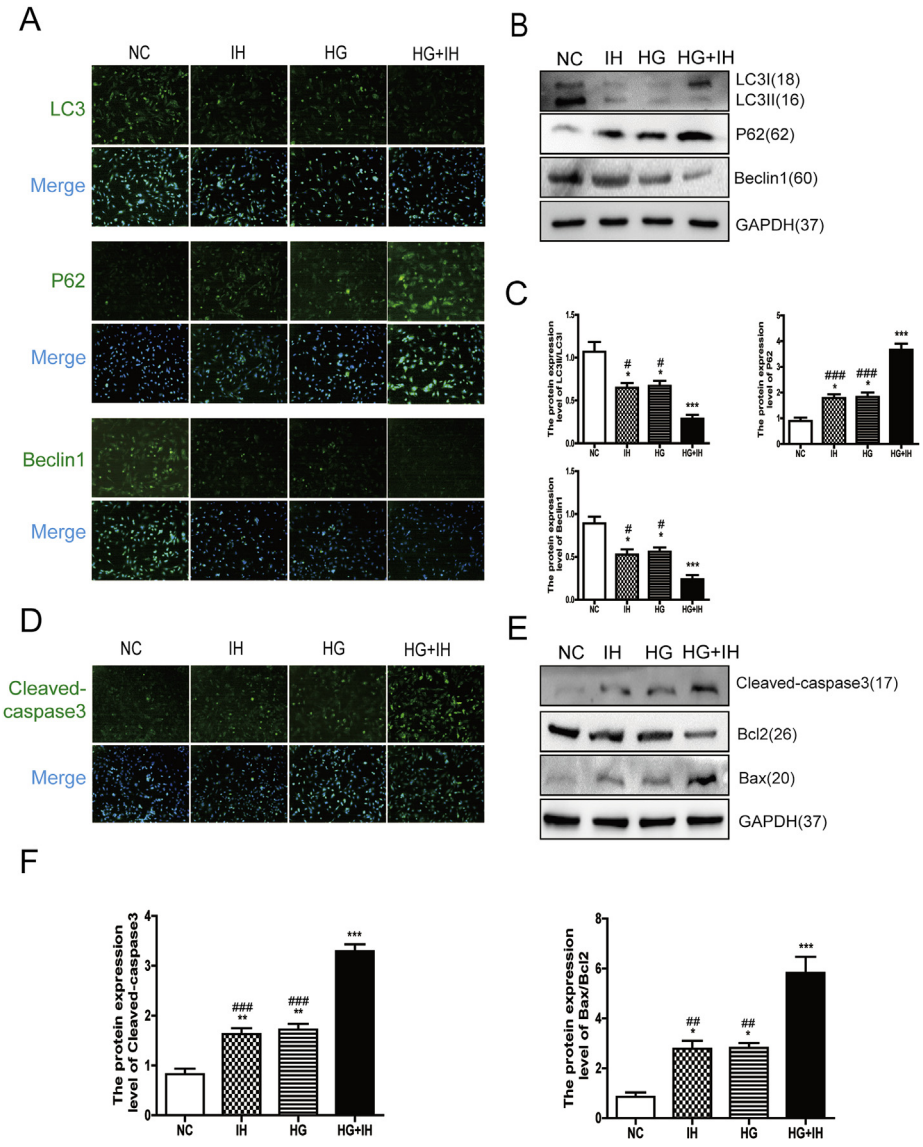


**Fig. 2. IH aggravates apoptosis and reduces autophagy in HG-treated HT22 cells.** (A and D) Representative images of immunofluorescence are shown. LC3, p62, Beclin 1 are autophagy indicators, and cleaved caspase-3 is used as an apoptotic indicator. All indicators in the images are labeled with green. The nuclear images are labeled with blue. (B and C) LC3, p62, Beclin 1 and GAPDH proteins were detected by Western blotting. (E and F) Cleaved caspase-3, Bcl2, Bax and GAPDH were detected by Western blotting. The data are presented as the mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001 versus the C57 group; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001 versus the KKAy + IH group.

effects on hippocampal neuronal apoptosis and learning and memory impairment [17]. Therefore, we focused on the effects of HG + IH exposure on hippocampal neuronal apoptosis and autophagy.

According to previous studies, KKAy mice exposed to IH exhibit high levels of HMGB1, which activates TLR4 to impair the autophagic function of hippocampal neurons and leads to apoptosis. Our HMGB1 siRNA transfection experiments further showed that the HMGB1/TLR4 signaling pathway is inhibited in HT22 cells, and restores apoptosis to basal levels and enhances autophagy.

KKAy mice were established by transferring the yellow obese gene (Ay allele) to KK mice [49]. KKAy mice spontaneously develop type 2 diabetes during the first 8 weeks of life [50]. The KKAy mouse model is considered similar to human type 2 diabetes [51]. KKAy mouse are characterized by hyperglycemia, obesity and insulin resistance [9]. Therefore, KKAy mice are considered to be typical models of T2DM [49]. C57BL/6J mice were used as homologous control mice for KKAy mice [52]. A study showed that KKAy mice suffer from spatial learning impairment and memory defects [49]. Other experiments have shown that KKAy mice have cognitive impairment caused by hippocampal neuronal injury(Y [52].

According to our previous study, the HMGB1 protein expression level in IH-exposed KKAy mice is high. HMGB1 is an evolutionarily highly preserved nuclear nonhistone DNA-binding protein that is a highly mobile protein in the nucleus [53]. Under normal conditions, HMGB1 is mainly located in the nucleus [15]. Upon exposure to various stresses, HMGB1 is passively released into the extracellular space by dying and damaged cells [54,55]. The role of extracellular HMGB1 is mediated by the receptor and its redox state; nuclear HMGB1 always appears in a fully reduced form and cannot activate the TLR4 signaling pathway; extracellular HMGB1 is slightly oxidized to bind to the TLR4 receptor with high affinity [53]. Extracellular HMGB1 activates the HMGB1 receptor and potentiates the production and translocation of HMGB1 [54]. HMGB1 causes brain damage to be aggravated by activating TLR4 [56]. Studies have shown that learning and memory impairment induced by elevated levels of HMGB1 in brain tissue may be mediated by TLR4 [57]. HMGB1 is released into the extracellular space of neurons after ischemic injury to induce neuronal death and aggravate brain tissue damage in rats [58]. HG-mediated oxidative stress can also induce the expression of HMGB1 and its receptors [59,60]. HMGB1-mediated inflammation and angiogenesis also contribute to the onset of T2DM. A vicious cycle is formed between HMGB1 and the onset of diabetes. The protein sequence of HMGB1 has 99% homology between rodents and humans(R [61]. HG also contributes to the increased protein and mRNA expression of TLR4 [62]. Therefore, we exposed T2DM mice to IH and found that the protein expression level of HMGB1 was significantly increased, and the expression of TLR4 was

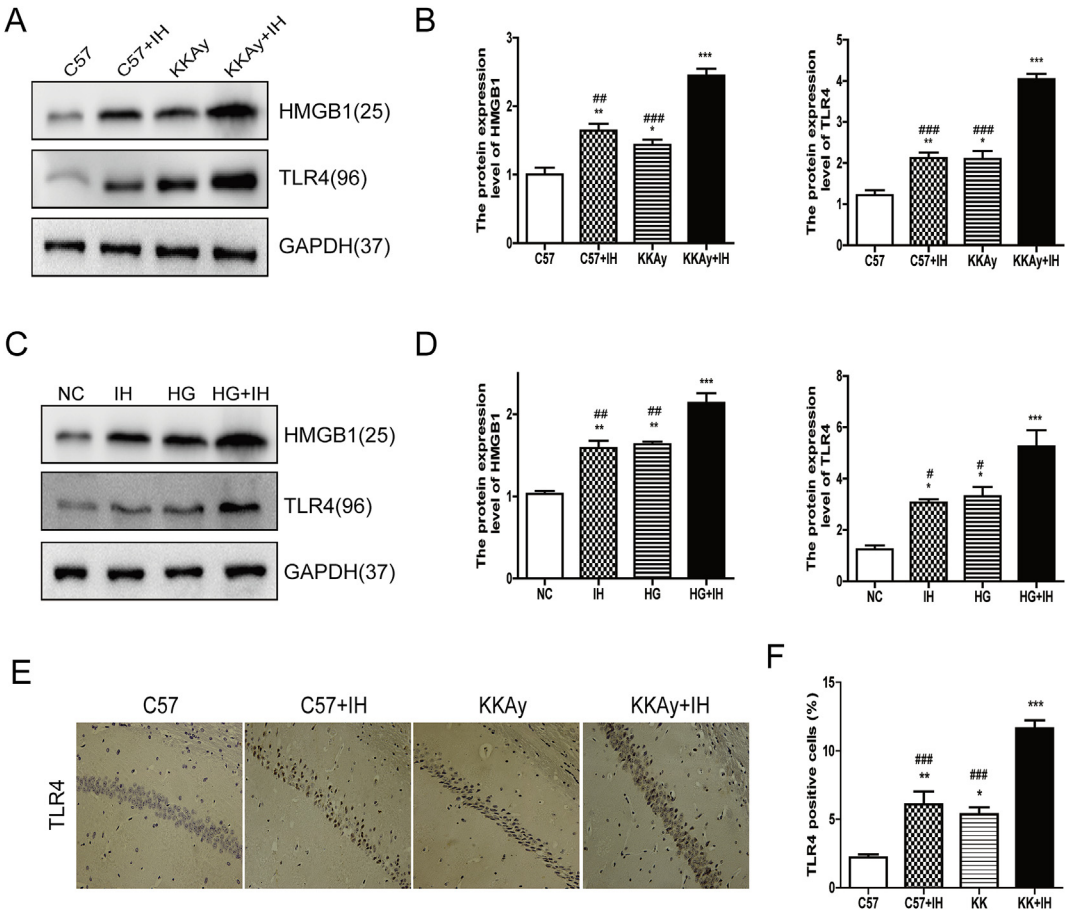


**Fig. 3. HMGB1 and TLR4 are significantly expressed in KKAy mice exposed to IH and in HT22 cells treated with HG + IH.** (A and B) The expression of HMGB1, TLR4 and GAPDH in HT22 cells were detected by Western blotting. (C and D) The protein levels of HMGB1, TLR4 and GAPDH in hippocampus tissue from KKAy mice exposed to IH was determined by Western blotting. (E and F) The expression of TLR4 was observed by immunohistochemistry. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the C57 group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus the KKAy + IH group.

also increased accordingly; the combination of these two promoted the role of the downstream signaling pathway. Meanwhile, the protein expression levels of HMGB1 and TLR4 in HG + IH-treated HT22 hippocampal neurons were also increased.

HMGB1 plays different roles inside and outside of cells. HMGB1 not only participates in inflammation but also participates in apoptosis and plays a role in the process of autophagy impairment. HMGB1 in the cytoplasm is a key autophagy protein that regulates the relationship between protective autophagy and apoptosis in cells [63]. The reduction of HMGB1 can promote the Beclin 1-dependent autophagy pathway [64]. Autophagy defects may increase the proportion of oxidized HMGB1 [65]. Oxidized HMGB1 leads to the activation of caspase-3 and apoptosis in the mitochondrial pathway [64]. Cleaved caspase-3 increases mitochondrial ROS production and the subsequent release of HMGB1, which provides an explanation of apoptosis-mediated HMGB1 release. ROS are major signals that reduce nuclear HMGB1-DNA binding to promote cytoplasmic translocation and release(R [61]. A previous study showed that LPS inhibits autophagy in a TLR4-dependent manner, while mTOR is strongly activated as a key inhibitor of autophagy [16]. Extracellular HMGB1 interacts with TLR and activates nuclear factor kappa B (NF-κB), thereby activating TNF-α, IL-1 and other inflammatory cytokines [66]. HMGB1 mediates apoptosis and accelerates damage through TLR4/NF-κB in cardiomyocytes [67]. Overstimulated neurons may passively release HMGB1 into the extracellular space, resulting in the increased expression of this protein [12,15]. There is evidence that TLR4 can mediate apoptosis [68,69]. Other experiments have shown that inhibition of TLR4 can reduce inflammation and apoptosis [21].Our previous experimental results indicated that IH exposure aggravates apoptosis of hippocampal neurons and cognitive deficits in T2DM mice [17]. Chronic hyperglycemia may eventually lead to hippocampal neuronal apoptosis; therefore, the prevention of hippocampal neuronal apoptosis may delay the onset of diabetes-related cognitive deficits [7]. Our experiments showed that the hippocampus of IH-exposed T2DM mice exhibited more obvious apoptosis and autophagy defects, and the expression of HMGB1 and TLR4 was further increased. Apoptosis was aggravated, autophagy was significantly reduced, and the expression of HMGB1 and TLR4 was significantly increased in HT22 cells exposed to HG + IH. This further supports the idea that the activation of the HMGB1/TLR4 signaling pathway leads to hippocampal neuronal apoptosis and the down-regulation of autophagy. Exposure to HG + IH caused the release of a large amount of HMGB1, and cell apoptosis was activated and autophagy was reduced by binding to TLR4. After HT22 cells transfected with HMGB1 siRNA were exposed to HG + IH; we found that apoptosis was inhibited and autophagy was enhanced. The release of HMGB1 into the extracellular space exacerbated neuronal damage, further increasing the release of HMGB1, thus forming a vicious cycle. Therefore, HMGB1 caused sustained damage to neurons by activating TLR4 under HG + IH conditions. This study used HMGB1 siRNA to inhibit the intracellular and extracellular release of HMGB1 and block the apoptotic pathway, thereby completely breaking the HMGB1-mediated malignant cycle.

High levels of HMGB1 are involved in the development of various diseases [70]. Direct binding to HMGB1 by small natural or synthetic molecules or HMGB1-specific antibodies that target HMGB1 can prevent HMGB1-related pathogenesis [71]. HMGB1 siRNA has been shown to be useful for the treatment of diabetic retinopathy [72],

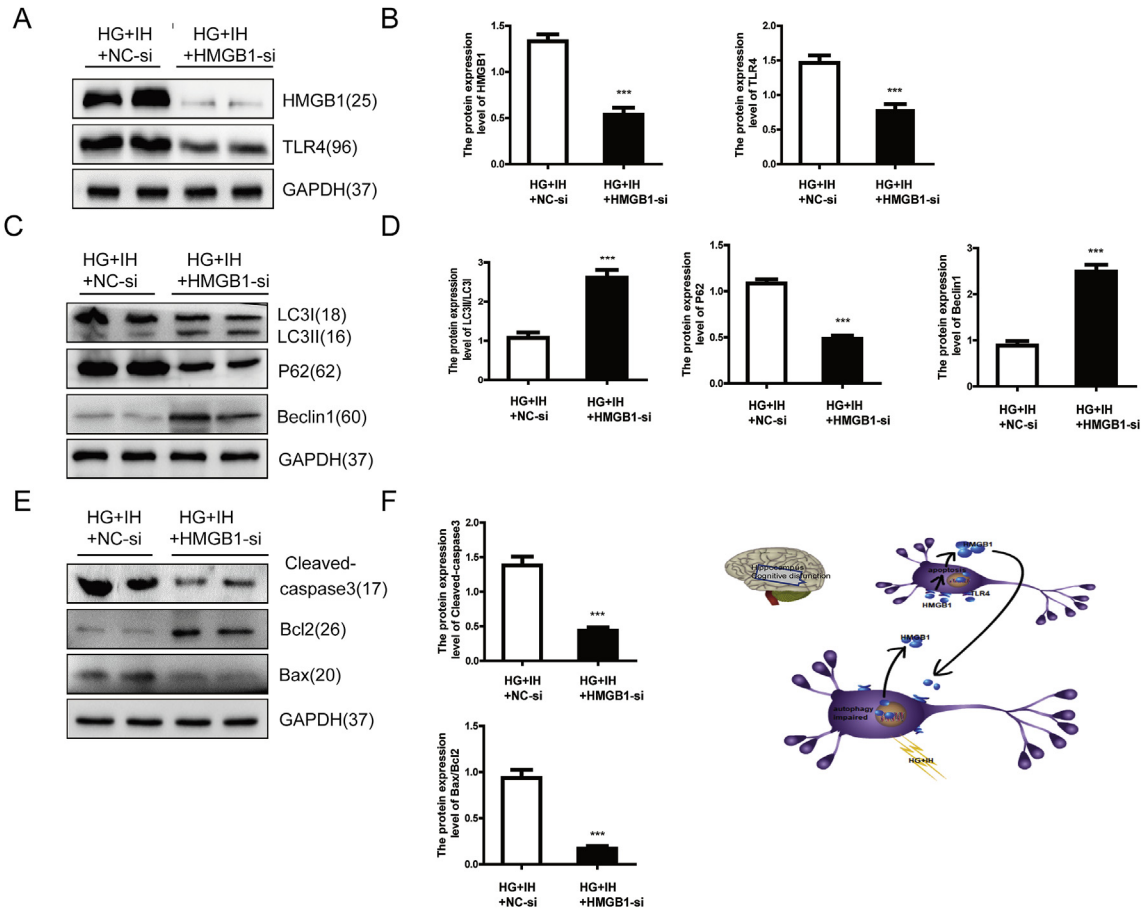


**Fig. 4. HMGB1 siRNA reinforces autophagy and reduces apoptosis in HT22 cells.** (A and B) HMGB1 and TLR4 protein expression. (C and D) LC3, Beclin1, p62 protein expression. (E and F) Cleaved caspase-3, Bcl2, and Bax protein expression. An HMGB1-dependent mechanism is involved in hippocampal neuronal apoptosis and impairs autophagy induced by HG + IH. The data are presented as the mean ± S.E.M. ***p < 0.001 versus the HG + IH + NC-si group.

atherosclerosis [73] and other diseases. Our HMGB1 siRNA transfection assay further demonstrated that the HMGB1/TLR4 signaling pathway was inhibited in HT22 cells, this prevented to prevent the occurrence of apoptosis and enhance cell-protective autophagy.

## 5. Conclusion

In conclusion, HMGB1-TLR4 signaling regulates autophagy dysfunction and promotes apoptosis in OSA complicated with T2DM model hippocampal neurons. Therefore, HMGB1 siRNA targeted therapy may be a potential method of blocking neuronal damage and cognitive impairment in this combined disease mode of T2DM and OSA.

## Author contributions

Xiangyu Guo and Yu Shi performed all experiments, analyzed data, writed the manuscript. Ping Du was responsible for accessing literature and the establishment of the mouse model. Jiahui Wang and Yelei Han edited the manuscript. Bei Sun and Jing Feng designed study, directed and supervised the study. All authors have endorsed the final version of the manuscript.

## Declaration of competing interest

The author declares that the study does not have any potential conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.117020.

## References

[1] R.S. Aronsohn, H. Whitmore, E. Van Cauter, E. Tasali, Impact of untreated obstructive sleep apnea on glucose control in type 2 diabetes, Am. J. Respir. Crit. Care Med. 181 (5) (2010) 507–513, https://doi.org/10.1164/rccm.200909-1423OC.
[2] B. Mokhlesi, D. Grimaldi, G. Beccuti, V. Abraham, H. Whitmore, F. Delebecque, E. Van Cauter, Effect of one week of 8-hour nightly continuous positive airway pressure treatment of obstructive sleep apnea on glycemic control in type 2 diabetes: a proof-of-concept study, Am. J. Respir. Crit. Care Med. 194 (4) (2016) 516–519, https://doi.org/10.1164/rccm.201602-0396LE.
[3] A. Goldbart, B.W. Row, L. Kheirandish, A. Schurr, E. Gozal, S.Z. Guo, ... D. Gozal, Intermittent hypoxic exposure during light phase induces changes in cAMP response element binding protein activity in the rat CA1 hippocampal region: water maze performance correlates, Neuroscience 122 (3) (2003) 585–590, https://doi.org/10.1016/j.neuroscience.2003.08.054.
[4] F. Stanke-Labesque, J.L. Pepin, E. Gautier-Veyret, P. Levy, M. Back, Leukotrienes as a molecular link between obstructive sleep apnoea and atherosclerosis, Cardiovasc. Res. 101 (2) (2014) 187–193, https://doi.org/10.1093/cvr/cvt247.
[5] L.H. Xu, H. Xie, Z.H. Shi, L.D. Du, Y.K. Wing, A.M. Li, W.H. Yung, Critical role of endoplasmic reticulum stress in chronic intermittent hypoxia-induced deficits in synaptic plasticity and long-term memory, Antioxidants Redox Signal. 23 (9) (2015) 695–710, https://doi.org/10.1089/ars.2014.6122.

[6] J. Cha, J.A. Zea-Hernandez, S. Sin, K. Graw-Panzer, K. Shifteh, C.R. Isasi, ... R. Arens, The effects of obstructive sleep apnea syndrome on the dentate gyrus and learning and memory in children, J. Neurosci. 37 (16) (2017) 4280–4288, https://doi.org/10.1523/JNEUROSCI.3583-16.2017.

[7] J.P. Klein, S.G. Waxman, The brain in diabetes: molecular changes in neurons and their implications for end-organ damage, Lancet Neurol. 2 (9) (2003) 548–554, https://doi.org/10.1016/S1474-4422(03)00503-9.

[8] I.K. Hwang, I.Y. Kim, E.J. Joo, J.H. Shin, J.W. Choi, M.H. Won, J.K. Seong, Metformin normalizes type 2 diabetes-induced decrease in cell proliferation and neuroblast differentiation in the rat dentate gyrus, Neurochem. Res. 35 (4) (2010) 645–650, https://doi.org/10.1007/s11064-009-0115-5.

[9] Y. Liu, J. Zhang, Y. Wang, X. Zeng, Apelin involved in progression of diabetic nephropathy by inhibiting autophagy in podocytes, Cell Death Dis. 8 (8) (2017) e3006, , https://doi.org/10.1038/cddis.2017.414.

[10] B. Liu, H. Zhang, C. Xu, G. Yang, J. Tao, J. Huang, J. Dong, Neuroprotective effects of icariin on corticosterone-induced apoptosis in primary cultured rat hippocampal neurons, Brain Res. 1375 (2011) 59–67, https://doi.org/10.1016/j.brainres.2010.12.053.

[11] G.P. Sims, D.C. Rowe, S.T. Rietdijk, R. Herbst, A.J. Coyle, HMGB1 and RAGE in inflammation and cancer, Annu. Rev. Immunol. 28 (2010) 367–388, https://doi.org/10.1146/annurev.immunol.021908.132603.

[12] U. Andersson, K.J. Tracey, HMGB1 is a therapeutic target for sterile inflammation and infection, Annu. Rev. Immunol. 29 (2011) 139–162, https://doi.org/10.1146/annurev-immunol-030409-101323.

[13] S. Jovanovic Stojanov, V. Martinovic, D. Bogojevic, G. Poznanovic, A. Petrovic, S. Ivanovic-Matic, I. Grigorov, Modulation of diabetes-related liver injury by the HMGB1/TLR4 inflammatory pathway, J. Physiol. Biochem. 74 (2) (2018) 345–358, https://doi.org/10.1007/s13105-018-0626-0.

[14] M. Lin, W.H. Yiu, H.J. Wu, L.Y. Chan, J.C. Leung, W.S. Au, S.C. Tang, Toll-like receptor 4 promotes tubular inflammation in diabetic nephropathy, J. Am. Soc. Nephrol. 23 (1) (2012) 86–102, https://doi.org/10.1681/ASN.2010111210.

[15] M. Maroso, S. Balosso, T. Ravizza, J. Liu, E. Aronica, A.M. Iyer, A. Vezzani, Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures, Nat. Med. 16 (4) (2010) 413–419, https://doi.org/10.1038/nm.2127.

[16] J.W. Lee, H. Nam, L.E. Kim, Y. Jeon, H. Min, S. Ha, S.W. Yu, TLR4 (toll-like receptor 4) activation suppresses autophagy through inhibition of FOXO3 and impairs phagocytic capacity of microglia, Autophagy (2018), https://doi.org/10.1080/15548627.2018.1556946.

[17] Y. Shi, X. Guo, J. Zhang, H. Zhou, B. Sun, J. Feng, DNA binding protein HMGB1 secreted by activated microglia promotes the apoptosis of hippocampal neurons in diabetes complicated with OSA, Brain Behav. Immun. 73 (2018) 482–492, https://doi.org/10.1016/j.bbi.2018.06.012.

[18] C. Li, X. Yang, J. Feng, P. Lei, Y. Wang, Proinflammatory and prothrombotic status in emphysematous rats exposed to intermittent hypoxia, Int. J. Clin. Exp. Pathol. 8 (1) (2015) 374–383.

[19] V.V. Makarenko, P.V. Usatyuk, G. Yuan, M.M. Lee, J. Nanduri, V. Natarajan, N.R. Prabhakar, Intermittent hypoxia-induced endothelial barrier dysfunction requires ROS-dependent MAP kinase activation, Am. J. Physiol. Cell Physiol. 306 (8) (2014) C745–C752, https://doi.org/10.1152/ajpcell.00313.2013.

[20] Q. He, Q.C. Yang, Q. Zhou, H. Zhu, W.Y. Niu, J. Feng, B.Y. Chen, Effects of varying degrees of intermittent hypoxia on proinflammatory cytokines and adipokines in rats and 3T3-L1 adipocytes, PLoS One 9 (1) (2014) e86326, , https://doi.org/10.1371/journal.pone.0086326.

[21] L. Hu, H. Yang, M. Ai, S. Jiang, Inhibition of TLR4 alleviates the inflammation and apoptosis of retinal ganglion cells in high glucose, Graefes Arch. Clin. Exp. Ophthalmol. 255 (11) (2017) 2199–2210, https://doi.org/10.1007/s00417-017-3772-0.

[22] Y. Zheng, S.H. Ley, F.B. Hu, Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, Nat. Rev. Endocrinol. 14 (2) (2018) 88–98, https://doi.org/10.1038/nrendo.2017.151.

[23] K. Iglay, H. Hannachi, P. Joseph Howie, J. Xu, X. Li, S.S. Engel, S. Rajpathak, Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus, Curr. Med. Res. Opin. 32 (7) (2016) 1243–1252, https://doi.org/10.1185/03007995.2016.1168291.

[24] S. Reutrakul, B. Mokhlesi, Obstructive sleep apnea and diabetes: a state of the art review, Chest 152 (5) (2017) 1070–1086, https://doi.org/10.1016/j.chest.2017.05.009.

[25] E.R. Chasens, C.W. Atwood, L.E. Burke, M. Korytkowski, R. Stansbury, P.J. Strollo, S.M. Sereika, Diabetes sleep treatment trial: premise, design, and methodology, Contemp. Clin. Trials 76 (2019) 104–111, https://doi.org/10.1016/j.cct.2018.11.014.

[26] Y. Teng, S. Wang, N. Wang, Muhuyati, STOP-Bang questionnaire screening for obstructive sleep apnea among Chinese patients with type 2 diabetes mellitus, Arch. Med. Sci. 14 (5) (2018) 971–978, https://doi.org/10.5114/aoms.2018.73984.

[27] A. Subramanian, N.J. Adderley, A. Tracy, T. Taverner, W. Hanif, K.A. Toulis, K. Nirantharakumar, Risk of incident obstructive sleep apnea among patients with type 2 diabetes, Diabetes Care 42 (5) (2019) 954–963, https://doi.org/10.2337/dc18-2004.

[28] N.M. Punjabi, M.M. Ahmed, V.Y. Polotsky, B.A. Beamer, C.P. O'Donnell, Sleep-disordered breathing, glucose intolerance, and insulin resistance, Respir. Physiol. Neurobiol. 136 (2–3) (2003) 167–178, https://doi.org/10.1016/S1569-9048(03)00079-X.

[29] A. Briancon-Marjollet, M. Weiszenstein, M. Henri, A. Thomas, D. Godin-Ribuot, J. Polak, The impact of sleep disorders on glucose metabolism: endocrine and molecular mechanisms, Diabetol. Metab. Syndrome 7 (2015) 25, https://doi.org/10.1186/s13098-015-0018-3.

[30] P. Rajan, H. Greenberg, Obstructive sleep apnea as a risk factor for type 2 diabetes mellitus, Nat. Sci. Sleep 7 (2015) 113–125, https://doi.org/10.2147/NSS.S90835.

[31] P. Bottini, M.L. Dottorini, M. Cristina Cordoni, G. Casucci, C. Tantucci, Sleep-disordered breathing in nonobese diabetic subjects with autonomic neuropathy, Eur. Respir. J. 22 (4) (2003) 654–660, https://doi.org/10.1183/09031936.03.00070402.

[32] M. Chakhtoura, S.T. Azar, Continuous positive airway pressure and type 2 diabetes mellitus, Diabetes Metab Syndr 6 (3) (2012) 176–179, https://doi.org/10.1016/j.dsx.2012.09.013.

[33] Y. Fang, Q. Zhang, J. Tan, L. Li, X. An, P. Lei, Intermittent hypoxia-induced rat pancreatic beta-cell apoptosis and protective effects of antioxidant intervention, Nutr. Diabetes 4 (2014) e131, https://doi.org/10.1038/nutd.2014.28.

[34] D. Bhattacharya, M. Mukhopadhyay, M. Bhattacharyya, P. Karmakar, Is autophagy associated with diabetes mellitus and its complications? A review, EXCLI J 17 (2018) 709–720, https://doi.org/10.17179/excli2018-1353.

[35] P. Nilsson, T.C. Saido, Dual roles for autophagy: degradation and secretion of Alzheimer's disease Abeta peptide, Bioessays 36 (6) (2014) 570–578, https://doi.org/10.1002/bies.201400002.

[36] D. Gozal, J.M. Daniel, G.P. Dohanich, Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat, J. Neurosci. 21 (7) (2001) 2442–2450, https://doi.org/10.1523/JNEUROSCI.21-07-02442.2001.

[37] Y. Li, Y. Zhang, L. Wang, P. Wang, Y. Xue, X. Li, C. Chen, Autophagy impairment mediated by S-nitrosation of ATG4B leads to neurotoxicity in response to hyperglycemia, Autophagy 13 (7) (2017) 1145–1160, https://doi.org/10.1080/15548627.2017.1320467.

[38] C. Infante-Garcia, J. Jose Ramos-Rodriguez, Y. Marin-Zambrana, M. Teresa Fernandez-Ponce, L. Casas, C. Mantell, M. Garcia-Alloza, Mango leaf extract improves central pathology and cognitive impairment in a type 2 diabetes mouse model, Brain Pathol. 27 (4) (2017) 499–507, https://doi.org/10.1111/bpa.12433.

[39] J.J. Ramos-Rodriguez, O. Ortiz, M. Jimenez-Palomares, K.R. Kay, E. Berrocoso, M.I. Murillo-Carretero, M. Garcia-Alloza, Differential central pathology and cognitive impairment in pre-diabetic and diabetic mice, Psychoneuroendocrinology 38 (11) (2013) 2462–2475, https://doi.org/10.1016/j.psyneuen.2013.05.010.

[40] F.J. Kong, L.L. Ma, J.J. Guo, L.H. Xu, Y. Li, S. Qu, Endoplasmic reticulum stress/autophagy pathway is involved in diabetes-induced neuronal apoptosis and cognitive decline in mice, Clin. Sci. (Lond.) 132 (1) (2018) 111–125, https://doi.org/10.1042/CS20171432.

[41] C. Mirescu, E. Gould, Stress and adult neurogenesis, Hippocampus 16 (3) (2006) 233–238, https://doi.org/10.1002/hipo.20155.

[42] V.S. Hambrecht, P.E. Vlisides, B.W. Row, D. Gozal, H.A. Baghdoyan, R. Lydic, Hypoxia modulates cholinergic but not opioid activation of G proteins in rat hippocampus, Hippocampus 17 (10) (2007) 934–942, https://doi.org/10.1002/hipo.20312.

[43] O. Busquets, M. Ettcheto, M. Pallas, C. Beas-Zarate, E. Verdaguer, C. Auladell, ... A. Camins, Long-term exposition to a high fat diet favors the appearance of beta-amyloid depositions in the brain of C57BL/6J mice. A potential model of sporadic Alzheimer's disease, Mech. Ageing Dev. 162 (2017) 38–45, https://doi.org/10.1016/j.mad.2016.11.002.

[44] J. Beauquis, F. Homo-Delarche, M.H. Giroix, J. Ehses, J. Coulaud, P. Roig, ... F. Saravia, Hippocampal neurovascular and hypothalamic-pituitary-adrenal axis alterations in spontaneously type 2 diabetic GK rats, Exp. Neurol. 222 (1) (2010) 125–134, https://doi.org/10.1016/j.expneurol.2009.12.022.

[45] J.E. Owen, B. BenediktsdOttir, T. Gislason, S.R. Robinson, Neuropathological investigation of cell layer thickness and myelination in the hippocampus of people with obstructive sleep apnea, Sleep 42 (1) (2019), https://doi.org/10.1093/sleep/zsy199.

[46] N.R. Kreisman, S. Soliman, D. Gozal, Regional differences in hypoxic depolarization and swelling in hippocampal slices, J. Neurophysiol. 83 (2) (2000) 1031–1038, https://doi.org/10.1152/jn.2000.83.2.1031.

[47] G. He, W. Xu, L. Tong, S. Li, S. Su, X. Tan, C. Li, Gadd45b prevents autophagy and apoptosis against rat cerebral neuron oxygen-glucose deprivation/reperfusion injury, Apoptosis 21 (4) (2016) 390–403, https://doi.org/10.1007/s10495-016-1213-x.

[48] L.P. Reagan, B.S. McEwen, Controversies surrounding glucocorticoid-mediated cell death in the hippocampus, J. Chem. Neuroanat. 13 (3) (1997) 149–167, https://doi.org/10.1016/S0891-0618(97)00031-8.

[49] H. Yin, W. Wang, W. Yu, J. Li, N. Feng, L. Wang, X. Wang, Changes in synaptic plasticity and glutamate receptors in type 2 diabetic KK-ay mice, J. Alzheimer's Dis. 57 (4) (2017) 1207–1220, https://doi.org/10.3233/JAD-160858.

[50] P.H. Yu, M. Wang, Y.L. Deng, H. Fan, L. Shira-Bock, Involvement of semicarbazide-sensitive amine oxidase-mediated deamination in atherogenesis in KKAy diabetic mice fed with high cholesterol diet, Diabetologia 45 (9) (2002) 1255–1262, https://doi.org/10.1007/s00125-002-0903-9.

[51] J. Zhang, J. Yin, Y. Wang, B. Li, X. Zeng, Apelin impairs myogenic response to induce diabetic nephropathy in mice, FASEB J. 32 (8) (2018) 4315–4327, https://doi.org/10.1096/fj.201701257R.

[52] Y. Li, Q. Li, C.S. Pan, L. Yan, B.H. Hu, Y.Y. Liu, J.Y. Han, Bushen huoxue attenuates diabetes-induced cognitive impairment by improvement of cerebral microcirculation: involvement of RhoA/ROCK/moesin and src signaling pathways, Front. Physiol. 9 (2018) 527, https://doi.org/10.3389/fphys.2018.00527.

[53] U. Andersson, H. Yang, H. Harris, High-mobility group box 1 protein (HMGB1) operates as an alarmin outside as well as inside cells, Semin. Immunol. 38 (2018) 40–48, https://doi.org/10.1016/j.smim.2018.02.011.

[54] P. Feldman, M.R. Due, M.S. Ripsch, R. Khanna, F.A. White, The persistent release of HMGB1 contributes to tactile hyperalgesia in a rodent model of neuropathic pain, J.

Neuroinflammation 9 (2012) 180, https://doi.org/10.1186/1742-2094-9-180.
[55] Q. Lin, J. Fang, D. Fang, B. Li, H. Zhou, S.B. Su, Production of recombinant human HMGB1 and anti-HMGB1 rabbit serum, Int. Immunopharmacol. 11 (6) (2011) 646–651, https://doi.org/10.1016/j.intimp.2011.01.005.
[56] M.D. Laird, J.S. Shields, S. Sukumari-Ramesh, D.E. Kimbler, R.D. Fessler, B. Shakir, K.M. Dhandapani, High mobility group box protein-1 promotes cerebral edema after traumatic brain injury via activation of toll-like receptor 4, Glia 62 (1) (2014) 26–38, https://doi.org/10.1002/glia.22581.
[57] A. Mazarati, M. Maroso, V. Iori, A. Vezzani, M. Carli, High-mobility group box-1 impairs memory in mice through both toll-like receptor 4 and Receptor for Advanced Glycation End Products, Exp. Neurol. 232 (2) (2011) 143–148, https://doi.org/10.1016/j.expneurol.2011.08.012.
[58] K. Kikuchi, K. Kawahara, K.K. Biswas, T. Ito, S. Tancharoen, Y. Morimoto, I. Maruyama, Minocycline attenuates both OGD-induced HMGB1 release and HMGB1-induced cell death in ischemic neuronal injury in PC12 cells, Biochem. Biophys. Res. Commun. 385 (2) (2009) 132–136, https://doi.org/10.1016/j.bbrc.2009.04.041.
[59] P.C. Ren, Y. Zhang, X.D. Zhang, L.J. An, H.G. Lv, J. He, X.D. Sun, High-mobility group box 1 contributes to mechanical allodynia and spinal astrocytic activation in a mouse model of type 2 diabetes, Brain Res. Bull. 88 (4) (2012) 332–337, https://doi.org/10.1016/j.brainresbull.2012.03.002.
[60] D. Yao, M. Brownlee, Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands, Diabetes 59 (1) (2010) 249–255, https://doi.org/10.2337/db09-0801.
[61] R. Kang, R. Chen, Q. Zhang, W. Hou, S. Wu, L. Cao, D. Tang, HMGB1 in health and disease, Mol. Asp. Med. 40 (2014) 1–116, https://doi.org/10.1016/j.mam.2014.05.001.
[62] M.R. Dasu, S. Devaraj, S. Park, I. Jialal, Increased toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects, Diabetes Care 33 (4) (2010) 861–868, https://doi.org/10.2337/dc09-1799.
[63] D. Tang, R. Kang, C.W. Cheh, K.M. Livesey, X. Liang, N.E. Schapiro, M.T. Lotze, HMGB1 release and redox regulates autophagy and apoptosis in cancer cells, Oncogene 29 (38) (2010) 5299–5310, https://doi.org/10.1038/onc.2010.261.
[64] D. Tang, M.T. Loze, H.J. Zeh, R. Kang, The redox protein HMGB1 regulates cell death and survival in cancer treatment, Autophagy 6 (8) (2010) 1181–1183, https://doi.org/10.4161/auto.6.8.13367.
[65] D. Tang, R. Kang, K.M. Livesey, C.W. Cheh, A. Farkas, P. Loughran, M.T. Lotze, Endogenous HMGB1 regulates autophagy, J. Cell Biol. 190 (5) (2010) 881–892, https://doi.org/10.1083/jcb.200911078.
[66] N. Kang, Y. Hai, J. Yang, F. Liang, C.J. Gao, Hyperbaric oxygen intervention reduces secondary spinal cord injury in rats via regulation of HMGB1/TLR4/NF-kappaB signaling pathway, Int. J. Clin. Exp. Pathol. 8 (2) (2015) 1141–1153.
[67] J. Xue, H. Ge, Z. Lin, H. Wang, W. Lin, Y. Liu, Q. Zhao, The role of dendritic cells regulated by HMGB1/TLR4 signalling pathway in myocardial ischaemia reperfusion injury, J. Cell Mol. Med. (2019), https://doi.org/10.1111/jcmm.14192.
[68] D.D. Yuan, X.J. Chi, Y. Jin, X. Li, M. Ge, W.L. Gao, Z.Q. Hei, Intestinal injury following liver transplantation was mediated by TLR4/NF-kappaB activation-induced cell apoptosis, Mol. Med. Rep. 13 (2) (2016) 1525–1532, https://doi.org/10.3892/mmr.2015.4719.
[69] X. Zhang, W. Jiang, A.L. Zhou, M. Zhao, D.R. Jiang, Inhibitory effect of oxymatrine on hepatocyte apoptosis via TLR4/PI3K/Akt/GSK-3beta signaling pathway, World J. Gastroenterol. 23 (21) (2017) 3839–3849, https://doi.org/10.3748/wjg.v23.i21.3839.
[70] M.T. Lotze, K.J. Tracey, High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal, Nat. Rev. Immunol. 5 (4) (2005) 331–342, https://doi.org/10.1038/nri1594.
[71] D. Musumeci, G.N. Roviello, D. Montesarchio, An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies, Pharmacol. Ther. 141 (3) (2014) 347–357, https://doi.org/10.1016/j.pharmthera.2013.11.001.
[72] S. Jiang, X. Chen, HMGB1 siRNA can reduce damage to retinal cells induced by high glucose in vitro and in vivo, Drug Des. Dev. Ther. 11 (2017) 783–795, https://doi.org/10.2147/DDDT.S129913.
[73] N. Hu, L. Kong, A. Qian, Q. Meng, C. Li, X. Yu, X. Li, HMGB1 silencing potentiates the anti-inflammatory effects of sodium ferulate in ox-LDL-stimulated vascular smooth muscle cells, Cell Biochem. Biophys. 72 (1) (2015) 297–304, https://doi.org/10.1007/s12013-014-0455-x.