

# TMAO promotes apoptosis and oxidative stress of pancreatic acinar cells by mediating IRE1 $\alpha$ -XBP-1 pathway

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## Abstract

**Background:** Acute pancreatitis caused by hyperlipidemia is a severe life-threatening condition. Therefore, it is urgent to develop new therapeutic methods to treat this disease.

**Methods:** Cell viability was determined by the Cell Counting Kit-8 (CCK-8) assay. Western blotting (WB) was used to detect the expression levels of apoptotic and endoribonuclease inositol-requiring enzyme 1 $\alpha$  (IRE1 $\alpha$ )/X-box binding protein 1 (XBP-1) pathway-associated proteins. The induction of cell apoptosis was determined using flow cytometry. The expression levels of the oxidative stress indicators were measured by an enzyme-linked immunosorbent assay.

**Results:** WB analysis and the CCK-8 assay demonstrated that trimethylamine-N-oxide (TMAO) decreased cell viability and facilitated apoptosis of MPC-83 cells in a dose-dependent manner. Furthermore, the induction of oxidative stress was assessed by evaluating the levels of specific markers, including hydrogen peroxide, reactive oxygen species, nitric oxide, and superoxide dismutase. The levels of the aforementioned markers were increased in the TMAO-treated group. Subsequently, the IRE1 $\alpha$ /XBP-1 pathway-associated proteins were analyzed by WB analysis and the data demonstrated that the regulatory effects of TMAO on MPC-83 cells were mediated by the IRE1 $\alpha$ /XBP-1 signaling pathway. Subsequently, rescue experiments were performed to further assess the effects of TMAO.

**Conclusion:** The present study provides evidence on the application of TMAO as a potential diagnostic and therapeutic strategy for the therapeutic intervention of hyperlipidemic acute pancreatitis.

**Keywords:** Apoptosis, hyperlipidemic acute pancreatitis, IRE1 $\alpha$ /XBP-1, oxidative stress, trimethylamine oxide

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## INTRODUCTION

Acute pancreatitis (AP) is one of the most common acute abdominal diseases encountered worldwide.<sup>[1]</sup> AP is caused by numerous factors, such as excessive alcohol consumption and cholelithiasis. Certain studies have suggested that hyperlipidemia is an additional major cause of AP.<sup>[2]</sup> AP caused by hyperlipidemia is termed

hyperlipidemic acute pancreatitis (HLAP). In recent years, the incidence of HLAP has increased and this condition has become the secondary cause of AP under biliary AP.<sup>[3]</sup> Patients with HLAP are prone to recurrence and present occasionally with uncontrolled seizures.<sup>[4,5]</sup> Therefore, this disease has gained unprecedented attention from

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physicians, researchers, and other members of the medical community.<sup>[6]</sup>

The presence of intestinal bacteria has been closely associated with several biological processes that regulate the processing of various nutrients and drugs, including lipids and amino acids.<sup>[7]</sup> The most widely known pathway of microbial-mammalian co-metabolism is the synthesis of bile acid and the enterohepatic circulation. Furthermore, the production of trimethylamine-N-oxide (TMAO) is a significant pathway that has not been explored in detail.<sup>[8]</sup> The intestinal flora uses choline and carnitine derived from food to produce trimethylamine, which is subsequently oxidized to TMAO by liver enzymes. The reduction in TMAO levels can stimulate macrophages to reverse cholesterol transport and inhibit atherosclerosis.<sup>[9]</sup> TMAO-generating flavin-containing monooxygenase 3 (FMO3) is an enzyme involved in cholesterol metabolism and reverse cholesterol transport. Downregulation of FMO3 expression can reduce the secretion of bile in the gallbladder, delay the absorption of cholesterol in the intestine, and limit the synthesis of oxidized cholesterol and cholesterol esters.<sup>[10]</sup> FMO3 is the major isoform expressed in human liver tissues.<sup>[11]</sup> Mice fed on a western diet, which is considered a risk factor for cardiovascular diseases, exhibited higher plasma TMAO concentrations and developed cardiac dysfunction and heart fibrosis.<sup>[12]</sup> It has been shown that TMAO is associated with atherosclerosis, cardiovascular disease, and neurological disorders. TMAO is involved in the development of atherosclerosis and has recently been associated with inflammation and obesity.<sup>[13,14]</sup> Therefore, this compound plays an important role in the obesity-associated lipid metabolism disorders and inflammation.

At present, the diagnosis of HLAP is primarily based on the clinical signs, blood urine amylase levels, and serum triacylglycerol levels. However, these diagnostic criteria exhibit a number of limitations, which result in the difficult clinical diagnosis and treatment of HLAP.<sup>[15,16]</sup> Therefore, the identification of specific biomarkers for the diagnosis of HLAP is imperative. It has been reported that TMAO is associated with the occurrence and development of specific diseases, such as colorectal cancer.<sup>[17,18]</sup> TMAO may be a potential biomarker for the diagnosis of early-stage AP.<sup>[19]</sup> However, the association of TMAO with HLAP has not been investigated in detail. Furthermore, previous studies have shown that pancreatic cells are prone to external stimuli following endoplasmic reticulum stress (ERS), suggesting that the latter plays an indispensable role in the development and progression of AP.<sup>[20,21]</sup> It is important to note that ERS is a key component of oxidative stress and

it induces gene expression changes and activates various cellular processes, which ultimately lead to pancreatitis. Therefore, based on these two molecular processes (ERS and oxidative stress), the present study aimed to explore the effects of TMAO on pancreatic cells and its underlying mechanism of action.

In the present study, TMAO was shown to induce apoptosis of pancreatic acinar cells by activation of oxidative stress and regulation of the ERS-associated endoribonuclease inositol-requiring enzyme 1 $\alpha$  (IRE1 $\alpha$ )/X-box binding protein 1 (XBP-1) pathway. This was verified by a series of experimental techniques. In addition, the data suggested that TMAO acted as a specific biomarker for the diagnosis of HLAP by promoting cell apoptosis and oxidative stress.

## MATERIALS AND METHODS

### Cell culture

Rat pancreatic acinar MPC-83 cells were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Germany) supplemented with 3.7 g/L sodium bicarbonate, 10% fetal calf serum, 100 IU/mL penicillin, and 100  $\mu$ g/mL streptomycin. The cells were cultured in a 37°C incubator with 5% CO<sub>2</sub>.

### Cell proliferation assay

The Cell Counting Kit-8 (CCK-8) assay was used to measure the effects of different concentrations of TMAO (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) on MPC-83 cells. Briefly, MPC-83 cells at the logarithmic growth phase were seeded at a density of  $5 \times 10^3$  cells/mL in a pre-warmed medium (100  $\mu$ L) and incubated in a 96-well cell culture plate. Following cell adherence, the medium was aspirated and the MPC-83 cells were incubated with the following concentrations of TMAO: 0.01, 0.1, 1, 10, 100, and 200  $\mu$ M, phosphate buffered saline (PBS) was used as a control. The following day, 10  $\mu$ L CCK-8 solution was added to each well and the cells were incubated for 4 h. The absorbance of each well was measured at 450 nm. The experiments were performed in triplicate.

### Flow cytometry analysis

To analyze the effects of TMAO on cell apoptosis, pancreatic cells ( $5 \times 10^5$  cells) were seeded into a 6-well cell culture plate and a complete RPMI-1640 medium (GIBCO, Germany) was added. Different concentrations of TMAO were added and the cells were cultured for an additional 24 h. Subsequently, MPC-83 cells were digested with trypsin,

collected, and prepared into single-cell suspension, which was washed three times with pre-cooled PBS. Subsequently, the cells (200  $\mu$ L cell suspension) were stained with 10  $\mu$ L Annexin V-APC and incubated at 4°C in the dark. A total of 10  $\mu$ L propidium iodide was also added to each tube and the samples were immediately analyzed by a Fluorescence activating cell sorter (FACS Calibur, BD Bioscience, USA).

### Estimation of reactive oxygen species

The production of intracellular reactive oxygen species (ROS) in MPC-83 cells was examined using the ROS assay kit (Beyotime, China). The MPC-83 cells were cultured in 96-well plates, treated with different concentrations of TMAO, and incubated with 10  $\mu$ M 2,7-dichlorofluorescein diacetate (DCFH-DA; Sigma-Aldrich; Merck KGaA) at 37°C for 30 min. The fluorescence intensity was measured using a fluorescence plate reader (Falcon, Corning Life Sciences, Bedford, MA, USA) with an excitation and emission wavelength of 488 and 525 nm, respectively. The ROS production was assessed by fluorescence microscopy (Olympus Corporation, Tokyo, Japan) using an excitation and an emission wavelength of 488 and 525 nm, respectively (Ex/Em = 488/525 nm).

### Measurement of superoxide dismutase and nitric oxide levels

The activity levels of the antioxidant enzyme superoxide dismutase (SOD) were determined using a SOD assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentration of nitric oxide (NO) was measured using a NO assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Both the experiments were conducted according to the manufacturer's instructions. The absorbance was measured at 550 nm.

### Hydrogen peroxide detection

The activity levels of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were determined by the H<sub>2</sub>O<sub>2</sub> content detection kit (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. The absorbance of each well was measured at 415 nm.

### WB analysis

The cellular protein was extracted using radio-immunoprecipitation assay (RIPA) lysis buffer (Beyotime Institute of Biotechnology, Beijing, China) and the protein concentration levels were measured using a BCA Protein Assay Kit (Vazyme Biotech Co., Ltd., Nanjing, China) according to the manufacturer's protocol. The protein samples were separated by 10% dodecyl sulfate, sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on polyvinylidene fluoride (PVDF) membranes.

The PVDF membranes were blocked with 5% skimmed milk for 2 h at room temperature, and the membranes were then incubated overnight at 4°C with specific primary antibodies. The samples were washed three times and the blots were subsequently incubated with a goat horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. Following the washing of the membranes for 10 min (four times) in the Tris-buffered saline with Tween-20, the protein expression levels were detected using chemiluminescence. The intensity of the bands was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).  $\beta$ -actin served as the loading control.

### Statistical analysis

The measurement data were presented as the mean  $\pm$  SD. Statistical comparisons between experimental and control groups were performed using a one-way analysis of variance (ANOVA) followed by *post-hoc* tests. The statistical analysis was conducted using GraphPad Prism 9 software (GraphPad Software, Inc., La Jolla, CA, USA).  $P < 0.05$  was considered statistically significant. All the experiments were performed at least in triplicate.

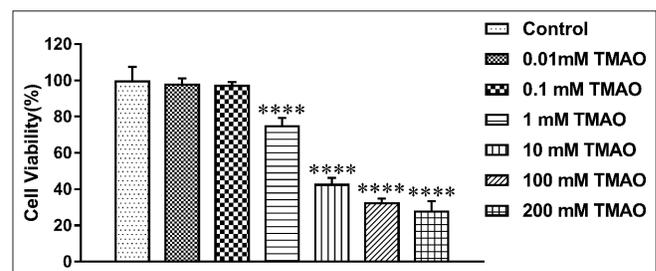
## RESULTS

### TMAO inhibits MPC-83 cell proliferation

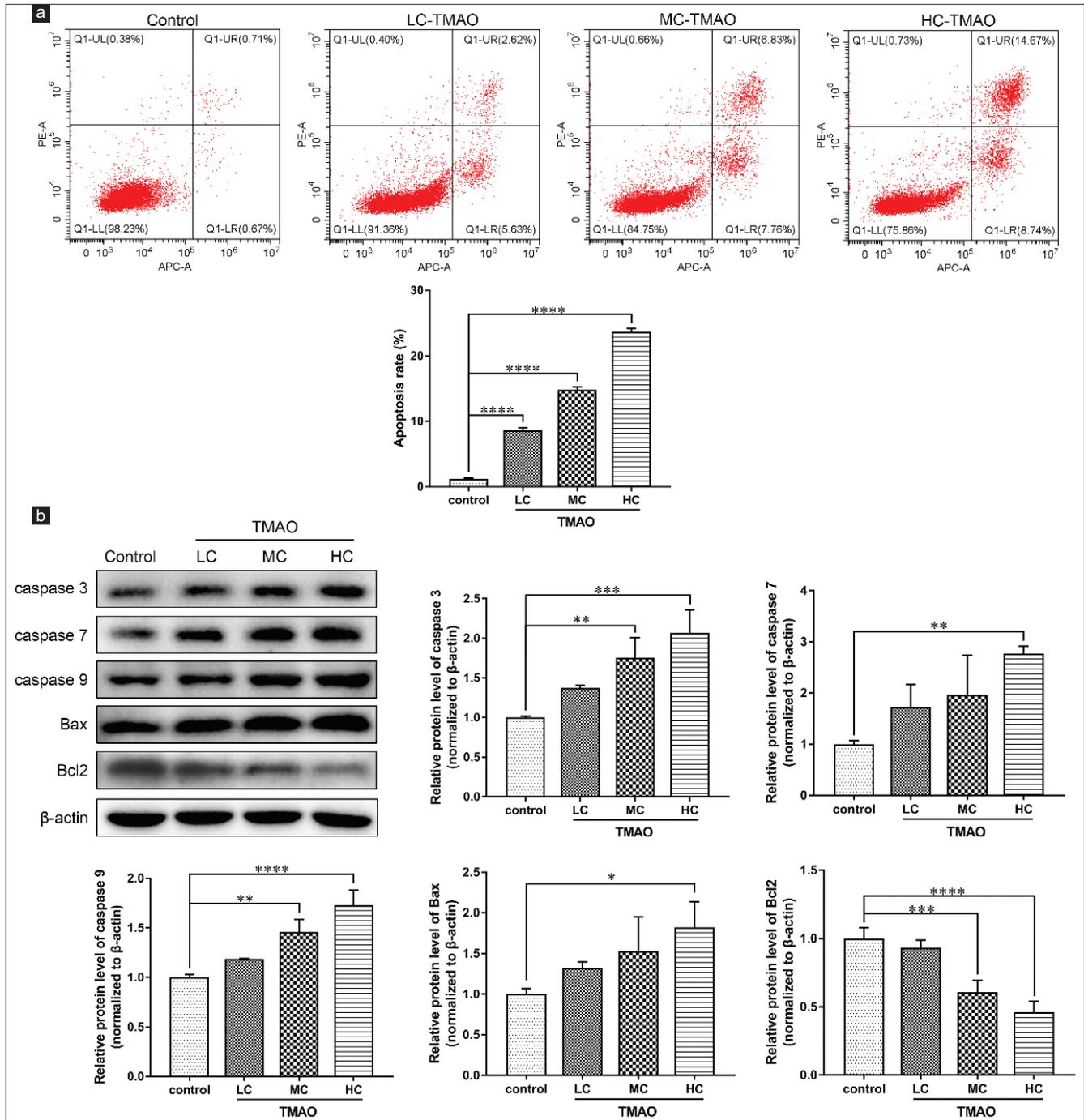
To analyze the effects of TMAO on MPC-83 cells, cell proliferation was measured using a CCK-8 assay. The results indicated that TMAO inhibited the proliferation of MPC-83 cells. Furthermore, the cell proliferation of MPC-83 cells treated with 1, 10, 100, and 200 mM TMAO was significantly decreased compared with that of the control group. This inhibitory effect of TMAO on MPC-83 cell proliferation occurred in a dose-dependent manner [Figure 1].

### TMAO promotes MPC-83 cell apoptosis

The induction of MPC-83 cell apoptosis was assessed following the treatment of the cells with



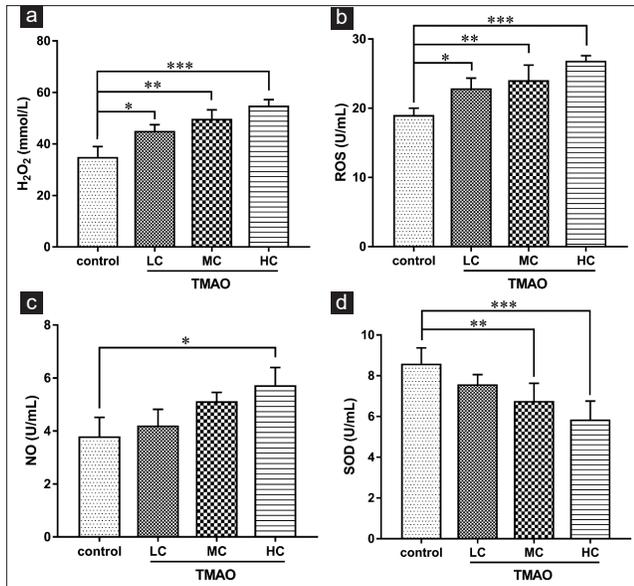
**Figure 1:** TMAO inhibits MPC-83 cell proliferation. MPC-83 cells were treated with or without TMAO (0.01, 0.1, 1, 10, 100, and 200 mM) for 48 h. Cell viability was measured using a Cell Counting Kit-8 assay. The bars represent the mean  $\pm$  SD from four independent experiments. \*\*\*\* $P < 0.0001$ . TMAO, trimethylamine oxide



**Figure 2:** TMAO promotes MPC-83 cell apoptosis. (a) Flow cytometry analysis demonstrated that TMAO increased MPC-83 cell apoptosis in a dose-dependent manner. The apoptotic rate of TMAO was significantly increased compared with that of the control group. (b) The expression levels of Bax, Bcl-2, and caspase-3, -7, and -9 were measured by the western blotting analysis;  $\beta$ -actin served as a loading control. The relative protein levels are shown. The bars represent the mean  $\pm$  SD from three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . TMAO, trimethylamine oxide

low-concentration (LC, 1 mM), middle-concentration (MC, 10 mM), and high-concentration (HC, 100 mM) TMAO. Subsequently, flow cytometry and WB analysis were performed. The results indicated that TMAO promoted MPC-83 cell apoptosis. The pro-apoptotic effect was gradually enhanced following an increase in the

concentration of TMAO [Figure 2a]. The apoptotic rates of the LC-TMAO, MC-TMAO, and HC-TMAO groups were 8.63, 14.8, and 23.7%, respectively. Furthermore, the expression levels of the apoptotic proteins, Bax, Bcl-2, caspase-3, -7, and -9 were measured by WB analysis and the intensity of the WB bands was assessed using ImageJ



**Figure 3:** TMAO induces oxidative stress in MPC-83 cells. The levels of H<sub>2</sub>O<sub>2</sub> (a), ROS (b), NO (c), and SOD (d) were detected following treatment of MPC-83 cells with different concentrations of TMAO for 24 h compared with those of the control group. H<sub>2</sub>O<sub>2</sub>, ROS, NO, and SOD are specific markers used to represent oxidative stress levels in the cells. The bars represent the mean  $\pm$  SD from three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . TMAO, trimethylamine oxide; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; ROS, reactive oxygen species; NO, nitric oxide; SOD, superoxide dismutase

software. Bax and caspase-3, -7 and, -9 are pro-apoptotic proteins, while Bcl-2 is an anti-apoptotic protein. In general, TMAO exhibited pro-apoptotic effects. The changes in the protein expression levels were noted in a dose-dependent manner [Figure 2b]. These results demonstrated that TMAO accelerated the induction of apoptosis of the MPC-83 cells. Furthermore, the MC-TMAO group caused significant changes in the expression levels of the apoptotic proteins compared with those noted in the control group.

### TMAO induces oxidative stress in MPC-83 cells

To investigate the induction of oxidative stress by TMAO, MPC-83 cells were treated with different concentrations of this compound. The levels of the specific oxidative stress indices, H<sub>2</sub>O<sub>2</sub>, ROS, and NO were measured. In addition, the expression levels of SOD were measured. The results indicated that the levels of H<sub>2</sub>O<sub>2</sub>, ROS, and NO were significantly increased in TMAO-treated MPC-83 cells compared with those of the control group [Figure 3a-c]. In contrast to these observations, SOD levels were markedly decreased in the TMAO-treated group [Figure 3d]. Therefore, these results indicated that TMAO induced oxidative stress in MPC-83 cells by increasing H<sub>2</sub>O<sub>2</sub>, ROS, and NO levels and by decreasing SOD levels.

### TMAO activates the IRE1 $\alpha$ /XBP-1 signaling pathway in MPC-83 cells

The IRE1 $\alpha$ /XBP-1 signaling pathway is one of the ERS-associated pathways.<sup>[22]</sup> It was hypothesized that the biological function of TMAO was mediated by the IRE1 $\alpha$ /XBP-1 pathway. To assess this hypothesis, the expression levels of IRE1 $\alpha$ , phospho-IRE1 $\alpha$  (p-IRE1 $\alpha$ ), total XBP-1, and glucose-regulated protein (GRP 78) were analyzed by WB analysis [Figure 4a]. The expression levels of p-IRE1 $\alpha$  and GRP78 were significantly increased in the MPC-83 cells treated with TMAO compared with those of the control cells [Figure 4c and e]. However, the expression levels of IRE1 $\alpha$  and XBP-1 were not altered [Figure 4b and d]. All these results indicated that the IRE1 $\alpha$ /XBP-1 signaling pathway was activated by TMAO in the MPC-83 cells.

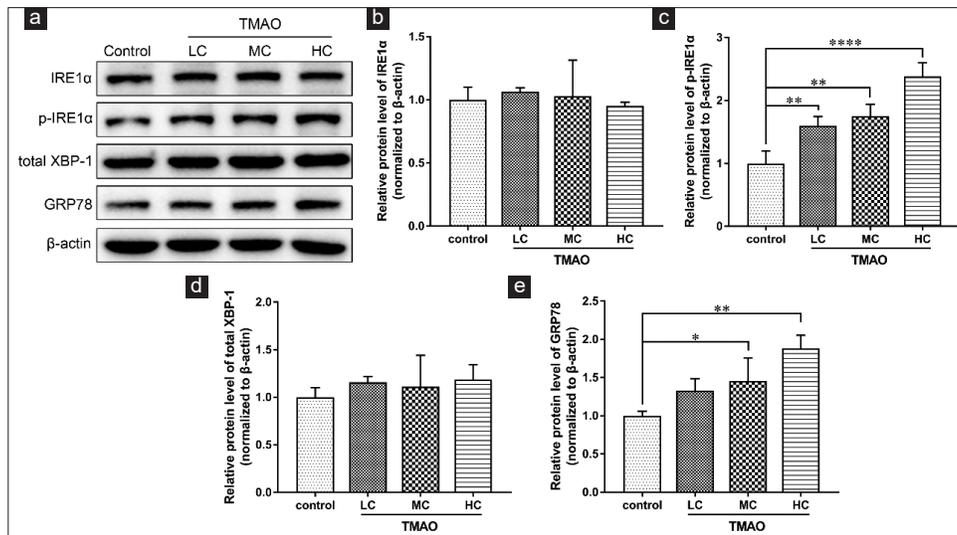
### The effect of TMAO on MPC-83 cells is mediated by the IRE1 $\alpha$ /XBP-1 signaling pathway

To demonstrate whether the function of TMAO in the MPC-83 cells is mediated by the IRE1 $\alpha$ /XBP-1 pathway, the IRE1 $\alpha$ /XBP-1 pathway inhibitor STF-083010 (MedChemExpress, Monmouth Junction, NJ, USA) was used in rescue experiments. The expression levels of IRE1 $\alpha$ , p-IRE1 $\alpha$ , total XBP-1, and GRP78 were partly reversed by STF-083010 treatment [Figure 5a]. WB analysis indicated that the induction of MPC-83 cell apoptosis following co-incubation with TMAO and STF-083010, was reduced, compared with that noted in the TMAO-treated cells [Figure 5b]. Subsequently, the levels of H<sub>2</sub>O<sub>2</sub>, ROS, NO, and SOD were measured. The results indicated that the effects noted on the oxidative stress status of MPC-83 cells, which were incubated with TMAO, were partially reversed when the cells were co-incubated with TMAO and STF-083010 [Figure 5c]. All these results suggested that the effects of TMAO on the pancreatic acinar cells were mediated by the IRE1 $\alpha$ /XBP-1 pathway.

### DISCUSSION

To date, a limited number of studies have assessed the association between TMAO and AP. Therefore, it is imperative to identify specific biomarkers in order to accurately diagnose HLAP. TMAO is associated with the occurrence and development of specific diseases and its application as a biomarker or target for the diagnosis and treatment of HLAP in humans is of considerable value. In the present study, the effects of TMAO on the MPC-3 cell viability and on the induction of MPC-3 cell apoptosis were investigated.

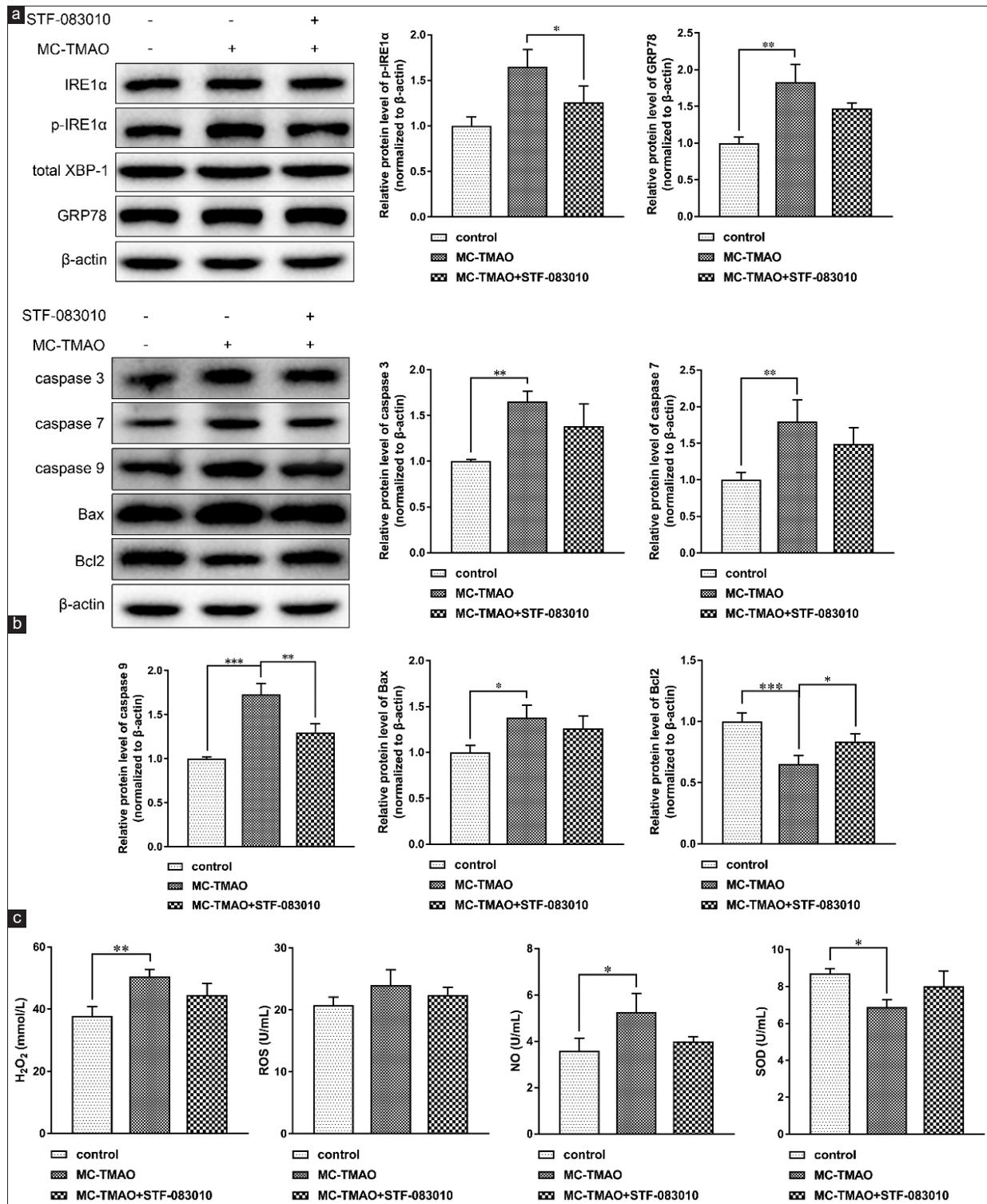
The endoplasmic reticulum (ER) stress is an important compartment regulating several cellular functions. However,



**Figure 4:** TMAO activates the IRE1 $\alpha$ /XBP-1 signaling pathway in MPC-83 cells. (a) The expression levels of IRE1 $\alpha$ , p-IRE1 $\alpha$ , total XBP-1, and GRP78 were analyzed using western blotting. The relative protein levels of IRE1 $\alpha$  (b), p-IRE1 $\alpha$  (c), total XBP-1 (d), and GRP78 (e) were semi-quantified using the ImageJ software. The bars represent the mean  $\pm$  SD from three independent experiments. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\*\* $P$  < 0.0001. TMAO, trimethylamine oxide; IRE1 $\alpha$ , inositol-requiring enzyme 1 $\alpha$ ; XBP-1, X-box binding protein 1; GRP78, glucose-regulated protein 78

excessive ER stress can induce a number of pathological and cellular changes, including apoptosis.<sup>[23]</sup> ER stress-induced apoptosis is mediated by the activation of three specific pathways. One of these is the caspase-12-dependent pathway.<sup>[24]</sup> Caspase-12 is specifically localized on the cytoplasmic side of the ER membrane and is activated by cleavage under ER stress conditions. Subsequently, caspase-12 activates caspase-9 and caspase-3, potentially eliminating the requirement of the mitochondria to undergo ER stress-induced apoptosis.<sup>[25]</sup> In addition, the imbalance between the levels of ROS and the concentrations of the endogenous antioxidants lead to oxidative stress and possibly apoptosis.<sup>[26]</sup> ROS are the most effective activators of apoptosis signal-regulating kinase 1.<sup>[27]</sup> Therefore, it is well accepted that oxidative stress can induce apoptosis. Previous studies have reported that ER stress and oxidative stress may play major roles in all types of pancreatitis.<sup>[28-30]</sup> Furthermore, TMAO is associated with increased oxidative stress and may induce deficits in synaptic plasticity through the ER stress-mediated PKR-like ER kinase (PERK) signaling pathway.<sup>[31,32]</sup> Based on the aforementioned evidence, it was hypothesized that the pro-apoptotic effect of TMAO on pancreatic acinar cells may be associated with ER stress and oxidative stress. In the present study, the expression levels of ROS, H<sub>2</sub>O<sub>2</sub>, and NO, which are positive regulators of oxidative stress, were upregulated in the TMAO group. In contrast to these observations, the expression levels of SOD, which is a negative regulator of oxidative stress, were decreased. These data demonstrated that TMAO accelerated oxidative stress in the pancreatic acinar cells.

Several physiological conditions and pathological perturbations disrupt protein folding in the ER lumen causing ER stress.<sup>[33,34]</sup> The cells developed a transcriptional signaling pathway termed the unfolded protein response (UPR), that is activated in the ER, and exerts its main function in the nucleus in order to inhibit the development of ER stress. UPR is mediated by these three sensors: IRE1, PERK, and activating transcription factor 6 (ATF6).<sup>[35,36]</sup> Under non-stress conditions, GRP78 is bound to IRE1 and prevents its oligomerization in order to inhibit IRE1 activation. Following induction of ER stress, ATF6 translocates from the ER to the Golgi apparatus and is successively cleaved from a specific protease, which releases the 50 kDa fragment of ATF6. This fragment enters the nucleus following a complex formation with the nuclear factor Y. Subsequently, it binds to the ER stress-response element (ERSE) and induces the expression of molecular chaperones and XBP-1.<sup>[37]</sup> The RNase of IRE1 facilitates XBP-1 mRNA splicing by removing the 97-amino acid site at the C-terminal of XBP-1 mRNA from ORF1, which adds the 212-amino acid site of open reading frame (ORF) 2 to the N-terminal of ORF1 and results in the formation of the splicing XBP-1 form (XBP-1s). Finally, the binding of XBP-1s to ERSE or UPR element activates the genes associated with the protein degradation pathway of ER, promotes the accumulation of unfolded proteins, and induces the expression of molecular chaperones, such as GRP78 and GRP94.<sup>[38]</sup> It has been previously reported that IRE1 is one of the ER stress transducers that serves as an ER stress marker.<sup>[39]</sup> GRP78 is a heat shock protein 70 family member that is produced during the AP process



**Figure 5:** The effects of TMAO on MPC-83 cells are mediated by the IRE1 $\alpha$ /XBP-1 signaling pathway. (a) MPC-83 cells with TMAO or control were incubated with or without STF-083010 and subsequently the expression levels of IRE1 $\alpha$ , p-IRE1 $\alpha$ , total XBP-1, and GRP78 were detected using WB. (b) The expression levels of the apoptosis-associated proteins were detected using WB in MPC-83 cells with TMAO or in control cells cultured in medium with or without STF-083010. (c) The indices associated with the oxidative stress were measured in MPC-83 cells with TMAO or control cells cultured in medium with or without STF-083010. The bars represent the mean  $\pm$  SD from three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . TMAO, trimethylamine oxide; IRE1 $\alpha$ , inositol-requiring enzyme 1 $\alpha$ ; XBP-1, X-box binding protein 1; p-IRE1 $\alpha$ , phosphor-IRE1 $\alpha$ ; GRP78, glucose-regulated protein 78; WB, western blotting

and ER stress has been shown to cause an increase in the GRP78 levels in hyperlipidemic pancreatitis.<sup>[40]</sup> In order to demonstrate the association between ER stress and TMAO, the levels of IRE1 $\alpha$ , p-IRE1 $\alpha$ , total XBP-1, and GRP78 were analyzed by WB analysis. The data indicated that the expression levels of p-IRE1 $\alpha$  and GRP78 were increased in the TMAO-treated group, which resulted in the induction of ERS stress in pancreatic acinar cells via the activation of the GRP78 and IRE1 $\alpha$ /XBP-1 pathways.

ER stress can generate excessive production of ROS, such as O<sup>2-</sup> and OH<sup>-</sup>, via the interaction between UPR and mitochondria, which in turn induces oxidative stress in the local tissues and cells.<sup>[41]</sup> To further elucidate the role of ER stress in the induction of apoptosis in TMAO-treated pancreatic acinar cells, an inhibitor of the IRE1 $\alpha$ /XBP-1 pathway was used. STF-083010 is a novel molecule, which was initially identified using high-throughput screening. This reagent can inhibit IRE1 endonuclease activity during ER stress both *in vitro* and *in vivo*.<sup>[42]</sup> In the present study, the TMAO-mediated induction of apoptosis and oxidative stress were decreased following the treatment of pancreatic acinar cells with STF-083010. This was demonstrated by the decreased levels of p-IRE1 $\alpha$ , GRP78, ROS, H<sub>2</sub>O<sub>2</sub>, and NO and by the increased expression levels of SOD. Therefore, the roles of TMAO in inducing apoptosis and oxidative stress of pancreatic acinar cells were mediated by the IRE1 $\alpha$ /XBP-1 pathway.

The IRE1 $\alpha$ /XBP-1 pathway has been reported to participate in the development of certain diseases. For example, it has been shown that targeting XBP-1 can be used as a treatment strategy for multiple myeloma, mature B-cell leukemia, and lymphoma.<sup>[43]</sup> In addition, it was shown that the degree of inflammation during the development of cystic fibrosis (CF)-associated pulmonary disease correlated with UPR. Targeting of the IRE1 $\alpha$ /XBP-1 pathway may be used as a therapeutic strategy for the treatment of CF-associated airway disease.<sup>[44]</sup> Similarly, the estrogen receptor  $\beta$ 1 has been shown to repress the IRE1 pathway of the UPR by inducing degradation of IRE1 $\alpha$  in breast cancer. This evidence suggests that the IRE1 $\alpha$ /XBP-1 pathway is indirectly associated with the pathogenesis of breast cancer.<sup>[45]</sup> Taken together, the data suggest the potential application of the IRE1 $\alpha$ /XBP-1 pathway in the development of therapeutic targets for HLAP.

## CONCLUSION

In summary, the present study indicated that TMAO decreased the viability and facilitates apoptosis of MPC-83 cells by inducing oxidative stress and ER stress.

The noxious effect of TMAO on the pancreatic acinar cells is described for the first time in the present study. The findings may provide a potential diagnostic and therapeutic strategy for the treatment of HLAP.

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## Conflicts of interest

There are no conflicts of interest.

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