

## Original Research Article

# Dexmedetomidine protects gastric mucosal epithelial cells against ischemia/reperfusion-induced apoptosis by inhibiting HMGB1-mediated inflammation and oxidative stress

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### Abstract

**Purpose:** To investigate the role of dexmedetomidine in gastric ischemia/reperfusion injury using gastric mucosal epithelial cell (GES-1) model.

**Methods:** GES-1 were subjected to oxygen-glucose deprivation conditions, followed by increasing dexmedetomidine concentrations (0.5, 1.0, or 1.5  $\mu\text{M}$ ) for 4 h of reoxygenation. Cell viability and apoptosis were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide and flow cytometry, respectively. Oxidative stress and inflammation were analyzed by enzyme-linked immunosorbent assay (ELISA).

**Results:** Oxygen-glucose deprivation conditions induced cytotoxicity in GES-1 by decreasing cell viability and increasing apoptosis. Dexmedetomidine treatment significantly increased the cell viability of hypoxia/reoxygenation-induced GES-1 ( $p < 0.01$ ) but reduced apoptosis. Dexmedetomidine also attenuated the hypoxia/reoxygenation-induced increase in malondialdehyde and myeloperoxidase, but the decrease in superoxide dismutase and glutathione in GES-1. Moreover, upregulated tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-18 in hypoxia/reoxygenation-induced GES-1 was downregulated by dexmedetomidine treatment. Dexmedetomidine also enhanced IL-10 levels and inhibited pro-inflammatory factor production ( $p < 0.01$ ). High-mobility group box 1 (HMGB1) protein in GES-1 was upregulated by hypoxia/reoxygenation but decreased by dexmedetomidine. HMGB1 over-expression attenuated the dexmedetomidine-induced increase in cell viability and the decrease in apoptosis, oxidative stress, and inflammation in hypoxia/reoxygenation-induced GES-1 ( $p < 0.01$ ).

**Conclusion:** Dexmedetomidine protects GES-1 against ischemia/reperfusion-induced apoptosis, inflammation, and oxidative stress by inhibiting HMGB1, thus providing a potential strategy for treating gastric ischemia/reperfusion injury.

**Keywords:** Dexmedetomidine, gastric mucosal epithelial cells, Ischemia/reperfusion, Apoptosis, Inflammation, Oxidative stress, High-mobility group box 1 (HMGB1)

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## INTRODUCTION

Gastric ischemia-reperfusion injury is a common clinical problem caused by distinct hemorrhagic diseases, such as vascular rupture, peptic ulcer bleeding, and hemorrhagic shock [1]. Gastric ischemia-reperfusion injury with significant morbidity and mortality lacks satisfactory treatment [2]. Pathophysiological manifestations of gastric ischemia-reperfusion injury include dysregulation of intracellular calcium homeostasis, increased mitochondrial permeability, and increased cytoskeletal and structural vulnerability [3]. Oxidative stress and the resultant inflammatory response that leads to cellular death and mucosal injury are implicated in the pathogenesis of gastric ischemia-reperfusion injury [4]. Therefore, strategies to suppress oxidative stress show promising effects against gastric ischemia-reperfusion injury [4].

Dexmedetomidine is a selective  $\alpha$ -2 adrenergic receptor agonist that exerts sedative, anti-anxiety, analgesic, and antihypertensive properties [5]. Pretreatment with dexmedetomidine has been reported to also attenuate ischemia-reperfusion-induced intestinal injury [6]. However, the role of dexmedetomidine in gastric ischemia-reperfusion injury remains unknown. High mobility group box 1 (HMGB1) participates in the immune process by binding to Toll-like receptors and activating NF- $\kappa$ B signaling [7]. HMGB1 is implicated in the pathogenesis of non-infectious inflammation-associated diseases, including trauma, cancer, and ischemia reperfusion injury [7]. For example, HMGB1 contributes to ischemia-reperfusion-induced injury in the heart [8], and inhibiting HMGB1 reduces ischemia-reperfusion-induced inflammation and apoptosis in the lung [9].

Dexmedetomidine reduces HMGB1 expression in oxygen and glucose deprivation/reoxygenation-treated primary microglia by downregulating small nucleolar RNA host gene 14 [10]. Therefore, dexmedetomidine might also attenuate gastric ischemia-reperfusion injury by regulating HMGB1. The effects of dexmedetomidine on hypoxia/reoxygenation-induced apoptosis, inflammation, and oxidative stress in gastric mucosal epithelial cells (GES-1) were investigated.

## EXPERIMENTAL

### Cell culture and treatments

GES-1 was acquired from ScienCell (San Diego, CA, USA) and cultured in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10 %

fetal bovine serum (Gibco). Cells were incubated in a 37 °C incubator with 5 % CO<sub>2</sub>. Hypoxic conditions were induced by culturing cells in glucose-free medium and incubating them in 1% O<sub>2</sub>, 5% CO<sub>2</sub>, and 94 % N<sub>2</sub> for 2 h. Cells in the control group were cultured in RPMI 1640 under normal conditions with 5 % CO<sub>2</sub> and 95 % atmosphere. Cells in the treatment groups were treated with increasing concentrations (0.5, 1.0, or 1.5 mM) of dexmedetomidine (Sigma-Aldrich, St. Louis, MO, USA) for 4 h of reoxygenation.

### Cell transfection

GES-1 was transfected with pcDNA-HMGB1 (Invitrogen, Carlsbad, CA, USA) using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) for 48 h. Cells were then subjected to hypoxia/reoxygenation conditions and treated with 1  $\mu$ M dexmedetomidine.

### Cell viability and apoptosis assays

GES-1 were seeded into 96 well plates and then was incubated with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (10 ml; Beyotime, Beijing, China) for 4 h. Absorbance at 490 nm was measured using a microplate reader (Thermo Fisher Scientific). For flow cytometry, GES-1 were resuspended in the binding buffer of the Annexin V FITC and PI Staining Kit (Thermo Fisher Scientific) and then stained with 5  $\mu$ L PI and 5  $\mu$ L FITC-labeled annexin V. The apoptosis ratio was evaluated using a FACS flow cytometer (Life Technologies, Gaithersburg, MD, USA).

### Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Cells were lysed in TRIzol kit (Invitrogen) to isolate RNAs. The RNAs were then synthesized into cDNAs, and the PreTaq II kit (Takara, Dalian, Liaoning, China) was used to determine the mRNA expression of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-18 and IL-10. The relative expression was calculated using the 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method with normalization to GAPDH. The primers used are shown in Table 1.

### Enzyme-linked immunosorbent assay

Culture supernatants and cell lysates of GES-1 were harvested, and the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), myeloperoxidase (MPO), TNF- $\alpha$ , IL-1 $\beta$ , IL-18, and IL-10 were detected using ELISA kits (Pharmingen, San Diego, CA, USA).

**Table 1:** Primers used in PCR

Gene	Forward	Reverse
GAPDH	5'-TCAACGACCACTTTGTCAAGCAGAGT-3'	5'-GCTGGTGGTCCAGGGGTCTTACT-3'
IL-18	5'-GGCATTTGCGAGCTGCCAAATTCC-3'	5'-CTTGTGTGTCTCTGGAACACG-3'
IL-10	5'-GCCTAACATGCTTCGAGATC-3'	5'-TGATGTCTGGGTCTTGGTTC-3'
IL-1 $\beta$	5'-CCAGCTTCAAATCTCACAGCAG-3'	5'-CTTCTTTGGGTATTGCTTGGGATC-3'
HMGB1	5'-CACCGTGGGACTATTAGGAT-3'	5'-GCTCACACTTTTGGGGATAC-3'
TNF- $\alpha$	5'-CCGAGTGACAAGCCTGTAGC-3'	5'-AGGAGGTTGACCTTGGTCTG-3'

### Western blotting

GES-1 was lysed in RIPA buffer (Beyotime), and the isolated proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples were transferred onto nitrocellulose membranes, which were then blocked by incubation with 5 % bovine serum albumin. The membranes were probed with specific antibodies anti-HMGB1 and anti- $\beta$ -actin (1:2000; Abcam, Cambridge, MA, USA). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:3000; Abcam). Immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich).

### Statistical analysis

All the data were expressed as means  $\pm$  standard error of the mean ( $n = 3$ ) and analyzed by Student's t-test or one-way analysis of variance (ANOVA) using SPSS 11.5. A  $p$  value of  $< 0.05$  was considered statistically significant.

## RESULTS

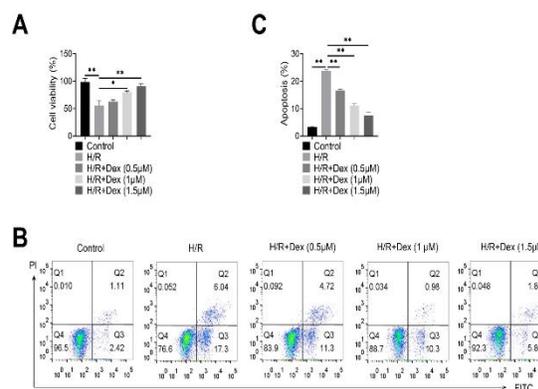
### Dexmedetomidine contributes to the cell survival of hypoxia/reoxygenation-induced GES-1

To induce ischemia/reperfusion-associated injury, GES-1 was treated under hypoxia/reoxygenation conditions. Hypoxia/reoxygenation induced decreased cell viability (Figure 1A) and increased cell apoptosis (Figure 1B and C) in GES-1. Moreover, hypoxia/reoxygenation-induced GES-1 was incubated with dexmedetomidine, which protected GES-1 against hypoxia/reoxygenation-induced cytotoxicity by increasing cell viability (Figure 1A) and decreasing cell apoptosis (Figure 1B and C), suggesting the anti-apoptotic effect of dexmedetomidine against gastric ischemia/reperfusion injury.

### Dexmedetomidine inhibits the oxidative stress of hypoxia/reoxygenation-induced GES-1

Hypoxia/reoxygenation promoted oxidative stress

in GES-1 by decreasing SOD (Figure 2A) and increasing MDA (Figure 2B). Moreover, GSH was downregulated (Figure 2C) while MPO was upregulated (Figure 2D) in GES-1 post-hypoxia/reoxygenation. However, dexmedetomidine reduced the levels of MDA (Figure 2B) and MPO (Figure 2D) but enhanced levels of SOD (Figure 2 A) and GSH (Figure 2 C) in a dose-dependent manner in hypoxia/reoxygenation-induced GES-1, demonstrating the antioxidant effect of dexmedetomidine against gastric ischemia/reperfusion injury.

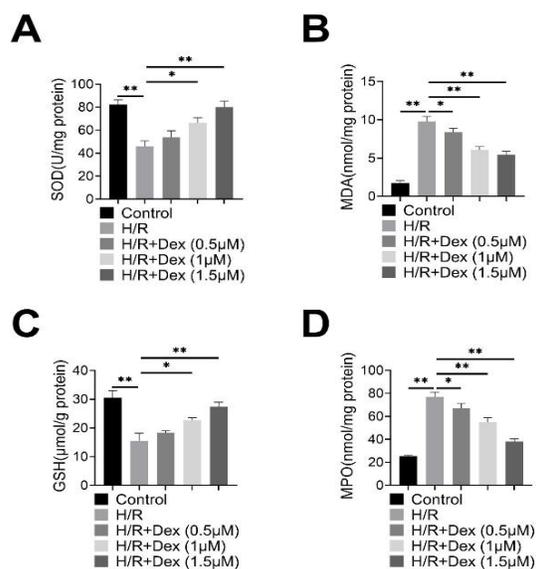


**Figure 1:** Dexmedetomidine contributes to the cell survival of hypoxia/reoxygenation-induced GES-1. (A) Incubation with dexmedetomidine increased the cell viability of hypoxia/reoxygenation-induced GES-1. (B) Incubation with dexmedetomidine decreased the cell apoptosis of hypoxia/reoxygenation-induced GES-1. (C) Relative apoptosis ratio of hypoxia/reoxygenation-induced GES-1. \* $P < 0.05$ , \*\* $p < 0.01$

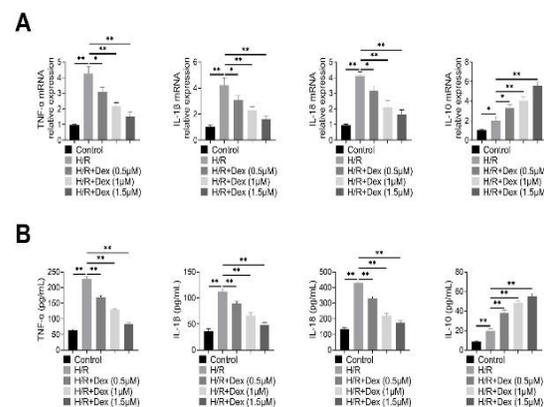
### Dexmedetomidine inhibits the inflammation of hypoxia/reoxygenation-induced GES-1

The mRNA expression of TNF- $\alpha$ , IL-1 $\beta$ , and IL-18 in GES-1 was upregulated by hypoxia/reoxygenation (Figure 3 A). Dexmedetomidine increased the mRNA expression of IL-10 in a dose-dependent manner to downregulate TNF- $\alpha$ , IL-1 $\beta$  and IL-18 in hypoxia/reoxygenation-induced GES-1 (Figure 3 A). Moreover, dexmedetomidine increased IL-10 protein to downregulate TNF- $\alpha$ , IL-1 $\beta$  and IL-18 proteins in hypoxia/reoxygenation-induced GES-1 (Figure 3 B), revealing the anti-inflammatory effect of

dexmedetomidine against gastric ischemia/reperfusion injury.



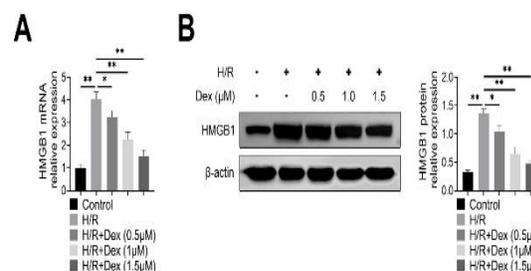
**Figure 2:** Dexmedetomidine inhibits the oxidative stress of hypoxia/reoxygenation-induced GES-1. (A) Incubation with dexmedetomidine enhanced the level of SOD in hypoxia/reoxygenation-induced GES-1. (B) Incubation with dexmedetomidine reduced the level of MDA in hypoxia/reoxygenation-induced GES-1. (C) Incubation with dexmedetomidine enhanced the level of GSH in hypoxia/reoxygenation-induced GES-1. (D) Incubation with dexmedetomidine reduced the levels of MPO in hypoxia/reoxygenation-induced GES-1. \* $P < 0.05$ , \*\* $p < 0.01$



**Figure 3:** Dexmedetomidine inhibits the inflammation of hypoxia/reoxygenation-induced GES-1. (A) Dexmedetomidine increased the mRNA expression of IL-10 to downregulate TNF- $\alpha$ , IL-1 $\beta$ , and IL-18 in hypoxia/reoxygenation-induced GES-1. (B) Dexmedetomidine increased the protein expression of IL-10 to downregulate TNF- $\alpha$ , IL-1 $\beta$  and IL-18 in hypoxia/reoxygenation-induced GES-1. \* $P < 0.05$ , \*\* $p < 0.01$

## Dexmedetomidine downregulates HMGB1 in hypoxia/reoxygenation-induced GES-1

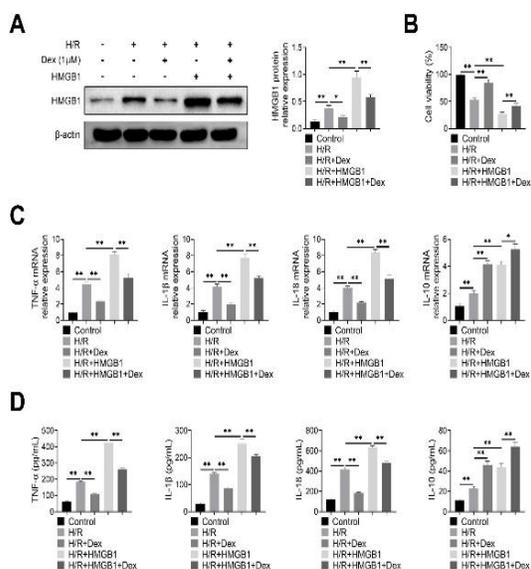
The expression of HMGB1 in GES-1 was enhanced by hypoxia/reoxygenation (Figure 4A and B). However, dexmedetomidine decreased HMGB1 expression in a dose-dependent manner in hypoxia/reoxygenation-induced GES-1 (Figure 4A and B).



**Figure 4:** Dexmedetomidine downregulates HMGB1 in hypoxia/reoxygenation-induced GES-1. (A) Dexmedetomidine decreased the mRNA expression of HMGB1 in hypoxia/reoxygenation-induced GES-1. (B) Dexmedetomidine decreased the protein expression of HMGB1 in a dose-dependent manner in hypoxia/reoxygenation-induced GES-1. \* $P < 0.05$ , \*\* $p < 0.01$

## Dexmedetomidine ameliorated gastric ischemia/reperfusion injury by regulating HMGB1

To investigate the role of dexmedetomidine/HMGB1 in gastric ischemia/reperfusion injury, GES-1 was transfected with pcDNA-HMGB1 and subjected to hypoxia/reoxygenation conditions. HMGB1 over-expression increased HMGB1 protein expression in hypoxia/reoxygenation-induced GES-1 and attenuated the dexmedetomidine-induced decrease in HMGB1 (Figure 5A). HMGB1 over-expression decreased the cell viability of hypoxia/reoxygenation-induced GES-1 and attenuated the dexmedetomidine-induced increase in cell viability (Figure 5B). Moreover, HMGB1 over-expression promoted the inflammation of hypoxia/reoxygenation-induced GES-1 by upregulating TNF- $\alpha$ , IL-1 $\beta$  and IL-18 (Figure 5C and D). HMGB1 over-expression attenuated the dexmedetomidine-induced increase in IL-10 and decrease in TNF- $\alpha$ , IL-1 $\beta$  and IL-18 in hypoxia/reoxygenation-induced GES-1 (Figure 5C and D). These results indicated that dexmedetomidine suppressed hypoxia/reoxygenation-induced inflammation in GES-1 by downregulating HMGB1.



**Figure 5:** Dexmedetomidine ameliorates gastric ischemia/reperfusion injury by regulating HMGB1. (A) HMGB1 over-expression increased HMGB1 protein expression in hypoxia/reoxygenation-induced GES-1 and attenuated the dexmedetomidine-induced decrease in HMGB1. (B) HMGB1 over-expression decreased the cell viability of hypoxia/reoxygenation-induced GES-1 and attenuated the dexmedetomidine-induced increase in cell viability. (C) HMGB1 over-expression promoted the mRNA expression of TNF- $\alpha$ , IL-1 $\beta$  and IL-18 in hypoxia/reoxygenation-induced GES-1 and attenuated the dexmedetomidine-induced increase in IL-10 and decrease in TNF- $\alpha$ , IL-1 $\beta$  and IL-18. (D) HMGB1 over-expression promoted protein expression of TNF- $\alpha$ , IL-1 $\beta$  and IL-18 in hypoxia/reoxygenation-induced GES-1 and attenuated the dexmedetomidine-induced increase in IL-10 and decrease in TNF- $\alpha$ , IL-1 $\beta$  and IL-18. \* $P < 0.05$ , \*\* $p < 0.01$

## DISCUSSION

$\alpha$ -2 Adrenergic receptors are widely distributed throughout the whole body and exert pathologic effects in various diseases [11]. The agonists of  $\alpha$ -2 adrenergic receptors are widely used to treat pain and panic disorders, hypertension, and alcohol withdrawal [11]. Dexmedetomidine, an  $\alpha$ -2 adrenergic receptor, protects against ischemia-reperfusion-induced intestinal injury [6]. The present study revealed that dexmedetomidine also attenuates gastric ischemia/reperfusion injury by inhibiting HMGB1-mediated inflammation and oxidative stress.

Hypoxia/reoxygenation induces cytotoxicity in gastric epithelial cells by decreasing cell viability and increasing inflammation and oxidative stress [12]. Therefore, hypoxia/reoxygenation-induced GES-1 was used as a cell model of gastric ischemia/reperfusion injury [12]. Here, GES-1

was also subjected to hypoxia/reoxygenation conditions. The cell viability of GES-1 was decreased, while cell apoptosis was increased, by hypoxia/reoxygenation. Moreover, hypoxia/reoxygenation reduced the levels of SOD and GSH and enhanced the levels of MDA and MPO to promote oxidative stress in GES-1. The levels of pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , and IL-18, were upregulated in hypoxia/reoxygenation-induced GES-1.

Suppression of hypoxia/reoxygenation-induced inflammation and oxidative stress in GES-1 provided potential value in preventing acute gastric mucosal lesions [12]. Here, dexmedetomidine increased the cell viability of hypoxia/reoxygenation-induced GES-1 and decreased cell apoptosis to protect against cytotoxicity. Moreover, a previous study has shown that dexmedetomidine attenuated ischemia/reperfusion-induced hepatic inflammation and oxidative stress [13]. Dexmedetomidine in this study enhanced the levels of MDA and MPO while reducing SOD and GSH in hypoxia/reoxygenation-induced GES-1. Dexmedetomidine also increased IL-10 to downregulate TNF- $\alpha$ , IL-1 $\beta$ , and IL-18, suggesting antioxidant and anti-inflammatory effects against gastric ischemia/reperfusion injury.

HMGB1 functions as a DNA-binding nuclear protein to promote inflammatory responses by binding to Toll-like receptors [7]. In addition, HMGB1 activity is regulated by oxidative stress during inflammation and cell death [14]. HMGB1 also contributed to ischemia/reperfusion-induced inflammation [15] and oxidative stress [16]. HMGB1 suppression reduced ischemia/reperfusion-induced inflammation and oxidative stress [16]. Hypoxia/reoxygenation increased HMGB1 expression in cardiomyocytes, and dexmedetomidine reduced HMGB1 to inhibit hypoxia/reoxygenation-induced inflammation and necroptosis [17]. Dexmedetomidine also attenuated cerebral ischemic/reperfusion by downregulating HMGB1-mediated inflammation and oxidative stress [18]. Our findings showed that dexmedetomidine reduced HMGB1 expression in hypoxia/reoxygenation-induced GES-1 and HMGB1 over-expression attenuated the dexmedetomidine-induced increase in cell viability and decrease in inflammation in hypoxia/reoxygenation-induced GES-1. Therefore, dexmedetomidine may attenuate gastric ischemia/reperfusion injury by inhibiting HMGB1-mediated inflammation and oxidative stress.

## CONCLUSION

Dexmedetomidine exerts anti-apoptotic, anti-inflammatory, and antioxidant effects against hypoxia/reoxygenation-induced GES-1 by downregulating HMGB1, providing a potential strategy to manage gastric ischemia/reperfusion injury. However, the role of dexmedetomidine in an animal model of gastric ischemia/reperfusion injury should be investigated in further studies.

## DECLARATIONS

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### Ethical approval

None provided.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Tianpin Liu and Bing Wang designed and performed the study. Qiong Han supervised the data collection, analyzed the data, and interpreted the data. Wansheng Gong and Juan Ye prepared the manuscript for publication and reviewed the draft of the manuscript. All the authors have read and approved the manuscript.

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