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ORIGINAL RESEARCH

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PSME3 promotes glycolysis and migration of gastric cancer cells *via* regulating the EGFR/c-myc pathway

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Abstract

Gastric cancer (GC) is a common malignant tumor that is pernicious to the health of patients. Proteasome activator subunit 3 (*PSME3*) has been shown to exhibit higher expression and aggravate tumorigenesis in cancer progression. A major finding is that *PSME3* is also highly expressed in GC tissues, which results in a worse prognosis. Nevertheless, the regulatory functions of *PSME3* in GC progression remain unclear. This study aimed to investigate the impacts of *PSME3* and related regulatory pathways on GC progression. From the Gene Expression Profiling Interactive Analysis (GEPIA) database, it was noted that *PSME3* expression was up-regulated in GC tissues. Our findings suggested that in GC, *PSME3* showed higher expression, resulting in a worse prognosis. Functional experiments revealed that *PSME3* accelerates cell growth, migration and abnormal glycolysis in GC. *PSME3* stimulates the epidermal growth factor receptor (EGFR)/c-myc pathway. In conclusion, GC cells exhibited higher *PSME3* expression, which modulated the EGFR/c-myc pathway to facilitate glycolysis and migration. *PSME3* might be an effective bio-target for GC treatment.

Keywords

PSME3; Glycolysis; Gastric cancer; EGFR/c-myc pathway

1. Introduction

Gastric cancer (GC) is a swart tumor that arises in the gastric mucosa epithelium, whose incidence rate occupies the top spot among diversified cancers in China [1, 2]. Changes in diet structure, increased work pressure, and Helicobacter pylori infection contribute to GC's inclination towards youth [3, 4]. Surgical resection is the dominant treatment for GC. As GC symptoms are difficult to observe in the early stages, most patients are diagnosed at a later stage, losing the opportunity to undergo resection [5]. Despite chemotherapy being a clinical therapy for GC, GC possesses strong metastatic ability, leading to a poor prognosis [6]. Therefore, understanding the mechanism and process of GC and finding effective molecular targets can be useful in GC treatment and diagnosis.

Proteasome activator subunit 3 (*PSME3*) is a subunit of 11S proteasome regulators referred to as REGγ and PA28gamma (PA28γ). It modulates the degradation of many key regulatory proteins in cancer [7, 8]. One study revealed that *PSME3* increases radiation sensitivity in colon cancer cells, while *PSME3* suppression can retard tumorigenesis by reducing the expression of cell cyclin-related proteins (cyclin B1 and cyclin-dependent kinase 1 (CKD1)) [9]. Moreover, *PSME3* displays high expression in breast cancer, and PSMES enhances the stem characteristics of tumors, strengthens epithelial-mesenchymal transitions, thereby aggravates the malignant phenotype of breast cancer [10]. Additionally, *PSME3* modulates c-myc degradation in pancreatic cancer cells, contributing to abnormal glycolysis and cell proliferation [11]. Importantly, *PSME3* is highly expressed in GC, as well as having a poor prognosis among GC patients [12]. However, the detailed regulatory functions of *PSME3* and its related molecular mechanism for GC progression remain unclear, calling for further research.

This study examines *PSME3*'s biological functions in GC progression. Results elucidated that *PSME3* exhibited higher expression and facilitated glycolysis and migration of GC cells through modulating the EGFR/c-myc pathway. Our findings may provide a novel target for effective GC therapy.

2. Materials and methods

2.1 Cell lines and cell culture

GC cell lines (AGS, SNU-1 and NCI-N87) and gastric mucosa cell line (GES-1) were obtained from the American Tissue Culture Collection (ATCC, USA). Cell culturing was conducted in Dulbecco's modified Eagle's medium (DMEM; Gibco, New York, NY, USA) containing 10% fetal bovine serum (FBS, 10099-141, Gibco, Grand Island, NY, USA) at 37 °C with 5% CO_2 in a moist incubator.

2.2 Cell transfection

PSME3 knockdown plasmids (shPSME3-1# and shPSME3-2#) and an empty plasmid (shNC) were synthesized from

GenePharma (Shanghai, China). Cell transfection was performed with Lipofectamine 2000 (11668019, Invitrogen, Carlsbad, CA, USA) in line with the manufacturer's instructions.

2.3 Western blot

The total protein in GC cells was isolated using a radioimmunoprecipitation assay (RIPA) solution (P0013B, Beyotime, Shanghai, China). Protein samples in each lane were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then migrated to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Primary antibodies in membranes were then cultured overnight at 4 °C. After washing, further cultivation of horseradish peroxidase (HRP)-conjugated secondary antibodies (ab6721, 1:1000 dilution, Abcam) in membranes was performed. The bands were assessed through a chemiluminescence detection kit (89880, Pierce, Rockford, lL, USA).

Primary antibodies: PSME3 (ab157157, 1/1000 dilution, Abcam, Shanghai, China), matrix metalloproteinase 9 (MMP9) (ab76003, 1/1000 dilution), MMP2 (ab92536, 1/1000 dilution), glucose transporter 1 (GLUT1) (ab115730, 1/100,000 dilution), hexokinase 2 (HK2) (ab209847, 1/1000 dilution), Lactate Dehydrogenase A (LDHA) (ab52488, 1/5000 dilution), EGFR (ab40815, 1/500 dilution), EGFR (ab52894, 1/1000 dilution), c-myc (ab32072, 1/1000 dilution) and β-actin (ab6276, 1/5000 dilution).

2.4 CCK-8 assay

The cell counting kit-8 (CCK8) assay was utilized to assess cell viability. AGS cells were seeded into 96-well plates. At 0, 24, 48 and 72 h, 20 μL of CCK8 solution (C0046, Beyotime, Shanghai, China) was added to each well and incubated for another 4 h. An optical density of 450 nm was confirmed.

2.5 EDU assay

The 5-ethynyl-20-deoxyuridine (EDU) assay kit (C10337, Thermo Fisher Scientific, Waltham, MA, USA) was adopted to examine cell proliferation. AGS cells (1×10^5) were seeded into 6-well plates. After 48 h, 100 μL of EDU was added and incubated for 2 h. Cells were mixed with 4% formaldehyde, followed by 0.3% Triton X-100. EDU-positive cells were captured under fluorescence microscopy (IX71, Olympus, Tokyo, Japan).

2.6 Transwell assay

For cell invasion, Transwell chambers (Corning, Tewksbury, MA, USA) coated with Matrigel (Bedford, MA, USA) were used. The upper chamber contained 200 μL of serum-free medium, while the lower chamber contained 15% FBS medium. After 48 h culturing, AGS cells were immobilized in 4% paraformaldehyde (P0899, Sigma-Aldrich, St. Louis, MO, USA) and stained with 0.1% crystal violet (C3886, Sigma-Aldrich, St. Louis, MO, USA). As in the above steps, the migration assay is performed in chambers without Matrigel. The stained, migrated and invaded cells were imaged under a microscope (E200, Nikon, Tokyo, Japan).

2.7 The detection of glucose consumption and lactate production

Measurements of glucose consumption and lactate production in AGS cells were performed using glucose assay kits (KA4086, Abnova, Taiwan, China) and lactate assay kits (ab65330, Abcam, Shanghai, China).

2.8 Statistical analysis

Data were displayed as mean ± standard deviation (SD). The statistical analysis was conducted using GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA). Two groups were compared using Student's *t*-test, and multiple groups were compared using one-way analysis of variance (ANOVA). $p < 0.05$ was deemed statistically significant.

3. Results

3.1 The higher *PSME3* expression was discovered in GC

From the GEPIA database, *PSME3* expression was up-regulated in GC tissues compared with normal tissues (Fig. 1A). Moreover, GC cell lines (AGS, SNU-1 and NCI-N87) had higher PSME3 protein expression than gastric mucosa cells (GES-1) (Fig. 1B). According to the kmplot database (OS and FP), the prognosis of GC patients with higher *PSME3* expression was worse (Fig. 1C). In GC, higher *PSME3* expression was observed.

3.2 *PSME3* accelerated cell growth in GC

Fig. 2A confirms PSME3's knockdown efficiency. *PSME3* inhibition reduced cell viability (Fig. 2B). Furthermore, cell proliferation ability was weakened after PSME3 suppression (Fig. 2C). In GC, PSME3 accelerated cell growth.

3.3 *PSME3* facilitated cell migration in GC

Silencing *PSME3* reduced cell migration and invasion abilities (Fig. 3A,B). In addition, MMP9 and MMP2 protein expressions were down-regulated after *PSME3* suppression (Fig. 3C). Overall, *PSME3* facilitated cell migration in GC.

3.4 *PSME3* strengthened abnormal glycolysis in GC

PSME3's regulatory effects on glycolysis were investigated. After *PSME3* knockdown, GLUT1, HK2 and LDHA protein expression were all reduced (Fig. 4A). Glucose consumption and lactate production decreased after *PSME3* repression (Fig. 4B,C). In brief, *PSME3* strengthened abnormal glycolysis in GC.

3.5 *PSME3* stimulated the EGFR/c-myc pathway

The protein expressions of p-EGFR/EGFR and c-myc were decreased after restraining *PSME3* (Fig. 5), suggesting that *PSME3* stimulated the EGFR/c-myc pathway.

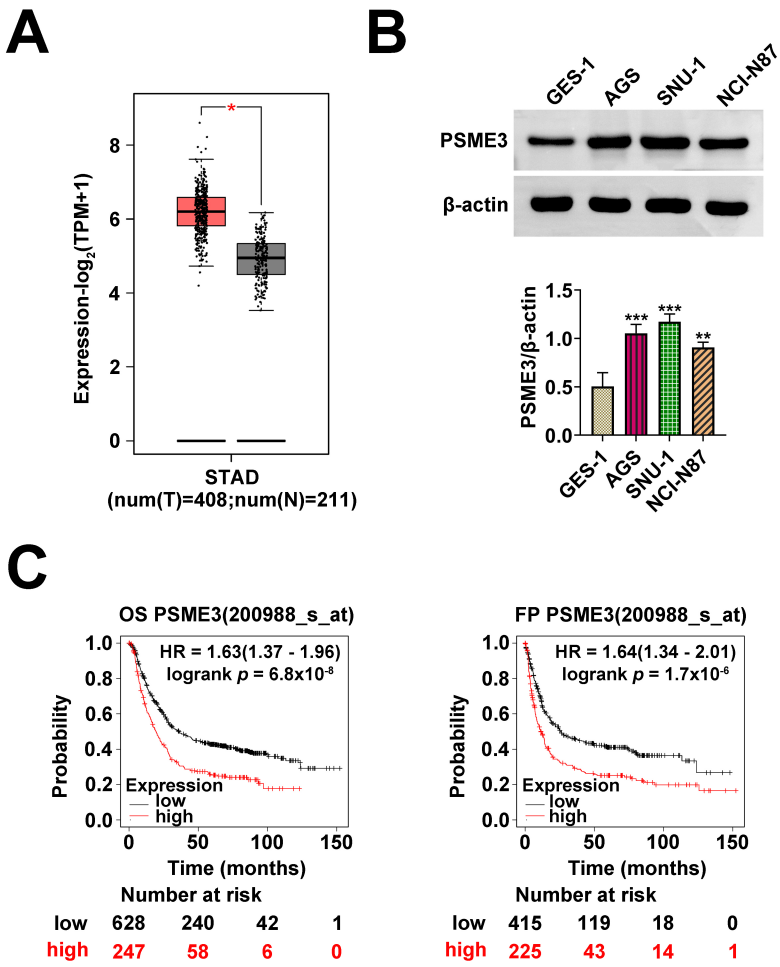

FIGURE 1. The higher *PSME3* expression was discovered in GC. (A) The expression of *PSME3* in normal and GC tissues was obtained from the GEPIA database. (B) By western blot, PSME3 protein expression in gastric mucosa cell line (GES-1) and GC cell lines (AGS, SNU-1 and NCI-N87) was examined. (C) The prognosis of GC patients was verified in the kmplot database (OS and FP). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. GC: Gastric cancer; PSME3: Proteasome activator subunit 3; GEPIA: Gene Expression Profiling Interactive Analysis.

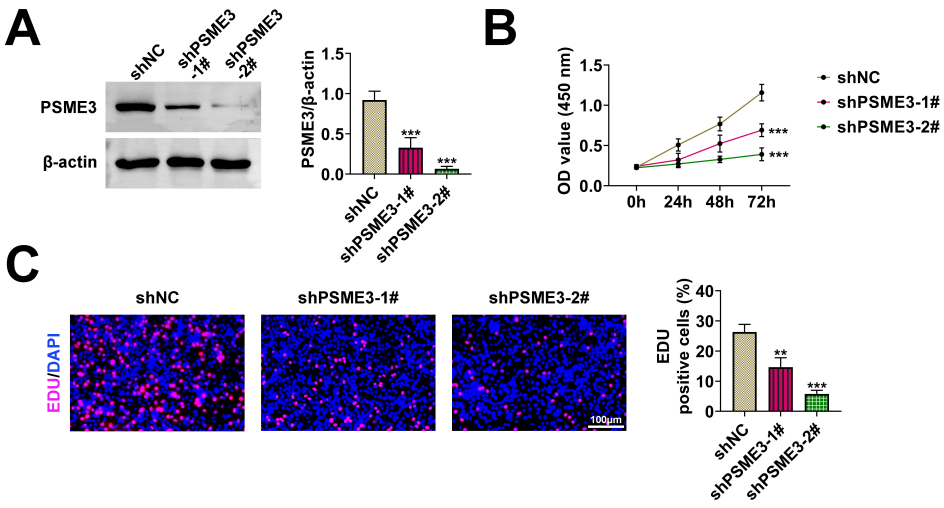

FIGURE 2. *PSME3* accelerated cell growth in GC. (A) PSME3 protein expression was examined after PSME3 knockdown. (B) Cell viability was tested after *PSME3* suppression through CCK-8 assay. (C) Cell proliferation was determined after *PSME3* inhibition through EDU assay. **$p < 0.01$, ***$p < 0.001$. PSME3: Proteasome activator subunit 3; shNC: short hairpin RNA negative control; OD: optical density; EDU: 5-Ethynyl-2′-deoxyuridine; DAPI: 4′,6-diamidino-2-phenylindole.

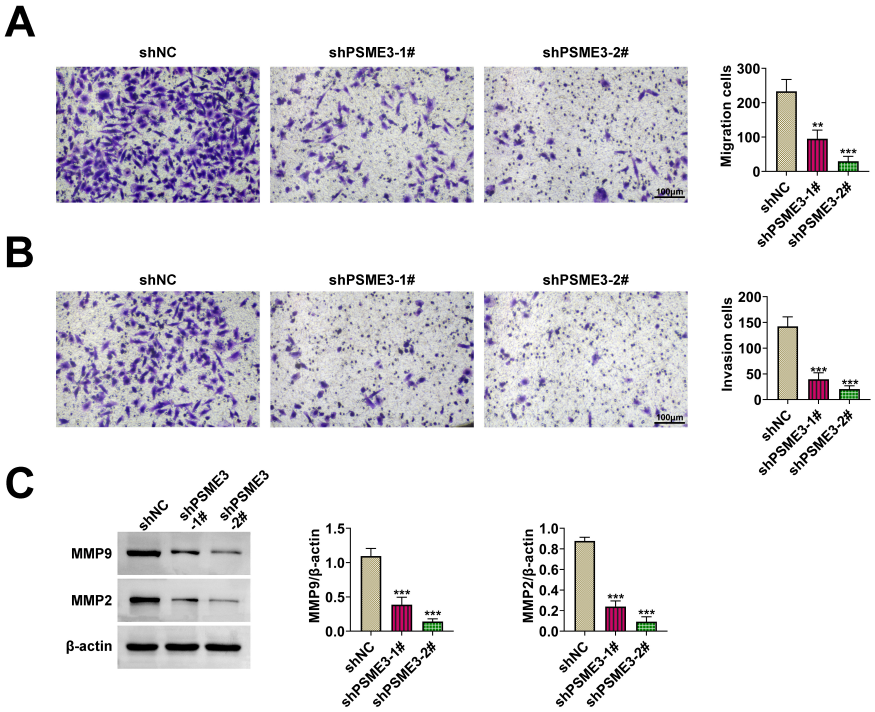

FIGURE 3. ***PSME3* facilitated cell migration in GC.** (A) Cell migration ability was evaluated after *PSME3* silencing through Transwell assay. (B) Cell invasion ability was tested after *PSME3* inhibition through Transwell assay. (C) MMP9 and MMP2 protein expressions were examined after *PSME3* suppression through western blot. $^{**}p < 0.01$, $^{***}p < 0.001$. PSME3: Proteasome activator subunit 3; shNC: short hairpin RNA negative control; MMP: Matrix Metalloproteinase.

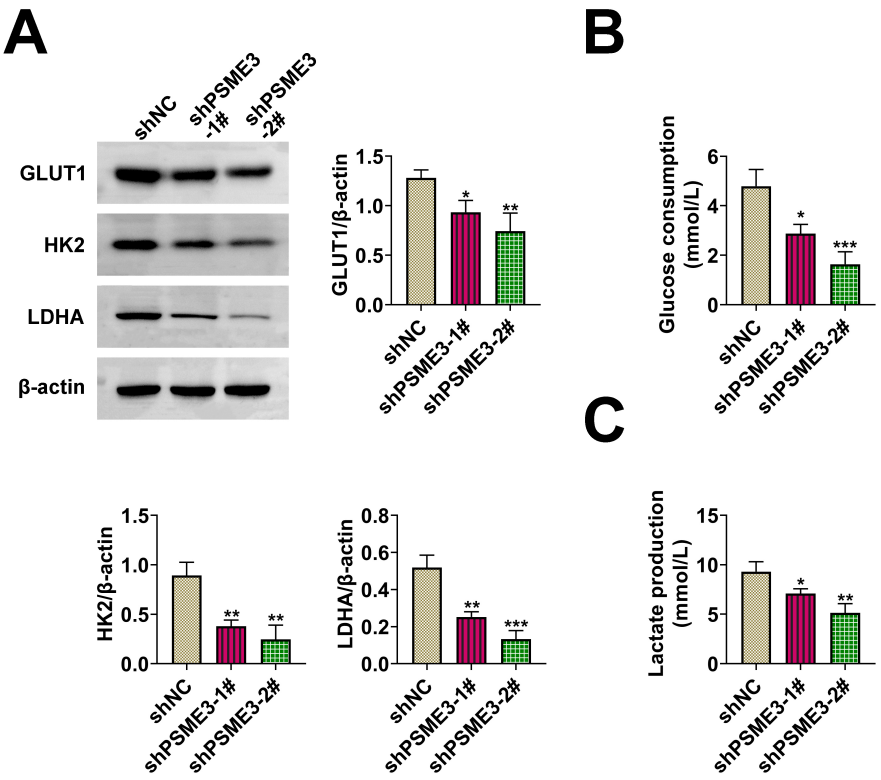

FIGURE 4. ***PSME3* strengthened abnormal glycolysis in GC.** (A) GLUT1, HK2 and LDHA protein expressions were assessed after *PSME3* inhibition through western blot. (B) Glucose consumption was detected after *PSME3* repression using the commercial kit. (C) Lactate production was evaluated after *PSME3* knockdown using the commercial kit. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. PSME3: Proteasome activator subunit 3; shNC: short hairpin RNA negative control; GLUT: glucose transporter; HK: hexokinase; LDHA: Lactate Dehydrogenase A.

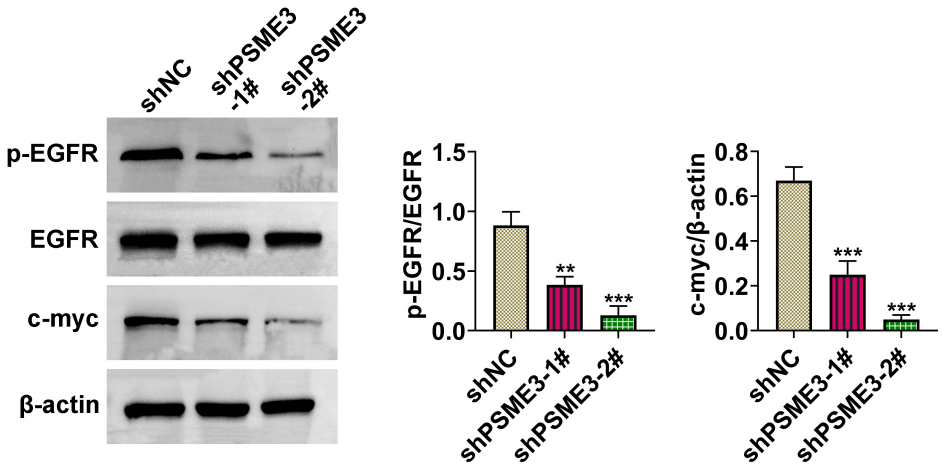

FIGURE 5. ***PSME3* stimulated the EGFR/c-myc pathway.** Protein expressions of p-EGFR, EGFR and c-myc were measured after inhibiting *PSME3* through western blot. $^{**}p < 0.01$, $^{***}p < 0.001$. PSME3: Proteasome activator subunit 3; shNC: short hairpin RNA negative control; EGFR: epidermal growth factor receptor.

4. Discussion

Increasing proteins have been claimed to contribute to GC progression. For example, PRMT1 stimulates the β-catenin pathway in GC to strengthen cell proliferation and metastasis through recruiting MLXIP [13]. Additionally, AFF3 serves as a novel prognostic biomarker that affects immunotherapy in GC [14]. Silencing RACK1 accelerates glutamine addiction and tumor growth by targeting the AKT/mTOR/ASCT2 axis in GC [15]. Besides, NSD1 modulates WNT10B to facilitate cell survival and migration in GC [16]. *PSME3* has been shown to display high expression and aggravate tumorigenesis in cancer progression [9–11]. Importantly, *PSME3* is highly expressed in GC tissues, resulting in a poor prognosis [12]. *PSME3*'s regulatory functions remain dim. In this work, a GEPIA database analysis confirmed upregulation of *PSME3* expression in GC tissues. Our findings suggested that *PSME3* expression was higher in GC, which led to a worse prognosis as a result.

As a metabolic hallmark of cancer, glycolysis refers to the prioritization of glucose conversion into lactate over oxidative phosphorylation to supply energy requirements [17]. Lactate is the end outcome of glycolysis; the accumulation of it is a feature of glycolysis and drives tumor development [18]. Glycolysis progress is implicated in GC progression regulation. Insulin gene enhancer protein 1 (ISL1) modulates glucose transporter 4 (GLUT4) in GC to enhance glycolysis and tumorigenesis [19]. In addition, protein phosphatase 2a (PP2A) retards MYC signaling to attenuate glycolysis in GC [20]. As a consequence, H19 accelerates aerobic glycolysis and immune escape by targeting the miR-519d-3p/lactate dehydrogenase A (LDHA) axis in GC [21]. The hexokinase domain containing protein-1 (HKDC1) aggravates glycolysis and strengthens chemoresistance in GC [22]. Therefore, it is necessary to explore *PSME3*'s regulatory functions in glycolysis in further detail. In this study, *PSME3* was found to accelerate cell growth, migration and abnormal glycolysis in GC.

A critical pathway in cancer progression is the EGFR/c-myc pathway. Gliomas, for example, display a decrease in miR-524 expression to retard the TGFβ/Hippo/Notch pathway following stimulation of the EGFR/c-myc axis [23]. Furthermore, dihydroconiferyl ferulate reduces breast cancer stemness by regulating nuclear EGFR/c-Myc signaling [24]. Yet, *PSME3*'s regulatory effects on the EGFR/c-myc pathway in GC progression are still unclear. In this study, *PSME3* stimulated the EGFR/c-myc pathway.

5. Conclusions

In conclusion, *PMSE3* was demonstrated for the first time to exhibit higher expression, and facilitate glycolysis and migration of GC cells by regulating the EGFR/c-myc pathway. Besides, this study has some limitations, including the need for more experiments on human samples, animal samples and other cytological processes. In the future, the regulatory roles of *PSME3* in GC progression will be further investigated.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

LH and SSX—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. LH, QFY and JCL—supervised the data collection, analyzed the data, interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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