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Original Research

NSD1 stimulated survival and migration of gastric cancer cells through WNT10B

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Abstract

Background and objective: To assess the expression of Nuclear receptor binding SET domain protein 1 (NSD1) in human gastric cancer tissues and cells and investigate its possible role in gastric cancer.

Methods: TCGA database was used to assess the expression levels of NSD1 in human gastric cancer tissues. Immunoblot assays were performed to detect NSD1 expression levels in gastric cancer cell lines. MTT and colony formation assays were conduced to detect its role in the survival of gastric cancer cells. Wound closure and transwell were performed to investigate the effects of NSD1 on the motility of gastric cancer cells. Immunoblot assays were also conducted to confirm its effects on WNT10B/β-catenin pathway.

Results: We found the high expression levels of NSD1 in human gastric cancer tissues and cell lines. NSD1 depletion suppressed the survival and motility of gastric cancer cells. Additionally, we revealed NSD1 activated the WNT10B/β-catenin pathway, therefore promoted gastric cancer progression.

Conclusion: We revealed the high NSD1 expression in gastric cancer tissues and cells, and thought NSD1 could serve as a promising gastric cancer target.

Keywords

Nuclear receptor binding SET domain protein 1 (NSD1); Gastric cancer; Survival; Motility; WNT10B/β-catenin

1. Introduction

Gastric cancer (GC) is one of the most common malignancies in China [1]. Its incidence ranks second with its highest morbidity and mortality. Currently, there is no effective and sensitive method for early detection and diagnosis of GC [2]. Therefore, it is of great significance to further understand the occurrence, development and metastasis of GC. The alterations in the expression and functions of oncogenes and tumor suppressor genes may indicate their important role in GC development [3]. Recently, targeted therapy becomes a promising therapeutic method for GC treatment, and it is urgently needed to identify novel molecular targets for GC treatment.

Nuclear receptor binding SET domain (NSD) proteins contain a family of three methyltransferases, including NSD1, NSD2 and NSD3, which were known as cancer regulators [4]. Among them, NSD1 can regulate gene expression through activating H3K27me3 [5]. Besides, NSD methyltransferase overexpression was found in various cancers, indicating their role as potential cancer diagnosis biomarkers [6]. In particular, NSD1 mutations were found in both head and neck cancer [4, 7]. Additionally, NSD1 expression was increased in metastatic pancreatic ductal carcinoma [8]. However, the possible role of NSD1 in GC progression is still unclear.

It is well-accepted that the alteration of WNT family protein expression is related to human carcinogenesis, targeting WNT signaling was thus considered to be a clinical treatment method for GC [9]. Among them, WNT10B, located on human chromosome 12q13, is a multiple-functional protein [10]. It has been reported that WNT10B could cause the activation of β-catenin transcription factor to participate in tumor development and progression, and is related to

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the down-regulation of cell growth by a mechanism independent of β-catenin [11]. Besides, WNT10B was highly overexpressed in GC tissues, and its knockdown suppressed the survival and migration of GC cells [9]. Interestingly, previous studies have confirmed that NSD1 could regulate the WNT10B signaling pathway in liver cancer and thus affect its progression. However, its effect in GC is still unknown.

Here, NSD1 expression level was found highly in human GC tissues and cell lines, and its roles in GC progression were investigated. The results revealed that NSD1 promoted the survival, migration and invasion of GC cells via WNT10B/β-catenin axis, and therefore indicated NSD1 as a promising target for GC.

2. Materials and methods

2.1 Bioinformatic analysis

Gene Expression Profiling Interactive Analysis (GEPIA) was used to assess NSD1 expression in GC tissues from the TCGA database (Cancer Genome Atlas).

2.2 Reagents, antibodies and siRNAs

Lipofectamine® 2000 was purchased from Invitrogen (Carlsbad, CA, USA). RIPA buffer was purchased from Cell Signaling (9800; Danvers, MA, USA). Crystal violet was purchased from Sigma-Aldrich (332488, St. Louis, MO, USA). Transwell chambers was purchased from Corning Inc. (NY, USA). The human gastric epithelial cell line GES-1 and three types of GC cells, including AGS, MKN45, and HGC-27, were all purchased from ATCC. Anti-NSD1 antibody (1 : 200 for Immunohistochemical (IHC), 1 : 1000 for Immunoblot, ab222145), anti-β-catenin antibody (1 : 2000, ab32572), anti-WNT10B antibody (1 : 1000, ab70816), anti-APC antibody (1 : 200, ab40778), and anti-GAPDH antibody (1 : 3000, ab8245) were all obtained from Abcam, Cambridge, MA, USA. The siRNAs of NSD1 and negative control (NC) were obtained from Riobio.

2.3 Cell culture and transfection

GES-1, AGS, MKN45, and HGC-27 cell lines were maintained in DMEM supplemented with FBS (10%) and incubated at an incubator (37 °C, 5% CO_2). The siRNAs were transfected into GC cells by Lipofectamine® 2000.

2.4 Immunoblot assay

GC cells were lysed with RIPA buffer. Cell and tissue samples were isolated to extract proteins, separated by SDS-PAGE, then transferred to PVDF membranes and subsequently blocked with non-fat milk (5%) in TBST buffer. All PVDF membranes were then treated with primary antibodies (1.5 h). Subsequently, the membranes were incubated with secondary antibodies (1 h). The signal was finally detected and analyzed.

2.5 MTT assays

MTT assay was used for detection of cell survival. After transfection of siRNAs (24 h), cells were incubated with MTT dye (3 h), followed by added with DMSO. The absorbance was captured at 490 nm using an Enzyme Immunoassay Analyzer (Bio-Rad, Hercules, CA, USA).

2.6 Colony formation assays

AGS cells were re-seeded into 6-well plates (1000 cells/well) and maintained for nearly 2 weeks, when the colonies were formation. Then, the colonies were fixed with methanol (-20 °C, 10 min) and stained with crystal violet (0.2%, 20 min). After washing, the stained colonies were then photographed.

2.7 Wound closure assays

Wound healing assay was performed to evaluate the cell migration capacity. 2×10^5 AGS cells with or without transfection were maintained overnight to induce 85% confluent monolayers. The monolayer cells were scratched mechanically with pipette tip (200-μL). The cell debris was washed (PBS), and the complete medium (serum-free medium) was added to induce healing. Wound was photographed at 0th hour and 24th hour after scratch, and cell images were captured and the extent percentage of wound closure was measured.

2.8 Cell invasion assays

Transwell assay was used for cell invasion assay. Briefly, after transfected with siRNAs (24 h), cells were seeded in the upper chamber of Transwell chambers (24-well 8-μm pore). The complete medium was then added into the bottom chamber of the well. After incubation, cells in the bottom of the Transwell membranes were stained using crystal violet (0.2%, 30 min, 37 °C). The invasive cell number in each well were quantified through images that captured using microscope.

2.9 Statistics

For statistical analysis, GraphPad 6.0 was used. Data were represented as mean $\pm$ SEM. Student's t-test was used for comparisons, and $p < 0.05$ was considered as significant.

3. Results

3.1 NSD1 was highly expressed in human GC tissues and cells

To uncover the possible effect of NSD1 on the progression of GC, we investigated NSD1 expression level in GC samples and the corresponding normal samples according to the TCGA database. We noticed the transcript per million of NSD1 in GC tissues was highly expressed than that in the normal samples, suggesting the overexpression of NSD1 in GC tissues (Fig. 1a). Additionally, NSD1 expression in GES-1 cell line, and three types of GC cell lines, including AGS, MKN45, and HGC-27. Through performing immunoblot assays, we found NSD1 was overexpressed in human GC cell

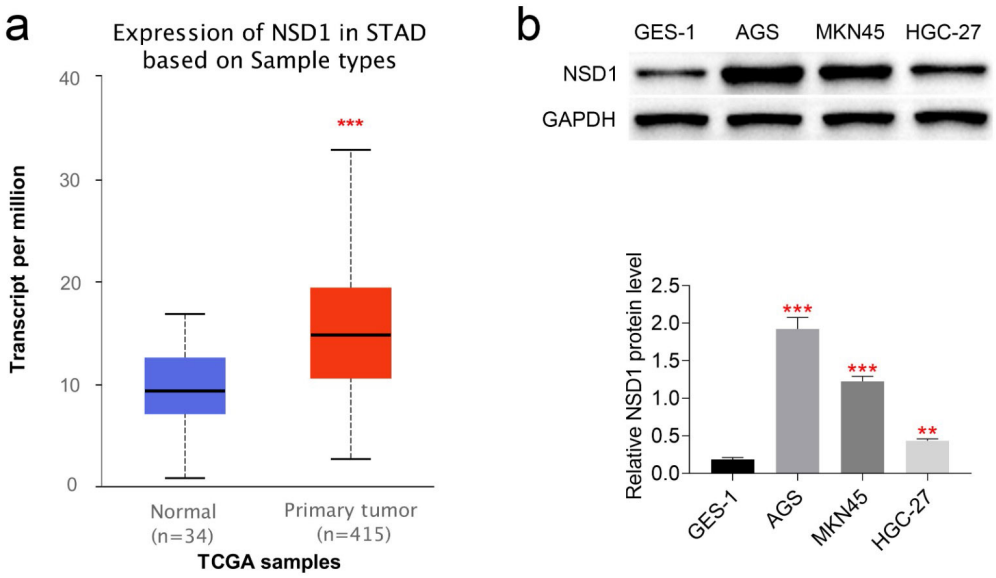

FIG. 1. NSD1 was highly expressed in human GC tissues and cell lines. (a) TCGA database showed the comparison of the transcript per million of NSD1 between human GC tissues and normal tissues. (b) Immunoblot assays revealed that NSD1 was overexpressed in AGS, MKN45, and HGC-27 cell lines than that in GES-1 cell line. Data were presented as mean ± SEM, *** $p < 0.001$.

lines, compared to that in GES-1 cell line (Fig. 1b). Collectively, NSD1 was highly expressed in human GC tissues and cells.

3.2 NSD1 depletion suppressed GC cell survival in vitro

Based on the fact that NSD1 was overexpressed in GC tissues and cells, its role was thus further investigated in GC progression. The siRNAs of NSD1 were transfected into GC cell line AGS to knock down its expression. Si-NC was non-sense siRNAs and used as a negative control. By performing immunoblot assays, decreased NSD1 expression was observed in NSD1 siRNA-transfected AGS cells, compared to that in si-NC group (Fig. 2a).

Subsequently, MTT assays showed that NSD1 depletion suppressed the viability of AGS cells, evidenced by the decreased OD values at 490 nm wavelength (Fig. 2b). The colony formation assays showed that the colony number of AGS cells in NSD1 depletion was obviously decreased, compared to that in si-NC group (Fig. 2c). In conclusion, these data showed that NSD1 depletion suppressed the survival of GC cells *in vitro*.

3.3 Knockdown of NSD1 suppressed the invasion and migration of GC cells

The wound closure assays showed that the ablation of NSD1 dramatically suppressed the wound healing of AGS cells, compared to the si-NC-transfected AGS cells (Fig. 3a). The transwell assays showed that NSD1 depletion suppressed the invasion of AGS cells as compared to the si-NC-transfected AGS cells (Fig. 3b).

3.4 NSD1 activated WNT10B/β-catenin axis in GC cells

In a previous study, NSD1 regulated the progression of hepatocellular carcinoma (HCC) through activating WNT10B-related signaling pathways. Therefore, the following study determined whether NSD1 also affected GC progression through this pathway. The immunoblot assays revealed that depletion of NSD1 decreased the expression of WNT10B in NSD1-depleted AGS cells, compared to the the si-NC-transfected AGS cells (Fig. 4). Additionally, the expression of downstream protein β-catenin was also decreased in NSD1-depleted AGS cells as compared to the si-NC-transfected AGS cells (Fig. 4). Further study showed that the expression of APC, a key factor in the WNT/β-catenin pathway, was upregulated in NSD1-depleted AGS cells as compared to the si-NC-transfected AGS cells (Fig. 4). Collectively, these investigations suggested that NSD1 activated WNT10B/β-catenin axis in GC cells.

4. Discussion

Globally, GC is the fourth most common cancer and the second leading cause of cancer-related deaths [12]. GC also has the highest incidence among all kinds of malignant tumors in China. The early diagnosis and precise treatment for GC become an important medical and social problem [13]. GC is often occult-onset with no specific symptoms, and patients are often in the advanced stage at the time of diagnosis [14]. Moreover, GC has high metastasis, and traditional radiotherapy and chemotherapy have little effects [15]. For GC, targeted therapy is undoubtedly emerged as a promising method, which need to be identified to effectively improve patient outcomes. In this study, NSD1 was highly expressed in human GC tissues and cells, indicating its potential role

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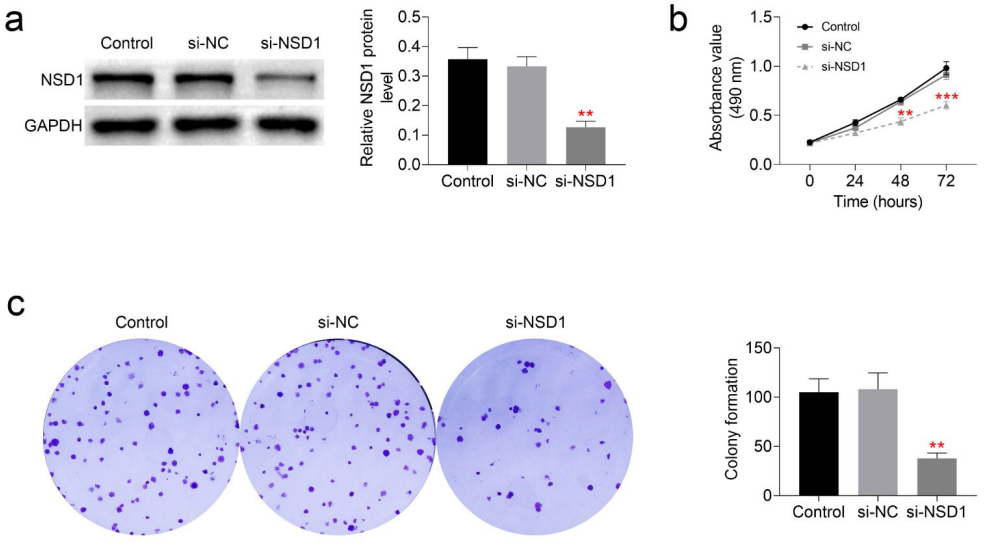

FIG. 2. NSD1 depletion suppressed the GC cell survival *in vitro*. (a) Immunoblot assays showed that NSD1 expression was decreased in NSD1 siRNA-transfected AGS cells, compared to the si-NC-transfected AGS cells. (b) MTT assays showed the survival capacity of AGS cells that transfected with NSD1 siRNA, control, or si-NC. (c) Colony formation assays revealed the AGS cell formation in cells transfected with NSD1 siRNA, control, or si-NC. Data were presented as mean ± SEM, ** $p < 0.01$ and *** $p < 0.001$.

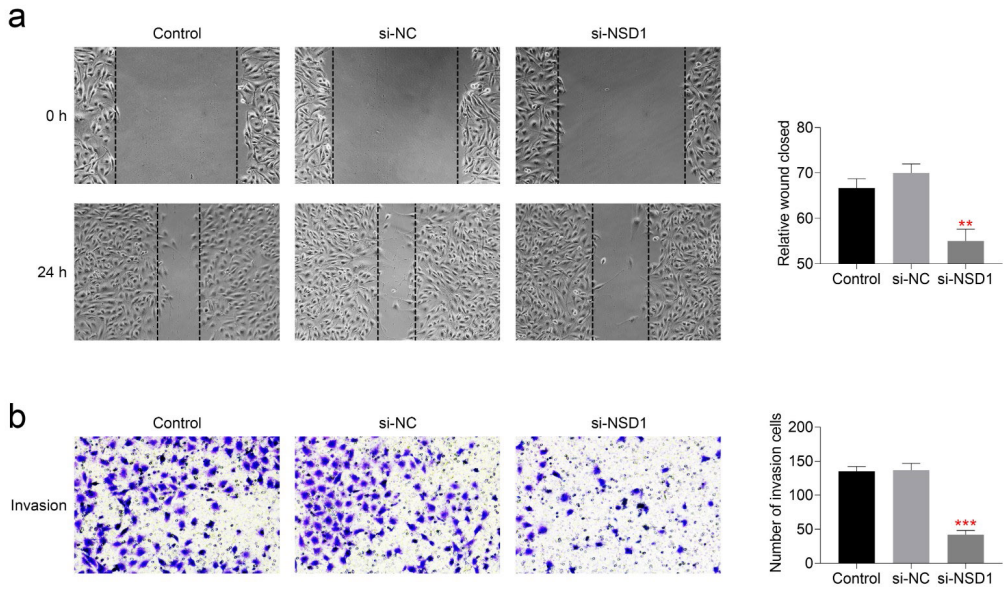

FIG. 3. The knockdown of NSD1 suppressed the migration and invasion of GC cells. (a) Wound closure assays revealed the migratory ability of AGS cells transfected with NSD1 siRNA, control, or si-NC. (b) Transwell assays revealed the invasive ability of AGS cells transfected with NSD1 siRNA, control, or si-NC. Data were presented as mean ± SEM, ** $p < 0.01$ and *** $p < 0.001$.

in the progression of GC. Further studies showed that NSD1 affected the survival, invasion and migration of GC cells. Therefore, this study indicated that NSD1 could serve as a promising target for GC treatment.

MTT and colony formation assays showed that NSD1 siRNAs suppressed the survival of GC cells. The wound healing and transwell assays showed the suppressive effects of NSD1 siRNAs on the migration and invasion of GC cells *in vitro*. These results therefore concluded that NSD1 affected the progression of GC. In fact, the important role of NSD1 in the progression and development of multiple types of tumors has been widely revealed [16]. For example, NSD1 suppressed HCC progression via the NSD1/WNT10B pathway [17]. NSD1 was also highly expressed in pancreatic ductal adenocarcinoma tissues and correlated with its prognosis [8]. In addition, NSD1 inactivation defined a type of DNA hypomethylated subtype in squamous cancer [18]. NSD1 damaging mutations also defined a type of laryngeal tumors with favorable prognosis [19]. These studies showed the key role of NSD1 in cancer progression.

Notably, the multiple biological functions of NSD1 have been previously reported. For example, NSD1-

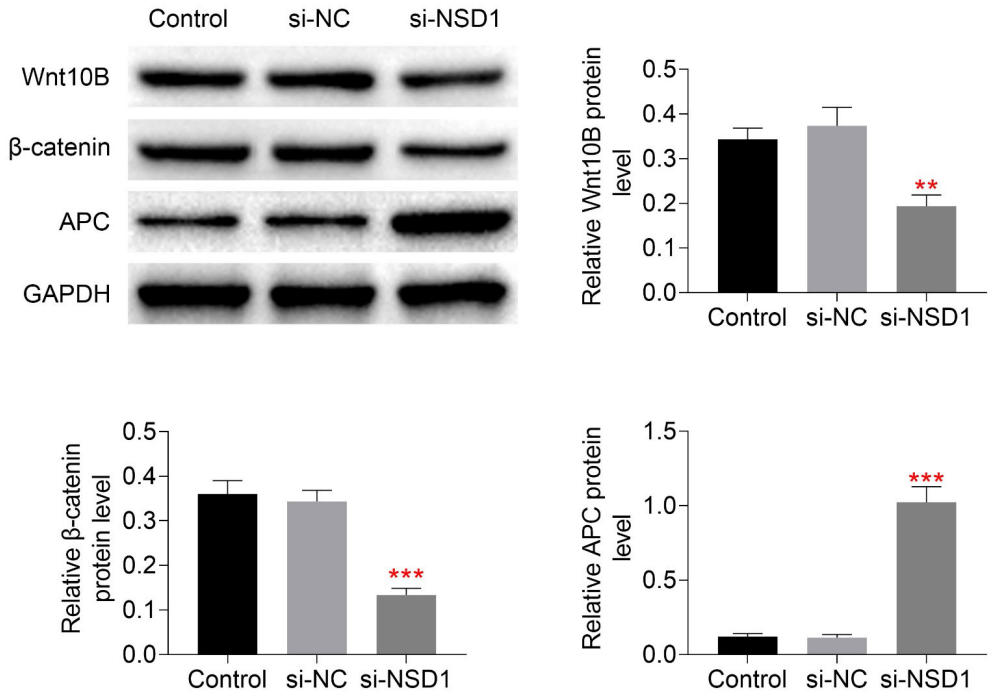

FIG. 4. NSD1 activated WNT10B/β-catenin axis in GC cells. Immunoblot assays revealed the expression of WNT10B, β-catenin, and APC in AGS cells transfected with NSD1 siRNA, control, or si-NC. Data were presented as mean $\pm$ SEM, ** $p < 0.01$ and *** $p < 0.001$.

deposited H3K36me2 directed de novo methylation in the male germline and counteracted Polycomb-associated silencing of mice [20]. NSD1 inactivation also impaired GATA1-regulated erythroid differentiation and led to the erythroleukemia [21]. Importantly, in embryonic stem cells, NSD1 demarcated PRC2-mediated H3K27me2 and H3K27me3 domains [22]. In this study, whether NSD1 stimulated the methylation of H3K36 and H3K27 and thus participated in the progression of GC needs further clarification.

It was widely reported that WNT10B expression was closely correlated with human carcinogenesis [23]. WNT10B can regulate WNT/β-catenin pathway, and it affected the expression of multiple types of downstream proteins including APC [24]. WNT10B affected the progression of multiple types of cancers, including GC [9]. It has also been reported that WNT10B affected the survival, migration, apoptosis, and EMT of GC cells [9]. In liver cancer, NSD1 was highly expressed, and knockdown of NSD1 could inhibit the cell proliferation, migration and invasion, and inhibited the expression of WNT10B [24]. NSD1 could also affect the progression of HCC through WNT10B [25]. Therefore, we speculated that, in GC, NSD1 may also inhibit the progression of GC through this signaling pathway. This hypothesis was verified by the western blotting results. Here, we found NSD1 activated the expression of WNT10B, and further affected β-catenin expression and APC, indicating that NSD1 promoted GC progression via this pathway. Thus, developing the inhibitors of WNT/β-catenin pathway may be a promising manner for GC treatment.

In conclusion, we found NSD1 was highly expressed in GC cells and tissues. The following results revealed that NSD1 promoted the survival and motility of GC cells via activating WNT/β-catenin pathway. This study therefore indicated that NSD1 could be served as a promising target for GC treatment.

Author contributions

BL and YH designed the study, supervised the data collection, BL analyzed the data, interpreted the data, YH prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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