

ORIGINAL RESEARCH

Knockdown of B3GNT3 inhibits colon cancer cell growth and migration

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Abstract

Colon cancer (CC) ranks among the most common malignant tumors globally. Providing theoretical support is essential to controlling CC and improving patients' prognoses. Beta-1,3-N-acetylglucosaminyltransferase 3 (B3GNT3), a protein-coding gene that encodes a member of the B3GNT family, affects multiple tumors. However, B3GNT3 remains largely unknown in terms of its role and mechanism in CC development and metastasis. This study explored B3GNT3's effect on CC progression. We revealed that B3GNT3 is highly expressed in human CC tissues. B3GNT3 depletion suppressed CC cell growth. Knocking down B3GNT3 restrained CC cells' motility. Mechanically, B3GNT3 knockdown blocks the nuclear factor kappa-B (NF- κ B) pathway in CC cells. In summary, B3GNT3 depletion restrained CC cells' growth and motility by targeting NF- κ B pathway. As a potential target for CC, B3GNT3 appears promising.

Keywords

Colon cancer (CC); Beta-1,3-n-acetylglucosaminyltransferase 3 (B3GNT3); Growth; Motility; NF- κ B pathway

1. Introduction

Colon cancer (CC) is one of the most prevalent malignant tumors worldwide. Early symptoms of CC are not obvious, so most patients are in the middle or late stages at the time of diagnosis [1, 2]. Further complicating matters, patients with CC often suffer recurrences and distant metastases, resulting in a survival rate of less than 50% [3]. This poses a major threat to patients' lives. CC manifests as a complex process involving the activation of oncogenes and the suppression of tumor suppressor genes. A comprehensive study of CC's pathogenesis, targeting early detection and prognostic markers, will aid in controlling CC's progress and improving patient prognosis.

B3GNT3 is an individual of the B3GNT family of protein-coding genes [4]. B3GNT3 triggers and progresses human malignant tumors. Overexpression of B3GNT3 overexpression promotes pancreatic cancer tumor progression and inhibits T cell infiltration [5]. B3GNT3 promotes cell growth, motility by regulating RhoA/RAC1 pathway-related markers, thus becoming a carcinogen of endometrial cancer [6]. B3GNT3 knockout can inhibit lung cancer cell growth and invasion *in vitro* [7]. In gynecological cancers, B3GNT3 silencing inhibits NF- κ B signaling activation [8]. However, remains unknown in terms of its potential role and mechanism in the development and metastasis of CC.

Nuclear factor- κ B transcription factor family regulates gene transcription and controls cell apoptosis and proliferation [9]. In most normal cells, NF- κ B remains inactive and resides in the cytoplasm, where it can be actively activated by tumor

necrosis factor- α (TNF- α) [10]. By inhibiting E-cadherin expression and regulating snail and zinc finger E-box-binding homeobox protein (ZEB) mRNA expression, NF- κ B enhances epithelial-mesenchymal transition (EMT) [11]. However, it is still unclear what role B3GNT3 plays in CC.

This study aimed to explore B3GNT3's role in CC progression and uncover its mechanism. Our findings demonstrated that B3GNT3 knockdown inhibits colon cancer cell growth and migration. B3GNT3 is therefore a possible target for CC.

2. Materials and methods

2.1 Samples

From patients undergoing surgical resection at our hospital, samples of colon cancer tissue sample and matched normal adjacent tissue were collected.

2.2 The Cancer Genome Atlas (TCGA) analysis

Preprocessing and extraction of data were performed using the TCGABiolinks package in R software (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

2.3 Cell culture and transfection

Human colon cancer cell line HCT116 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA, Catalog No. CCL-247). Using a CO₂ incubator (Model 3111, Thermo Fisher Scientific, Waltham, MA, USA), cells

were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island, NY, USA, Catalog No 11965-092), supplemented with 10% fetal bovine serum (FBS) (Gibco, Catalog No. 10082-147, Logan, UT, USA) and 1% penicillin-streptomycin (Gibco, Catalog No. 15140-122, Logan, UT, USA) at 37 °C in a humidified atmosphere containing 5% CO₂. Lipofectamine® 2000 (Catalog No. 11668019, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used according to the manufacturer's protocol to transfect siRNAs of negative control (NC) and B3GNT3 into CC cells.

2.4 5-Ethynyl-2'-deoxyuridine (Edu) assay

Incubation of HCT116 cells with Edu agent (C10337, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) for 2 h was followed by removal of the Edu agent. Fluorescence microscope (LSM710, Zeiss, Oberkochen, German) was used to photograph the cells.

2.5 Apoptosis assay

Cells were washed twice with cold phosphate-buffered saline (PBS, Catalog No. 10010-023, Gibco, Grand Island, NY, USA) and then resuspended in the 1× binding buffer provided in the kit. To each 100 liters of cell suspension, 5 μL of Annexin V-Fluorescein Isothiocyanate (FITC) and 5 μL of Propidium Iodide (PI, Catalog No. P3566, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The mixture was vortexed gently and incubated in the dark at room temperature for 15 minutes. After incubation, 400 μL of 1× binding buffer was added to each sample, which were analyzed using a FACSCalibur flow cytometer (BD Biosciences, Model: FACSCalibur, San Jose, CA, USA). Data were acquired and analyzed using CellQuest Pro software (5.1, BD Biosciences, Franklin Lake, NJ, USA).

2.6 Transwell assay

HCT116 cells were plated into the upper transwell chambers (3422, Corning Costar, Corning, NY, USA) with 20% matrigel (354234, BD Biosciences, San Jose, CA, USA) in culture medium. Staining and fixing were performed after 24 h after removal of cells in the upper layer.

2.7 Wound-healing assay

HCT116 cells were scratched with a 10-μL pipette tip, then washed twice. To determine the extent of wound closure, images were captured at 0 and 24 h.

2.8 Immunoblot

Using a Bicinchoninic Acid (BCA) Protein Assay Kit (Catalog No. 23225, Thermo Fisher Scientific, Waltham, MA, USA), protein concentrations were determined. Equal amounts of protein (30 μg) were separated by 10% Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto Polyvinylidene Fluoride (PVDF) membranes. After incubation with 5% Bovine serum albumin (BSA) in Tris Buffered Saline with Tween (TBST) for 1 h, PVDF was incubated with antibodies targeting E-cadherin (1:1000, ab231303,

Abcam, Cambridge, UK), Vimentin (1:500, ab92547, Abcam), Snail (1:500, ab216347, Abcam), anti-nuclear factor kappa B suppressor protein alpha (IκBα) (1:1000, sc-203, Santa Cruz, Finnell Street Dallas, TX, USA), anti-p-IκBα (Tyr42, 1:500, sc-101714, Santa Cruz), p65 (1:500, ab16502, Abcam), p-p65 (1:500, ab76302, Abcam), and anti-β-actin (1:3000; ab8226, Abcam) at 4 °C overnight. After washing for 15 minutes, a 1:1000 ratio of Horseradish peroxidase (HRP)-conjugated secondary antibodies was used to resolve membranes for 2 h. Signals were detected using Enhanced chemiluminescence (ECL) detection kit.

2.9 Statistics

Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). For triplicate experiments, data are presented as mean ± standard deviation (SD). Student's *t*-test were used to compare groups, or comparisons between multiple groups, one-way Analysis of Variance (ANOVA) was used followed by Tukey's *post-hoc* test for pairwise comparisons. Two-way ANOVA was used to assess the interaction and main effects of experiments involving more than two groups and multiple variables. *p* < 0.05 indicates statistically significant. Triplicates of all experiments were conducted.

3. Results

3.1 B3GNT3 is highly expressed in human CC tissues

B3GNT3's effects on CC progression were first investigated by investigating its expression in CC tissues. According to the TCGA database, B3GNT3 transcripts per million (TPM) are high in CC tissues (Fig. 1a), indicating high expression. Quantitative Real-time Polymerase chain reaction (qPCR) assays also confirmed high B3GNT3 mRNA levels in CC tissues compared to normal tissues (Fig. 1b). Immunoblots also revealed high B3GNT3 expression in 5 representative CC tissues (Fig. 1c). Analysis of clinical characteristics of CC patients revealed a correlation between B3GNT3 expression and metastasis of tumors (*p* < 0.001, Table 1), as well as clinical stage (*p* = 0.002, Table 1). Accordingly, B3GNT3 is highly expressed in human CC tissues.

3.2 The depletion of B3GNT3 suppressed CC cell growth

HCT116 cells were used as a cell model to investigate B3GNT3's role in CC progression. B3GNT3 siRNAs were then transfected into HCT116 cells to deplete its expression. Compared to si-NC transfection, transfection of B3GNT3 siRNAs effectively decreased its expression in HCT116 cells, according to immunoblots (Fig. 2a). Edu assays were also performed to investigate B3GNT3's effects on cell growth. Interestingly, a decrease in Edu-positive cells was observed after its depletion in HCT116 cells, with the decreased percentage of Edu-positive cells (Fig. 2b). In flow cytometry (FCM) assays, we observed that B3GNT3 depletion stimulated HCT116 cells' apoptosis, suggesting cell

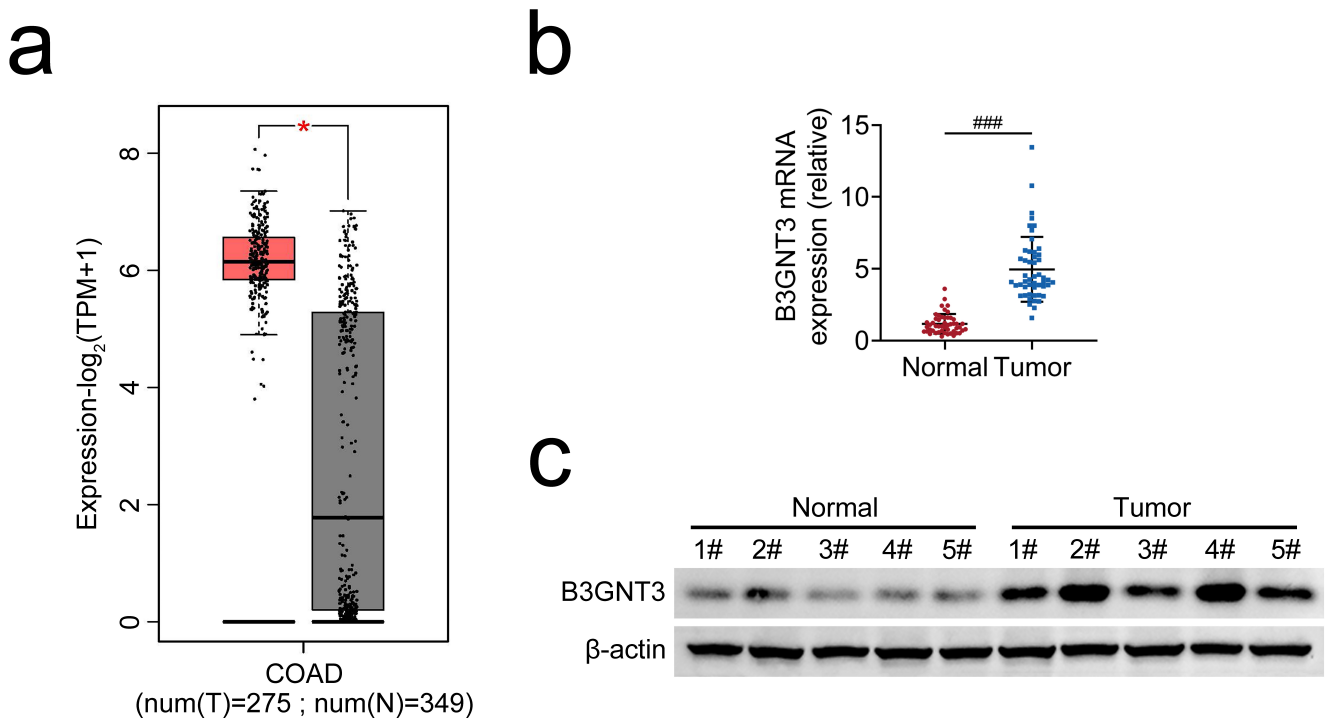


FIGURE 1. B3GNT3 is highly expressed in human CC tissues. (a) TCGA database indicated the transcripts per million (TPM) value of B3GNT3 in 349 tumor tissues and 275 normal tissues. $*p < 0.05$. (b) qPCR assays showed B3GNT3 mRNA levels in 30 CC tissues and 30 normal tissues. (c) Immunoblot assays indicated B3GNT3 expression levels in 5 representative tumor and normal tissues. The assay was repeated 3 times. $###p < 0.001$. CC: colon cancer; B3GNT3: Beta-1,3-n-acetylglucosaminyltransferase 3; COAD: Colorectal adenocarcinoma.

TABLE 1. Clinical characteristics of colon cancer patients.

Characteristic	B3GNT3 (-)	B3GNT3 (+)	<i>p</i> value
Age (yr)			
>60	12	10	0.945
<60	15	13	
Degree of tissue differentiation			
High	17	12	0.389
Moderate	7	5	
Low	3	6	
Metastasis			
Yes	10	20	<0.001
No	17	3	
Clinical stage			
I + II	22	9	0.002
III + IV	5	14	

B3GNT3: Beta-1,3-n-acetylglucosaminyltransferase 3.

apoptosis stimulation (Fig. 2c). Therefore, B3GNT3 depletion inhibited CC cell growth.

3.3 B3GNT3 knockdown restrained CC cells' motility

After transfection of siRNAs into CC cells, B3GNT3 was tested for its effects on motility. B3GNT3 depletion decreased HCT116 cell invasion numbers through transwell, suggesting a block in cell invasion (Fig. 3a). B3GNT3 ablation affected cell migration *via* wound healing assays. Depletion of B3GNT3 inhibits HCT116 cells' migration, resulting in a decreased migration ratio (Fig. 3b). Immunoblot assays further indicated increased E-cadherin expression and decreased Snail expression, suggesting cell motility suppression in HCT116 cells (Fig. 3c). Therefore, B3GNT3 knockdown restrained CC cells' motility.

3.4 Knocking down B3GNT3 blocked the NF- κ B pathway in CC cells

A possible mechanism underlying B3GNT3 depletion suppressing CC progression was then investigated *in vitro*. We detected the effects of its depletion on the NF- κ B axis, which is important in controlling cell motility and growth, through Immunoblotting. Interestingly, we noticed that B3GNT3 ablation decreased p65 and I κ B α phosphorylation levels, increased I κ B α expression, suggesting suppression of NF- κ B axis in CC cells (Fig. 4). So, knocking down B3GNT3 blocked the NF- κ B axis in CC cells.

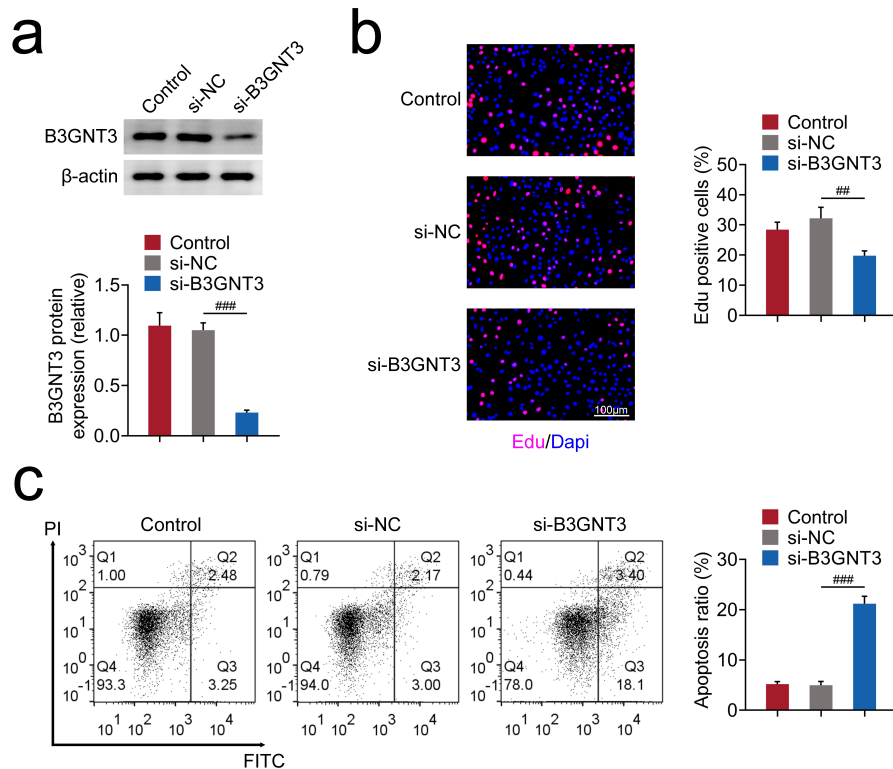


FIGURE 2. The depletion of B3GNT3 suppressed CC cell growth. (a) Immunoblot assays indicated B3GNT3 expression levels upon the transfection of B3GNT3 and NC siRNAs for 24 h in HCT116 cells. (b) Edu assays showed transfecting HCT116 cells with B3GNT3 and NC siRNAs for 24 h, resulted in cell growth. Edu-positive cells percentage was quantified. Scale bar, 100 μm . The assay was repeated 3 times. (c) FCM assays demonstrated apoptosis levels in HCT116 cells after transfection with B3GNT3 and NC siRNAs for 24 h. The quantification of apoptosis cell percentage was conducted. The assay was repeated 3 times. $\#\#p < 0.01$, $\#\#\#p < 0.001$. CC: colon cancer; NC: negative control; B3GNT3: Beta-1,3-n-acetylglucosaminyltransferase 3; Edu: 5-Ethynyl-2'-deoxyuridine; PI: Propidium Iodide; FITC: Fluorescein Isothiocyanate.

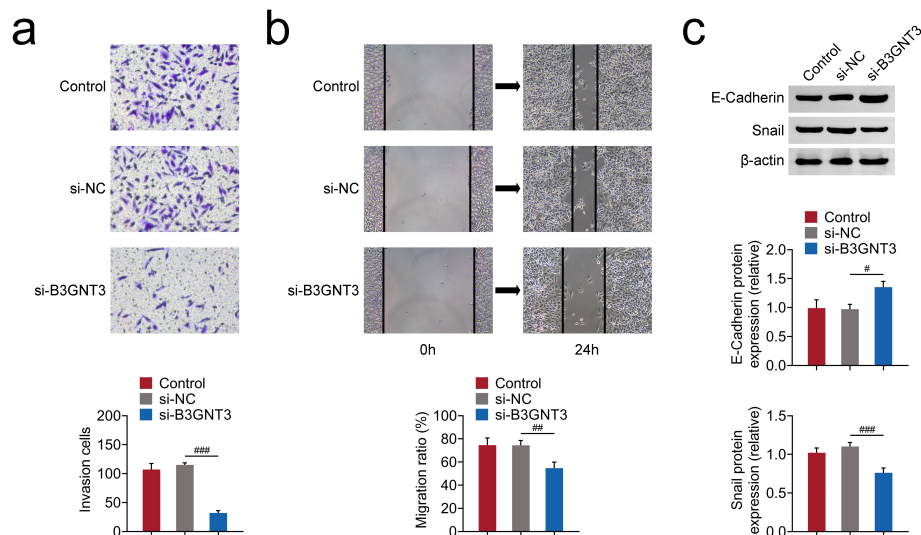


FIGURE 3. B3GNT3 knockdown restrained CC cells' motility. (a) Transwell assays demonstrated HCT116 cells invasion levels upon transfection of B3GNT3 and NC siRNAs for 24 h. Invasive cell numbers were counted. The assay was repeated 3 times. (b) Wound healing assays showed the migration levels of HCT116 cells upon the transfection of B3GNT3 and NC siRNAs for 24 h. Migration ratio was measured. The assay was repeated 3 times. (c) Immunoblot assays indicated E-cadherin and Snail expression level after transfection of B3GNT3 and NC siRNAs for 24 h in HCT116 cells. The assay was repeated 3 times. $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.001$. CC: colon cancer; NC: negative control; B3GNT3: Beta-1,3-n-acetylglucosaminyltransferase 3.

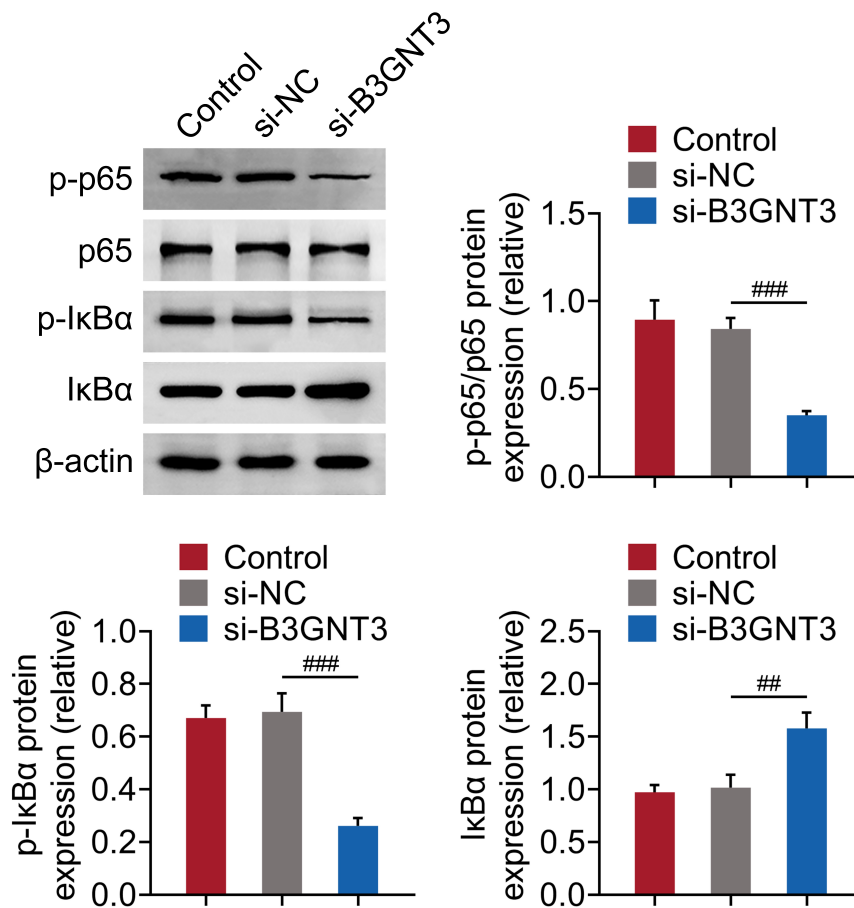


FIGURE 4. Knocking down B3GNT3 blocked the NF- κ B pathway in CC cells. Immunoblot assays indicated p65 and I κ B α phosphorylation levels after transfection of B3GNT3 and NC siRNAs for 24 h in HCT116 cells. Phosphorylation levels of the indicated proteins were quantified. The assay was repeated 3 times. $##p < 0.01$, $###p < 0.001$. CC: colon cancer; NC: negative control; B3GNT3: Beta-1,3-n-acetylglucosaminyltransferase 3; I κ B α : nuclear factor kappa B suppressor protein alpha.

4. Discussion

Globally, colon cancer is the most common type of malignant tumor of the digestive system, with a high incidence and mortality rate worldwide, especially in developed countries. Risk factors that contributed to CC include aging, family history, genetic predisposition, and lack of exercise. Surgery, chemotherapy, and radiotherapy are the main treatments. Targeted therapies such as epidermal growth factor receptor inhibitors and angiogenesis inhibitors are commonly used in advanced or metastatic CC. Various molecular mechanisms and signaling pathways are involved in CC development, such as the Wnt/ β -catenin signaling pathway and p53 gene mutations [12, 13]. Many patients are still ineffective or develop resistance to existing treatments despite certain effects. It is therefore crucial to develop new treatments and discover new targets to improve CC patients' prognosis. A constant state of innovation and research is necessary to research and treat CC. Importantly, our findings demonstrated that B3GNT3 is highly expressed in CC, and B3GNT3 knockdown blocks both CC cell growth and motility. Therefore, it could be a promising target for CC. A glycosyltransferase, B3GNT3 catalyzes the transfer of specific sugar moieties in the biosynthesis of cell surface glycoproteins and glycolipids. Cell signaling, adhe-

sion, and immune recognition are impacted by its expression in various cell types. Different pathological states are associated with aberrant B3GNT3 expression, including inflammation, autoimmune diseases, and cancer [14]. B3GNT3 expression is closely related to the development, progression and prognosis of cancers. For instance, several cancers exhibit increased tumor growth, invasion, and metastasis after overexpression of B3GNT3, possibly through the regulation of cell surface glycan structures and influencing the interactions between tumor cells and the microenvironment [15, 16]. Oncology considers B3GNT3 as a potential biomarker and therapeutic target. Studies on its expression and mechanisms in tumors contribute to a deeper understanding of cancer molecular mechanisms and to the development of new anti-tumor therapies.

Cell proliferation, migration, and invasiveness are closely associated with increased B3GNT3 expression in cancer [17]. In pancreatic and lung cancers, overexpression of B3GNT3 modulates the tumor microenvironment. In turn, this promotes intercellular interactions between tumor cells and their surroundings by altering cell surface glycan structures, accelerating tumor growth [18].

This study examined how B3GNT3 influences CC development by modulating the NF- κ B signaling pathway. It is a crucial pathway in cancer biology, as it plays a role in regulating

inflammatory responses, immune responses, and cell growth and apoptosis. CC cells' growth and migration capabilities were significantly reduced by B3GNT3 inhibition, suggesting that B3GNT3 influences the cells' biological characteristics through the NF- κ B pathway.

NF- κ B pathway is a crucial cell signaling pathway for regulating inflammation, immune responses, cell growth, apoptosis, and regeneration [19]. In colorectal cancer, the activation of the NF- κ B pathway is closely associated with tumor development and progression. By inducing anti-apoptotic genes, cell cycle-related genes, and growth factors, it supports tumor growth and survival. Also, it promoted the formation of inflammatory environments, which are favorable for tumor growth. In regards to motility, the NF- κ B pathway plays a significant role by regulating Matrix metalloproteinases (MMPs) expression, enhancing extracellular matrix degradation, and increasing tumor cell motility [20]. Tumor metastasis is also promoted by its effect on tumor cells and the surrounding microenvironment. Consequently, this pathway is crucial to CC growth and motility, making it a potential therapeutic target. Importantly, this study showed that knockdown of B3GNT3 suppressed CC cell growth and motility *via* targeting this pathway. The precise mechanism, however, requires further study.

While this study supports B3GNT3's potential role in CC progression, several limitations are present. Firstly, this study relies primarily on *in vitro* cell models, which may not accurately reflect *in vivo* conditions. Secondly, the molecular mechanism of the NF- κ B pathway that underlies B3GNT3 inhibition has not yet been comprehensively explored. This study's small sample size may affect statistical power and generalizability. The mechanism and therapeutic potential of B3GNT3 should be confirmed in a broader range of clinical samples in future studies.

5. Conclusions

In summary, knockdown of B3GNT3 blocks CC cell growth and motility. As a potential target for CC, B3GNT3 appears promising.

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

RTA—designed the study and carried them out. RTA, SP—supervised the data collection, analyzed the data, interpreted the data, prepared 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

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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