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Research Article

The Expression and Role of MAN2A1 in Triple-negative Breast Cancer

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Abstract

Background: Triple-negative breast (TNBC) cancer responds poorly to surgery, radiotherapy, chemotherapy, and endocrine therapy, it is considered the subtype of breast cancer with the worst prognosis. This study investigated MAN2A1's role in regulating the proliferation, invasion, and migration of TNBC cells and its clinical significance. *Methods*: We enrolled 220 TNBC patients treated at our institution from January 2020 to December 2022. MAN2A1 protein expression was detected, and its correlation with TNBC clinicopathological features was analyzed. Western blot and immunohistochemistry measured MAN2A1 protein levels. CCK-8, colony formation, and EdU assays evaluated MAN2A1's impact on TNBC cell proliferation. Wound healing and Transwell assays assessed its effects on migration and invasion. *Results*: MAN2A1 protein expression positively correlated with TNBC malignancy. MAN2A1 is overexpressed in larger tumors (≥2 cm), lymph node metastasis-positive cases, and advanced-stage (III-IV) patients. Functional assays demonstrated that MAN2A1 overexpression promoted TNBC cell growth, clonogenicity, migration, and invasion, while its knockdown suppressed these processes. *Conclusion*: This study systematically elucidates the role of MAN2A1 as a key glycosylation-modifying enzyme in promoting the malignant progression of TNBC. The experimental results demonstrate that MAN2A1 drives TNBC development by enhancing cellular proliferation, invasive capacity, and migratory potential, providing a theoretical basis for developing targeted therapeutic strategies against MAN2A1.

Keywords

Triple-negative Breast Cancer, MAN2A1, Proliferation, Invasion, Metastasis

1. Introduction

With its incidence rising annually, breast cancer has become the most common cancer among Chinese women. According to statistics, the incidence of breast cancer is growing at an annual rate of 2%-3%, making it one of the most rapidly increasing malignant tumors [1-3]. Among these, triple-negative breast cancer represents a specific subtype accounting for approximately 12-17% of cases. This subtype is characterized by the absence of ER, PR, and HER2 expression. Notorious for its aggressiveness, poor prognosis, and limited treatment options (lacking endocrine and targeted therapies), this subtype has become a global research focus [4].

Glycosylation, a crucial post-translational modification of proteins in eukaryotic cells, is involved in complex biological processes and is closely related to cell membrane recognition, intracellular substance transport, signal transduction, and cell migration [5]. Aberrant glycosylation has been reported in

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many tumors, and studies suggest that tumor-associated glycans or glycoproteins resulting from altered glycosylation can serve as biomarkers, helping predict tumor progression and clinical outcomes [6].

MAN2A1 is a glycosidase located in the Golgi apparatus of eukaryotes that is essential for N-glycan biosynthesis [7-9]. Studies show that N-glycans regulate E-cadherin-mediated abnormal cell adhesion in tumors, leading to tumor invasion and metastasis [10]. MAN2A1 plays a central role in the N-glycosylation pathway and may serve as a potential therapeutic target for triple-negative breast cancer [11]. This study reveals that MAN2A1 drives the malignant progression of triple-negative breast cancer.

2. Materials and Methods

2.1. Study Subjects

We retrospectively enrolled 220 consecutive breast cancer patients who received treatment at our hospital between January 2020 and December 2022. All cases were confirmed as triple-negative breast cancer through immunohistochemical analysis. Patients' age range was 38–69 years, with a mean age of 52.35 ± 4.39, mean gravidity of 2.69 ± 0.35, and mean parity of 1.23 ± 0.19. The cohort included 118 postmenopausal (53.6%) and 102 premenopausal women (46.4%). Inclusion criteria: 1) Histopathological confirmation diagnosis after surgical resection; 2) Complete clinical data; 3) Written informed consent obtained. Exclusion criteria: 1) Concurrent other cancers; 2) Poor compliance; 3) Other comorbidties.

2.2. Materials

The MDA-MB-231 tumor cell line was sourced from the Shanghai Cell Bank of the Chinese Academy of Sciences; fetal bovine serum and DMEM medium were obtained from GIBCO (USA); MAN2A1, OGT, and β-actin antibodies were procured from CST (USA); the EdU kit was purchased from Guangzhou RiboBio Co., Ltd.; tissue specimens were obtained from the Second Affiliated Hospital of Xuzhou Medical University.

3. Methods

3.1. Immunohistochemical Staining

Paraffin-embedded tissue sections were dewaxed and hydrated, followed by antigen retrieval in citrate buffer. Primary antibodies were added and incubated overnight at 4 ℃, followed by secondary antibody incubation at room temperature for 1 h. DAB was applied for chromogenic detection, and sections were counterstained with hematoxylin. Differentiation was performed with hydrochloric acid-alcohol for a few seconds, followed by bluing with saturated lithium carbonate solution. Dehydration was performed using an alcohol gradient, and sections were cleared in xylene and mounted with neutral resin for imaging under a Zeiss microscope.

3.2. Western Blot

Total protein was extracted from homogenized tissue samples, and protein concentration was measured using the BCA method. Equal amounts of protein were subjected to SDS-PAGE electrophoresis, transferred to PVDF membranes, and blocked with goat serum. Primary antibodies were incubated overnight at 4 ℃. After washing, secondary antibodies were added and incubated at room temperature for 2 h. Enhanced chemiluminescence (ECL) substrate was applied, and images were captured using a Bio-Rad imaging system.

3.3. CCK-8 Assay

Cells were seeded in 96-well plates at 5,000 cells per well, and 10 μL of CCK-8 working solution was added to each well. After mixing, the plates were incubated in the dark for 1 h, and absorbance readings were taken at 450 nm using a microplate reader.

3.4. Colony Formation Assay

Five hundred tumor cells were seeded in six-well plates. After three weeks, the medium was discarded, and cells were fixed with 4% paraformaldehyde, permeabilized with 20% methanol, and stained with crystal violet. Colony counts were recorded under an inverted microscope.

3.5. EdU Assay

Cells were seeded in 96-well plates and cultured until the logarithmic growth phase. Click-iT® EdU Apollo reaction cocktail was added and incubated for 2 h, followed by fixation with 4% paraformaldehyde in PBS. Cells were permeabilized with Triton X-100 for 10 min, incubated with DAPI (1 μg/mL) for 15 min, and imaged under a fluorescence microscope after PBS washes.

3.6. Wound Healing Assay

MDA-MB-231 cells were seeded in six-well plates until confluent. A scratch was made using a pipette tip, and detached cells were washed away with PBS. Serum-free DMEM was added, and cells were cultured for 24 h. Images were taken at 0 h and 24 h under a Zeiss microscope.

3.7. Transwell Assay

Matrigel (X μg/mL) was layered on the microporous membrane of Transwell chambers and polymerized at 37 ℃. A cell suspension (200 μL containing 4 × 10^4 cells) was added to

the upper chamber, while DMEM with 10% fetal bovine serum was added to the lower chamber. After 24 h, non-invading cells were removed with a cotton swab. Invading cells were fixed with 4% formaldehyde, stained with 0.1% crystal violet for 15 min, and imaged under a Zeiss microscope.

3.8. Statistical Analysis

Statistical analysis was performed using SPSS 12.0 software. Experiments were performed in triplicate. Group comparisons used Student's t-test; categorical data were expressed as percentages and analyzed with the χ^2 test. Statistical significance was set at $P < 0.05$.

4. Results

4.1. MAN2A1 is Highly Expressed in Triple-negative Breast Cancer

Western Blot analysis of MAN2A1 and glycosylation levels revealed lower MAN2A1 protein expression in normal breast tissues compared to tumor tissues (Figure 1 A&B). Additionally, glycosylation levels were significantly elevated in tumor tissues relative to non-tumor breast tissues (Figure 1A). These findings indicate increased MAN2A1 expression and glycosylation in triple-negative breast cancer.

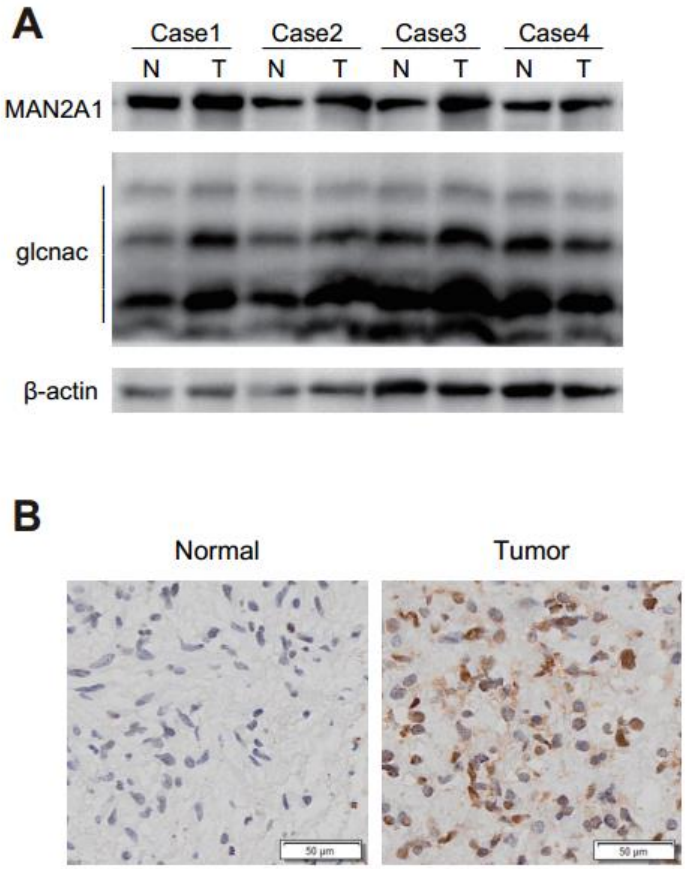

Figure 1. *Clinical importance of MAN2A1 in triple-negative breast cancer.*

A. The protein level of MAN2A1 and glycosylation in non-tumor and TNBC clinical specimens examined by Western blot.
B. The protein level of MAN2A1 in non-tumor and TNBC clinical specimens examined by immunohistochemistry. (Scale bar=50μm).

4.2. MAN2A1 Promotes Aggressive Behavior in Triple-negative Breast Cancer

Immunohistochemical analysis demonstrated elevated MAN2A1 expression in triple-negative breast cancer, with significantly greater immunoreactivity in tumor compared to normal tissues (Table 1). Clinicopathological correlation analysis revealed MAN2A1 overexpression in larger tumors (≥2 cm), lymph node metastasis-positive cases, and advanced-stage (III-IV) patients (Table 2).

Table 1. *MAN2A1 expression comparison (cases,%).*

Group	Cases	MAN2A1 Positive
Tumor tissue	220	150 (68.1%)

Group	Cases	MAN2A1 Positive
Normal tissue	220	35 (15.9%)
χ^2		55.143
P		<0.001

Table 2. MAN2A1 association with clinicopathological features.

Clinicopathological Feature	Cases	MAN2A1 Positive (n=150)	χ^2	*P*
Age				
≥50 years	120	78	0.231	0.123
<50 years	100	72		
Postmenopausal				
Yes	118	77	0.249	0.156
No	102	73		
Tumor size				
≥2 cm	131	115	41.534	<0.001
<2 cm	89	35		
Clinical stage				
Stage I-II	136	112	39.538	<0.001
Stage III-IV	84	38		
Lymph node metastasis				
Present	90	67	25.534	<0.001
Absent	120	83		

4.3. MAN2A1 Promotes Tumor Proliferation

Although MAN2A1 is highly expressed in TNBC tissues, its functional role in TNBC progression remains unexplored. CCK8 assays showed that MAN2A1-overexpressing cells exhibited significantly enhanced viability (Figure 2A). Similarly, MAN2A1 overexpression markedly increased colony formation (Figure 2 B&C). EdU assays revealed that MAN2A1 overexpression significantly elevated the proportion of EdU-positive cells (Figure 2 D&E). These results demonstrate that MAN2A1 promotes tumor growth.

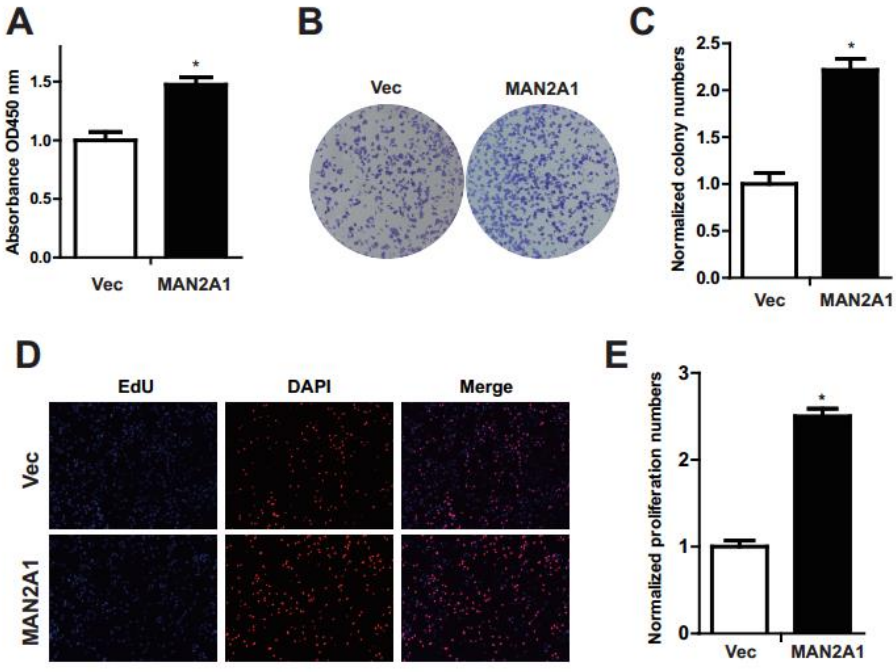

Figure 2. MAN2A1 Drives Triple-Negative Breast Cancer Cell Proliferation.

A. Cell viability was detected by CCK8 assays. ($p < 0.05$).
B&C. Representatives images (B) and quantitative analysis (C) of TNBC cell colonies.
D. Typical pictures EdU assay showed the cell proliferation rate of cells (Scale Bar=50μm).
E. Quantification results of D.

4.4. MAN2A1 Enhances Invasion and Migration

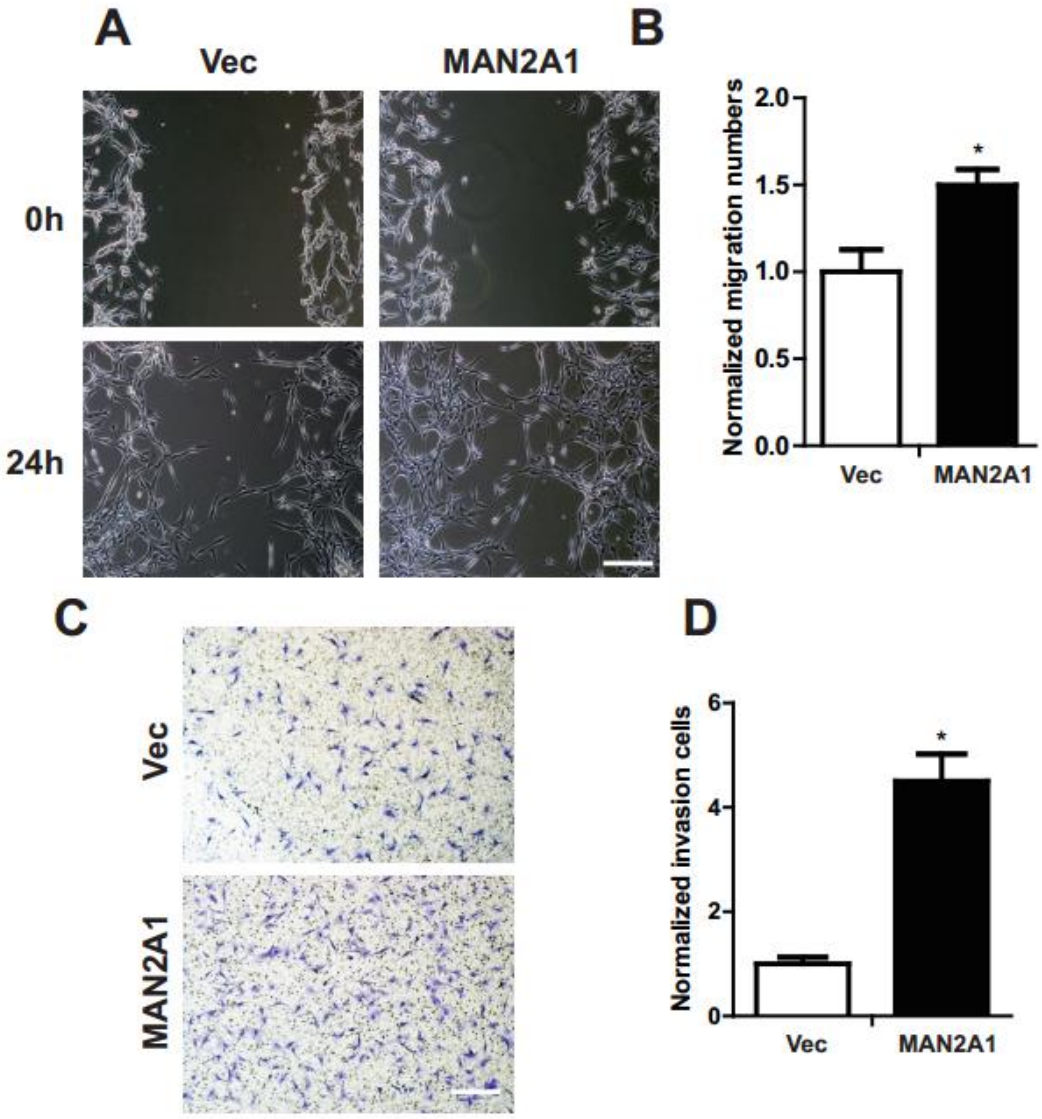

Figure 3. *MAN2A1 promotes cell invasion and migration.*

A. Representative digital pictures of wound healing assay were taken at 0 and 24h after scratching (Scale bar=200μm).
B. Quantification results of A.
C. Invasion ability was examined by transwell assay (Scale bar=200μm).
D. Quantification results of C.

Triple-negative breast cancer's aggressiveness involves rapid proliferation and frequent metastasis. We also observed that MAN2A1-overexpressing TNBC cells exhibited enhanced wound healing after 24 hours of scratching (Figure 3 A&B). Next, we performed a transwell assay with Matrigel to evaluate the effects of MAN2A1 overexpression on TNBC cell invasion. MAN2A1 upregulation significantly increased the number of cells that migrated through the Matrigel in TNBC cells (Figure 3 C&D). These results suggest that MAN2A1 may contribute to TNBC invasion and migration.

4.5. MAN2A1 Knockdown Inhibits Proliferation

To investigate the potential for clinical application, we downregulated MAN2A1 and assessed the proliferation capacity of TNBC cells. CCK-8 assays revealed that cell viability was reduced in MAN2A1-downregulated cells (Figure 4 A). Similarly, colony formation assays demonstrated significantly fewer colonies in MAN2A1-downregulated cells (Figure 4 B&C). EdU assays indicated that MAN2A1 downregulation led to a significant decrease in the percentage of

EdU-positive cells (Figure 4 D&E). These results suggest that MAN2A1 downregulation inhibits TNBC cell proliferation.

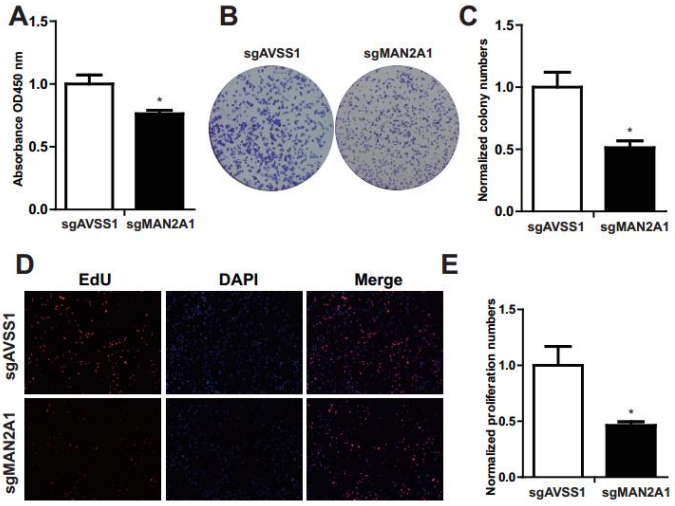

Figure 4. *Downregulation of MAN2A1 suppresses triple-negative breast cancer cell proliferation.*

A. Cell viability was detected by CCK8 assays.
B&C. Representatives images (B) and quantitative analysis (C) of TNBC cell colonies.
D. Typical pictures EdU assay showed the cell proliferation rate of cells (Scale Bar=50μm).
E. Quantification results of D.

4.6. MAN2A1 Knockdown Suppresses Invasion and Migration

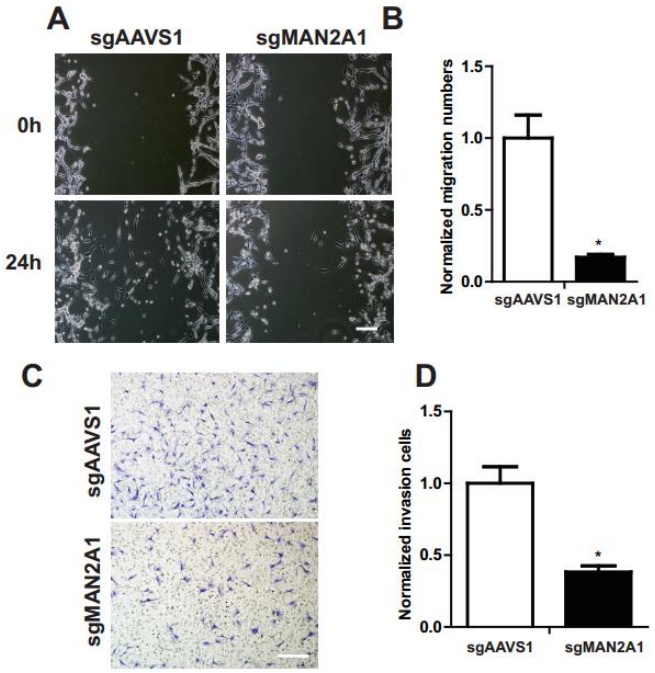

Figure 5. *Down-regulation of MAN2A1 inhibits cell invasion and migration.*

A. Representative digital pictures of wound healing assay were taken at 0 and 24h after scratching (Scale bar=200μm).
B. Quantification results of A.
C. Invasion ability was examined by transwell assay (Scale bar=200μm).
D. Quantification results of C.

We found that MAN2A1-downregulated TNBC cells exhibited reduced wound healing after 24h of scratching (Figure 5 A&B). Down-regulating MAN2A1 significantly decreased the number of cells that migrated through the Matrigel in TNBC cells (Figure 5 C&D). These results indicated that MAN2A1 represents a therapeutic target, and targeting MAN2A1 can inhibit the malignant progression of TNBC.

5. Discussion

Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer with an extremely poor prognosis. Characterized by early onset, high histological grade, rapid tumor growth, frequent recurrence, and a high propensity for distant metastasis, TNBC demonstrates significantly worse clinical outcomes compared to other breast cancer subtypes [12, 13]. Despite advances in multimodal treatment approaches, the survival rates for TNBC patients remain largely unchanged [14]. Consequently, there is an urgent need to identify novel therapeutic targets to improve TNBC prognosis.

Recent studies have established a strong association between aberrant glycosylation modifications and malignant tumor phenotypes. Notably, dysregulation of N-glycosylation pathways has been shown to play a pivotal role in tumor initiation and progression [6]. MAN2A1, a member of the glycoside hydrolase family, primarily catalyzes the cleavage of α-1, 6-mannose residues on glycoproteins during Golgi-mediated glycan processing. This enzymatic activity directly modulates glycoprotein structure and function, thereby influencing critical cellular processes including cell adhesion, signal transduction, and immune recognition. While MAN2A1 overexpression has been documented in various malignancies such as colorectal cancer [15], and is clinically associated with enhanced tumor survival, invasion, and metastasis, conflicting evidence shows its downregulation in prostate cancer. Despite these observations, research on MAN2A1 in breast cancer remains limited, and its precise pathogenic mechanisms are still unclear.

This study aims to investigate the expression and functional role of MAN2A1 in breast cancer. We present the first systematic evidence of the oncogenic role of MAN2A1 in TNBC. Immunohistochemical analysis revealed that MAN2A1 is significantly overexpressed in breast cancer tissues compared to normal tissues, with particularly higher expression in tumors larger than 2 cm, as well as in cases with lymph node metastasis and stage III-IV disease. Western blot analysis indicates that high MAN2A1 expression elevates glycosylation levels in TNBC, suggesting a potential role in regulating oncogenic pathways through glycosylation-dependent mechanisms. Functional studies demonstrate that MAN2A1 overexpression markedly enhances the proliferation, colony-forming capacity, and invasive and migratory abilities of MDA-MB-231 cells, while its knockout produces the opposite effect. These gain- and loss-of-function data suggest that MAN2A1 may drive tumor progression by modulating cell cycle progression and the epithelial-mesenchymal transition (EMT) process. Notably, the EdU assay indicates that MAN2A1 may accelerate DNA replication by promoting S-phase progression, while Transwell results suggest a potential enhancement of metastatic potential through increased ECM degradation activity. These findings broaden the current understanding of glycosylases in tumor cell plasticity and highlight MAN2A1 as a potential therapeutic target for TNBC prognosis.

Although this study does not directly investigate the immune microenvironment, MAN2A1-mediated glycosylation modifications may influence tumor immune evasion through multiple mechanisms. Our findings demonstrate that high MAN2A1 expression elevates glycosylation levels in TNBC. A 2020 study [16] revealed that MAN2A1-mediated aberrant glycosylation can obscure antigenic epitopes on tumor cell surfaces, impairing immune cell recognition, while also modifying immunosuppressive molecules such as PD-L1 to enhance their stability and promote tumor immune evasion. The MAN2A1-dependent glycosylation increase observed in this study may disrupt T-cell recognition by altering immune synapse-related proteins (e.g., MHC-I or co-stimulatory molecules). These potential mechanisms offer valuable insights for future investigations into MAN2A1's role in TNBC immunotherapy resistance and present novel opportunities for developing combination therapies targeting both glycosylation and immune pathways.

In summary, this study systematically elucidates the role of MAN2A1 as a key glycosylation-modifying enzyme in promoting the malignant progression of TNBC. The experimental results demonstrate that MAN2A1 drives TNBC development by enhancing cellular proliferation, invasive capacity, and migratory potential, providing a theoretical basis for developing targeted therapeutic strategies against MAN2A1. Despite the aforementioned limitations, this study represents the first to reveal the biological functions of MAN2A1 in TNBC, opening new avenues for exploring the mechanisms of glycosylation modifications in tumorigenesis and progression. Future research should focus on elucidating the precise molecular networks regulated by MAN2A1 and developing novel targeted inhibitors to achieve therapeutic breakthroughs in TNBC treatment.

Abbreviations

TNBC	Triple-negative Breast
MAN2A1	Mannosidase Alpha Class 2A Member 1
CCK-8	Cell Counting Kit-8
ER	Estrogen Receptor
PR	Progesterone Receptor
HER2	Human Epidermal Growth Factor Receptor-2
PVDF	Polyvinylidene Fluoride
ECL	Enhanced Chemiluminescence

DMEM	Dulbecco's Modified Eagle Media
EMT	Epithelial-mesenchymal Transition

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Conflicts of Interest

The authors declare no conflicts of interest.

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