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The Potential Role of SNRPD1 Stabilized by IGF2BP2 in the Progression of Triple-Negative Breast Cancer

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Background: Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), an RNA-binding protein with N6-methyladenosine (m6A) reader function, is associated with the poor prognosis of various tumors, including triple-negative breast cancer (TNBC). Small nuclear ribonucleoprotein D1 polypeptide (SNRPD1), a spliceosome member, exerts diagnostic and therapeutic functions in breast cancer by regulating the cell cycle and is a potential therapeutic target. However, the interaction between IGF2BP2 and SNRPD1 in the progression of TNBC remain unclear.

Objective: This study aimed to investigate the interaction between IGF2BP2 and SNRPD1 in TNBC and elucidate the underlying mechanisms.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were used to detect the expression levels of SNRPD1 and IGF2BP2 in human normal breast cells (MCF10A) and TNBC cells (MDA-MB-231). MDA-MB-231 cells were transfected with SNRPD1 interference or overexpression vectors, or co-transfected with SNRPD1 interference and IGF2BP2 overexpression vectors simultaneously. Cell viability, apoptosis, and invasion were assessed using MTT, flow cytometry, and Transwell assays. RNA stability, m6A levels, and the interaction between SNRPD1 and IGF2BP2 were evaluated using qRT-PCR, methylated RNA immunoprecipitation, and RIP assays.

Results: SNRPD1 was significantly up-regulated in TNBC cells, promoting cell viability and invasion while inhibiting apoptosis. IGF2BP2 was also up-regulated in TNBC cells and enhanced SNRPD1 mRNA stability via m6A modification. Furthermore, IGF2BP2 overexpression reversed the anti-tumor effect of SNRPD1 knockdown.

Conclusion: IGF2BP2 and SNRPD1 were significantly highly expressed in TNBC cells. IGF2BP2 might enhance the stability and protein expression of SNRPD1 through m6A-dependent mechanisms, potentially contributing to the progression of TNBC.

Keywords: triple-negative breast cancer, IGF2BP2, SNRPD1, m6A-dependent

Introduction

Breast cancer is a common malignant tumor, primarily caused by the abnormal proliferation of breast epithelial cells.[1] In China, breast cancer has the highest incidence among women and is showing a continuous upward trend. Moreover, breast cancer also exhibits a tendency towards younger age groups. Currently, breast cancer has overtaken lung cancer to become the leading cause of cancer worldwide.[2] Although breast cancer has a better prognosis compared to other malignant tumors, it remains one of the leading causes of cancer-related deaths among women due to factors such as low early detection rates, easily tumor metastasis, and drug resistance.[3] Triple-negative breast cancer (TNBC) is a subtype of breast cancer that accounts for approximately 15% to 20% of all breast cancer cases. TNBC is characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2), high level of specificity, and insensitive to endocrine therapy and targeted therapy. At present, the main treatment options for TNBC include surgery, chemotherapy, and radiotherapy, but the outcomes are not satisfactory.[4] Furthermore, TNBC is characterized by high invasiveness, high risk of distant metastasis, and poor prognosis, making it

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one of the most lethal subtypes of breast cancer. Currently, there is a lack of highly sensitive and specific biomarkers for the early diagnosis of TNBC. Additionally, high invasiveness of TNBC and the limitations of therapeutic drugs result in high 5-year mortality rates of TNBC patients, presenting a significant challenge in breast cancer treatment.[5] Therefore, there is an urgent need to discover more new therapeutic options and targets.

The m6A modification, also known as N6-methyladenosine modification, is a common and reversible chemical modification that occurs on RNA molecules. It is considered as the most abundant modification in mammalian messenger RNA (mRNA) and plays a significant role in cancer development. In recent years, m6A modification has received increasing attention and has become a hot topic in research.[6] The basic processes of m6A modification include writing, erasing, and reading. Of them, the recognition and reading of m6A, through identifying specific chemical modifications, play key roles in regulating mRNA nuclear export, splicing, translation, stability, and cytoplasmic localization.[7] Up to now, the identified m6A reader proteins mainly include YT521-B homology domain proteins, the heterogeneous nuclear ribonucleoprotein family, and eukaryotic initiation factor 3. The insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs, IMPs) are newly discovered RNA-binding proteins (RBPs) that exhibit m6A reader functions. Unlike the YT521-B homology domain family proteins that promote mRNA degradation, IGF2BPs contribute to the stabilization of mRNA.[8] Recent studies have found that, IGF2BP2, a member of the IGF2BP family, is not only overexpressed in various cancers but also associated with poor prognosis. For example, Hu et al[9] discovered that IGF2BP2 could promote the proliferation of pancreatic cancer by increasing the stability of m6A-modified differentiation antagonizing non-protein coding RNA mRNA; Yu et al[10] stated that IGF2BP2, acting as an m6A reader, could promote lymph node metastasis in head and neck squamous cell carcinoma. Additionally, some other studies have found that IGF2BP2 can also promote the proliferation of TNBC by down-regulating cyclin-dependent kinase 6 through direct regulation of m6A.[11] The spliceosome is a dynamic complex that catalyzes the splicing of precursor RNA into mRNA in eukaryotic cells. Accurate splicing is crucial for ensuring normal cellular functions such as cell proliferation, apoptosis, migration, and invasion. The spliceosome is composed of proteins encoded by seven genes, namely, small nuclear ribonucleoprotein polypeptide B (SNRPB), small nuclear ribonucleoprotein D1 polypeptide (SNRPD1), small nuclear ribonucleoprotein D2 polypeptide (SNRPD2), small nuclear ribonucleoprotein D3 polypeptide (SNRPD3), SNRPE, SNRPF, and SNRPG.[12] Previous studies have indicated that the expression of spliceosome genes is associated with the occurrence of cancer, and the SNRPD1 gene is considered as a potential therapeutic target for tumors such as ovarian cancer[13] and lung adenocarcinoma.[14] Recent studies have found that SNRPD1, through regulating the cell cycle, has diagnostic and therapeutic value for breast cancer and can serve as a therapeutic target for breast cancer.[15] However, the underlying mechanism of SNRPD1 remains unclear. Based on these findings, we hypothesized that IGF2BP2 may enhance the stability of SNRPD1 mRNA through m6A modification, thereby regulating the occurrence and development of TNBC. Therefore, this study aimed to investigate whether IGF2BP2 could regulate the stability of SNRPD1 mRNA through m6A modification, thereby mediating the occurrence and development of TNBC. Overall, this research provided theoretical support for SNRPD1 serving as a therapeutic target for breast cancer.

Materials and Methods

Cell Culture

MCF10A cells and MDA-MB-231 cells (American Type Culture Collection) were cultured in a high glucose Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin, and 100 U/mL penicillin. The cells were incubated at 37°C in a humidified incubator with 5% CO_2.

Cell Transfection and Grouping

MDA-MB-231 cells were seeded in a 6-well cell culture plate. After the cells had adhered and reached a confluence of 80%, each well was supplemented with 2 mL of basal medium. When the cells reached 90% confluence, transfection was then performed. The grouping of cell transfection was as follows: (1) Short hairpin (sh) RNA negative control (shNC) group: MDA-MB-231 cells were transfected with shNC. (2) sh-SNRPD1 group: MDA-MB-231 cells were transfected with SNRPD1 shRNA. (3) Overexpression negative control (OENC) group: MDA-MB-231 cells were transfected with an

OENC plasmid. (4) OE-SNRPD1 group: MDA-MB-231 cells were transfected with SNRPD1 overexpression plasmid. (5) sh-IGF2BP2 group: MDA-MB-231 cells were transfected with IGF2BP2 shRNA. (6) OE-IGF2BP2 group: MDA-MB-231 cells were transfected with IGF2BP2 overexpression plasmid. (7) NC group: MDA-MB-231 cells were transfected with both shNC and OENC plasmid. (8) sh-SNRPD1+OENC group: MDA-MB-231 cells were transfected with SNRPD1 shRNA and OENC plasmid. (9) sh-SNRPD1+OE-IGF2BP2 group: MDA-MB-231 cells were transfected with SNRPD1 shRNA and IGF2BP2 overexpression plasmid. All plasmids used for transfection were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The specific transfection procedures were carried out according to the manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction

Trizol reagent (Invitrogen, Cat#15596026CN) was added to the cells to extract total RNA. Subsequently, the A260/A280 absorbance ratio was measured using a NanodropTM spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific) to determine the concentration of RNA. The extracted RNA was then reverse transcribed into cDNA using a reverse transcription kit (Takara, Cat#RR037A), and the cDNA was used as a template for quantitative real-time polymerase chain reaction (qRT-PCR). The quantitative results were calculated using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as the internal control for the expression of SNRPD1 and IGF2BP2. The primer sequences for IGF2BP2 were as follows: F, 5′-ACCCTCTCGGGTAAAGTGGA-3′; R, 5′-TGTGTCTGTGTTGACTTGTTCC-3′. The primer sequences for SNRPD1 were: F, 5′-TGGAACAATCACAGACAGTTTACC-3′; R, 5′-GCAACAGCTTCCCTTTTCTTAGAT-3′. The primer sequences for β-actin were: F, 5′-AACAGTCCGCCTAGAAGCAC-3′; R, 5′-CGTTGACATCCGTAAAGACC-3′.

MTT Assay

After the MDA-MB-231 cells were cultured to the logarithmic growth phase, they were seeded into 96-well plates, transfected according to the experimental grouping, and then incubated for 24, 48, 72, and 96 hours, respectively. According to the kit's instructions, MTT solution (Abcam, Cat#ab211091) was added and the cells were incubated at 37°C for an additional 4 hours. Finally, dimethyl sulfoxide (Sigma, Cat#472301) was added to terminate the reaction, and the optical density at 490 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow Cytometry

After the MDA-MB-231 cells were cultured to the logarithmic growth phase, the cells were seeded into 6-well plates. Following an overnight culture, the cells were transfected according to the experimental grouping and then incubated for an additional 24 hours. Next, the cell supernatant was collected. After centrifugation, the supernatant was aspirated, and the cell pellet was pre-cooled with phosphate-buffered saline. After that, the centrifugation was performed and the supernatant was aspirated again. Subsequently, the cell pellet was resuspended in staining buffer, supplemented with propidium iodide and Annexin V-FITC, and mixed well. After incubation in the dark at room temperature for 5 minutes, the treated cells were analyzed using flow cytometry. The specific procedure was performed using the Annexin V-FITC Apoptosis Detection Kit (Beyotime Biotechnology, Cat#C1062S) according to the manufacturer's instructions.

Transwell Assay

According to the experimental grouping, the transfected cells were seeded into the upper chamber of a serum-free medium at a density of 2×10^5 cells per well. Then, a medium containing 10% FBS was added to the lower chamber. Next, the upper chambers of the 24-well Transwell plates were pre-coated with Matrigel diluted in DMEM at room temperature for 4 hours. Subsequently, the cells were incubated at 37°C with 5% CO_2 for 24 hours. Once the cells migrating to the underside of the membrane were fixed, they were stained with crystal violet solution, and then counted under a microscope.

RNA Stability Assay

MDA-MB-231 cells were treated with actinomycin D (Selleck, China) for 0, 2, 4, and 6 hours. At each time point, the treated cells underwent total RNA extraction, and the expression level of SNRPD1 in cells was measured using the qRT-PCR method as previous described.

Methylated RNA Immunoprecipitation Assay

The methylated RNA immunoprecipitation (MeRIP) analysis was performed according to the instructions of the Magna MeRIP m6A Kit (Shanghai Pufei Biotech Co., Ltd, Cat#17-10,499-2). Firstly, total RNA was extracted from MDA-MB -231 cells as previous described, and the extracted mRNA was fragmented into 100 nucleotide segments. The obtained RNA fragments were then incubated with m6A antibodies (anti-m6A, Abcam, Cat#ab208577) or mouse immunoglobulin G. Subsequently, the immunoprecipitated complexes were collected using protein A/G magnetic beads, and then purified and stored. Lastly, the purified MeRIP products were sent to RiboBio Co., Ltd (Guangzhou, China) for library construction and high-throughput sequencing.

RNA Immunoprecipitation Assay

The RNA immunoprecipitation (RIP) assay was performed according to the instructions provided in the Magna RIP Kit (Shanghai Pufei Biotechnology Co., Ltd, Cat#MAGNARIP01). Firstly, cell lysates were prepared using RIP lysis buffer and incubated with 5 μg of antibody at 4°C overnight. On the next day, RNA-protein immunocomplexes were collected using protein A/G magnetic beads. Subsequently, the purified RNA was analyzed by RT-PCR and qRT-PCR, and the amplified products were further analyzed and visualized using agarose gel electrophoresis. Finally, the purified RIP products were submitted to RiboBio Co., Ltd (Guangzhou, China) for library construction and high-throughput sequencing.

Western Blot Analysis

MDA-MB-231 cells were lysed with radio-immunoprecipitation assay lysis buffer (Beyotime, Cat#P0013C), and the total protein concentration was measured using a bicinchoninic acid protein assay kit (Abcam, Cat#ab287853). After separation by 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the proteins were transferred onto a polyvinylidene fluoride membrane. Next, the membrane was blocked with 5% non-fat milk. Following that, the membrane was incubated with primary antibodies at 4°C overnight. The used primary antibodies consisted of IGF2BP2 (Abcam, ab128175, 1:1000), SNRPD1 (Abcam, ab233115, 1:10,000), glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Beyotime, AF1186, 1:1000). On the next day, the membrane was incubated with the secondary antibody working solution (Abcam, ab6721, 1:2000) at room temperature for 1 hour. After 2 hours of incubation at room temperature, the membrane was developed using an enhanced chemiluminescence reagent (Sigma Aldrich, CPSOC), exposed by an imaging system, and then photographed (Thermo Fisher Scientific). The blot images were then semi-quantitatively analyzed using Image J software.

Statistical Analysis

The experimental data were statistically analyzed using SPSS 24.0 software. Differences between two groups were analyzed using the *T*-test, and comparisons among multiple groups were conducted using one-way ANOVA with Tukey's post-hoc test. Results were presented as mean ± standard deviation (SD). $P < 0.05$ indicated a statistical significance.

Results

Up-Regulation of SNRPD1 and Its Effects on Viability, Invasiveness, and Apoptosis in MDA-MB-231 Cells

To investigate the role of SNRPD1 in the development of TNBC, we first measured the mRNA and protein expression levels of SNRPD1 in MCF10A and MDA-MB-231 cells. qRT-PCR analysis revealed that the mRNA expression level of SNRPD1 was significantly higher in MDA-MB-231 cells than in MCF10A cells ($P < 0.01$) (Figure 1A). Similarly, Western blot analysis showed that compared to MCF10A cells, the protein expression level of SNRPD1 was also significantly higher in MDA-MB-231 cells ($P < 0.01$) (Figure 1B). These findings suggested that SNRPD1 may play an important role in the progression of TNBC.

To further elucidate the functional impact of SNRPD1 on TNBC cells, we constructed SNRPD1 overexpression (OE-SNRPD1) and silencing (sh-SNRPD1) vectors and transfected them into MDA-MB-231 cells. Western blot analysis

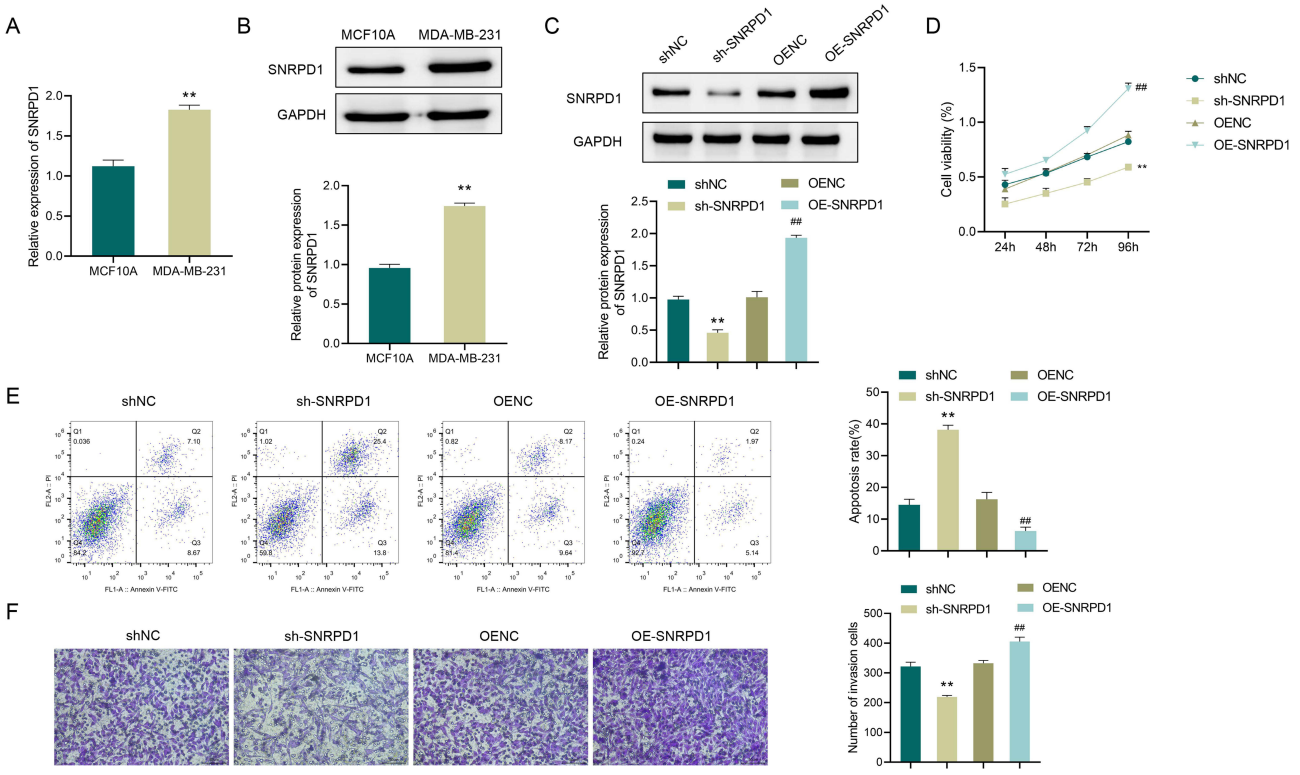

Figure 1 Up-regulation of SNRPD1 and its effects on viability, invasiveness, and apoptosis in MDA-MB-231 cells. (**A** and **B**), qRT-PCR and Western blot analyses showing the significant up-regulation of SNRPD1 mRNA and protein levels in MDA-MB-231 cells compared to MCF10A; Data were presented as mean ± SD; Statistical significance was determined using the *T*-test; $^{**}P < 0.01$ vs MCF10A group. (**C–F**), Functional analysis of MDA-MB-231 cells following SNRPD1 knockdown or overexpression: (**C**), Western blot analysis was performed to detect the protein expression levels of SNRPD1; (**D**), MTT assay was conducted to measure the viability of MDA-MB-231 cells; (**E**), Flow cytometry was used to assess the apoptosis levels of MDA-MB-231 cells; (**F**), Transwell assay was carried out to evaluate the invasiveness of MDA-MB-231 cells; Data were presented as mean ± SD; Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test; $^{**}P < 0.01$ vs shNC group; $^{\#\#}P < 0.01$ vs OENC group.
Abbreviations: qRT-PCR, quantitative real-time polymerase chain reaction; SNRPD1, small nuclear ribonucleoprotein D1 polypeptide; shNC, short hairpin RNA negative control; OENC, overexpression negative control.

confirmed that SNRPD1 expression was significantly reduced in the sh-SNRPD1 group and significantly increased in the OE-SNRPD1 group, compared to their respective controls ($P < 0.01$) (Figure 1C). Functional assays demonstrated that SNRPD1 silencing (sh-SNRPD1) significantly decreased cell viability and invasiveness, while increasing apoptosis compared to the shNC group ($P < 0.01$). Conversely, SNRPD1 overexpression (OE-SNRPD1) significantly increased cell viability and invasiveness, while decreasing apoptosis ($P < 0.01$) compared to the OENC group (Figure 1D–F). These results suggested that SNRPD1 enhanced the viability and invasiveness of MDA-MB-231 cells and inhibits apoptosis, contributing to the development and progression of TNBC.

Up-Regulation of IGF2BP2 in MDA-MB-231 Cells Enhances the Stability of SNRPD1 mRNA Through m6A Modification

Next, we examined the expression levels of the breast cancer susceptibility gene IGF2BP2 in MCF10A and MDA-MB-231 cells. The examination results revealed that compared to the MCF10A group, the mRNA and protein expression levels of IGF2BP2 were significantly raised in the MDA-MB-231 group ($P < 0.01$) (Figure 2A and B). Such outcome suggested that IGF2BP2 may also be involved in the development of TNBC. IGF2BP2 overexpression or silencing vectors were constructed to further investigate the role of IGF2BP2 in the development of TNBC. The investigation results showed that, compared to the shNC group, the protein levels of SNRPD1 and IGF2BP2 were significantly reduced in the sh-IGF2BP2 group of MDA-MB-231 cells. Conversely, compared to the OENC group, the protein expression levels of SNRPD1 and IGF2BP2 were significantly increased in the OE-IGF2BP2 group of MDA-MB-231 cells ($P < 0.01$) (Figure 2C). Additionally, the m6A sites of SNRPD1 were also predicted. The prediction results demonstrated that the m6A levels of SNRPD1 in MDA-MB-231 cells

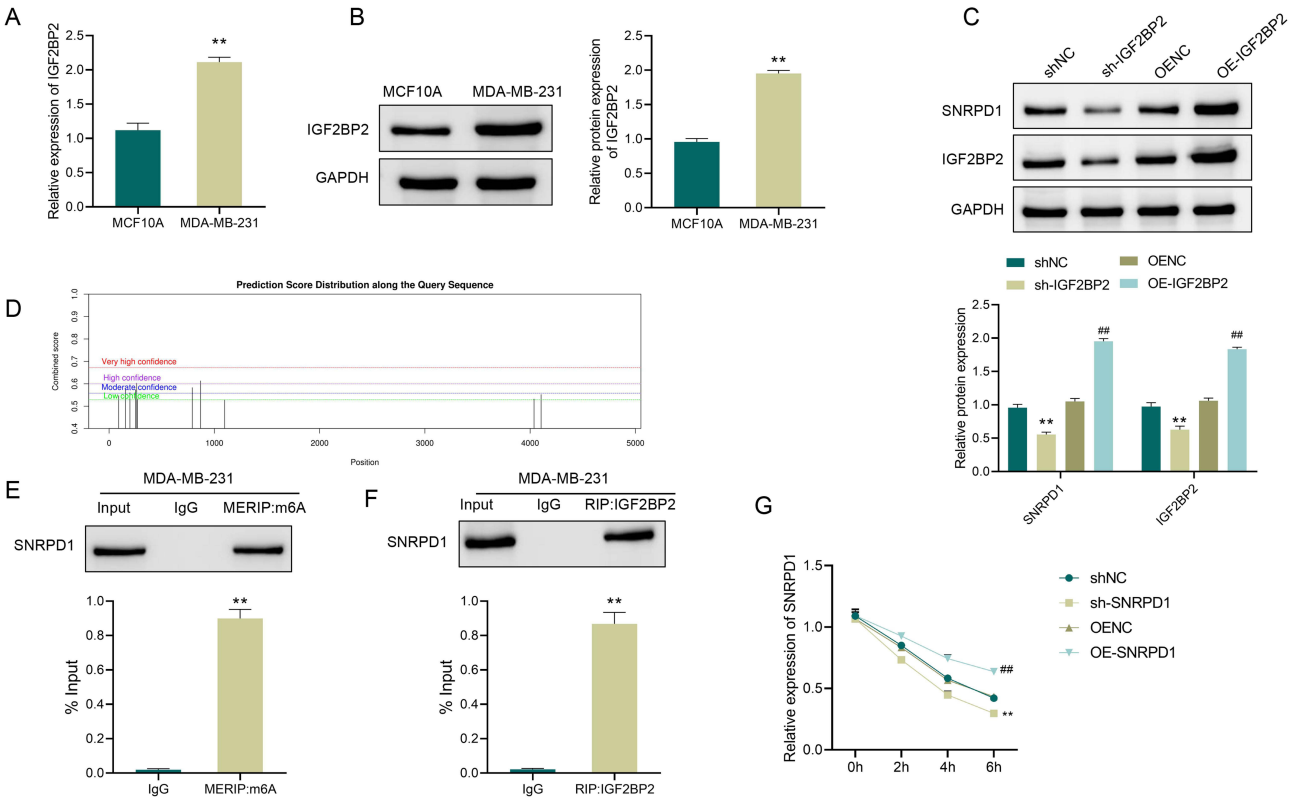

Figure 2 Up-regulation of IGF2BP2 in MDA-MB-231 cells increases the stability of SNRPD1 mRNA through m6A modification. (**A**), qRT-PCR analysis of IGF2BP2 expression levels in MCF10A and MDA-MB-231 cells; (**B**), Western blot analysis of IGF2BP2 protein expression levels in MCF10A and MDA-MB-231 cells; (**C**), Western blot analysis of SNRPD1 and IGF2BP2 expression levels in MDA-MB-231 cells transfected with SNRPD1 interference or overexpression vectors; (**D**), Prediction of m6A sites in SNRPD1; (**E**), MeRIP assay to detect the m6A levels of SNRPD1; (**F**), RIP assay to detect the interaction between SNRPD1 and IGF2BP2 in MDA-MB-231 cells; (**G**), RNA stability assay: After treatment with actinomycin D for 0, 2, 4, and 6 hours, qRT-PCR was used to measure the expression levels of SNRPD1 in shNC, sh-IGF2BP2, OENC, and OE-IGF2BP2 groups of MDA-MB-231 cells. Data were presented as mean ± SD; Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test; $^{**}P < 0.01$ vs shNC group; $^{\#\#}P < 0.01$ vs OENC group.

Abbreviations: IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2; SNRPD1, small nuclear ribonucleoprotein D1 polypeptide; mRNA, messenger RNA; m6A, N 6-methyladenosine; qRT-PCR, quantitative real-time polymerase chain reaction; MeRIP, methylated RNA immunoprecipitation; shNC, short hairpin RNA negative control; OENC, overexpression negative control.

were significantly elevated ($P < 0.01$) (Figure 2D and E). Subsequently, RIP assay results indicated that SNRPD1 could bind to IGF2BP2 in MDA-MB-231 cells ($P < 0.01$) (Figure 2F). Further analysis of RNA stability revealed that at 0 hour, there were no significant differences in SNRPD1 expression levels in MDA-MB-231 cells of each group. However, at 2, 4, and 6 hours, compared to the shNC group, the expression levels of SNRPD1 in the sh-IGF2BP2 group were significantly reduced; while in the OE-IGF2BP2 group, the expression levels of SNRPD1 were significantly increased compared to the OENC group. Furthermore, the levels of SNRPD1 in each group showed a decreasing trend with the increase in treatment time ($P < 0.01$) (Figure 2G). Hence, IGF2BP2 may enhance the stability of SNRPD1 mRNA through m6A modification, thereby contributing to the development of TNBC.

Overexpression of IGF2BP2 Reverses the Antitumor Effects of SNRPD1 Knockdown

SNRPD1 shRNA and IGF2BP2 overexpression plasmid were co-transfected into MDA-MB-231 cells, then the growth and progression of MDA-MB-231 cells were measured to further confirm the roles of IGF2BP2 and SNRPD1 in TNBC. The measurement results showed that, compared to the NC group, the viability and invasiveness of MDA-MB-231 cells in the sh-SNRPD1+OENC group were significantly reduced, while the level of apoptosis was significantly increased ($P < 0.01$). Conversely, relative to the sh-SNRPD1+OENC group, the viability and invasiveness of MDA-MB-231 cells in the sh-SNRPD1+OE-IGF2BP2 group were significantly increased, while the level of apoptosis was significantly decreased ($P < 0.01$) (Figure 3). Consequently, overexpression of IGF2BP2 could reverse the antitumor effects of SNRPD1 knockdown.

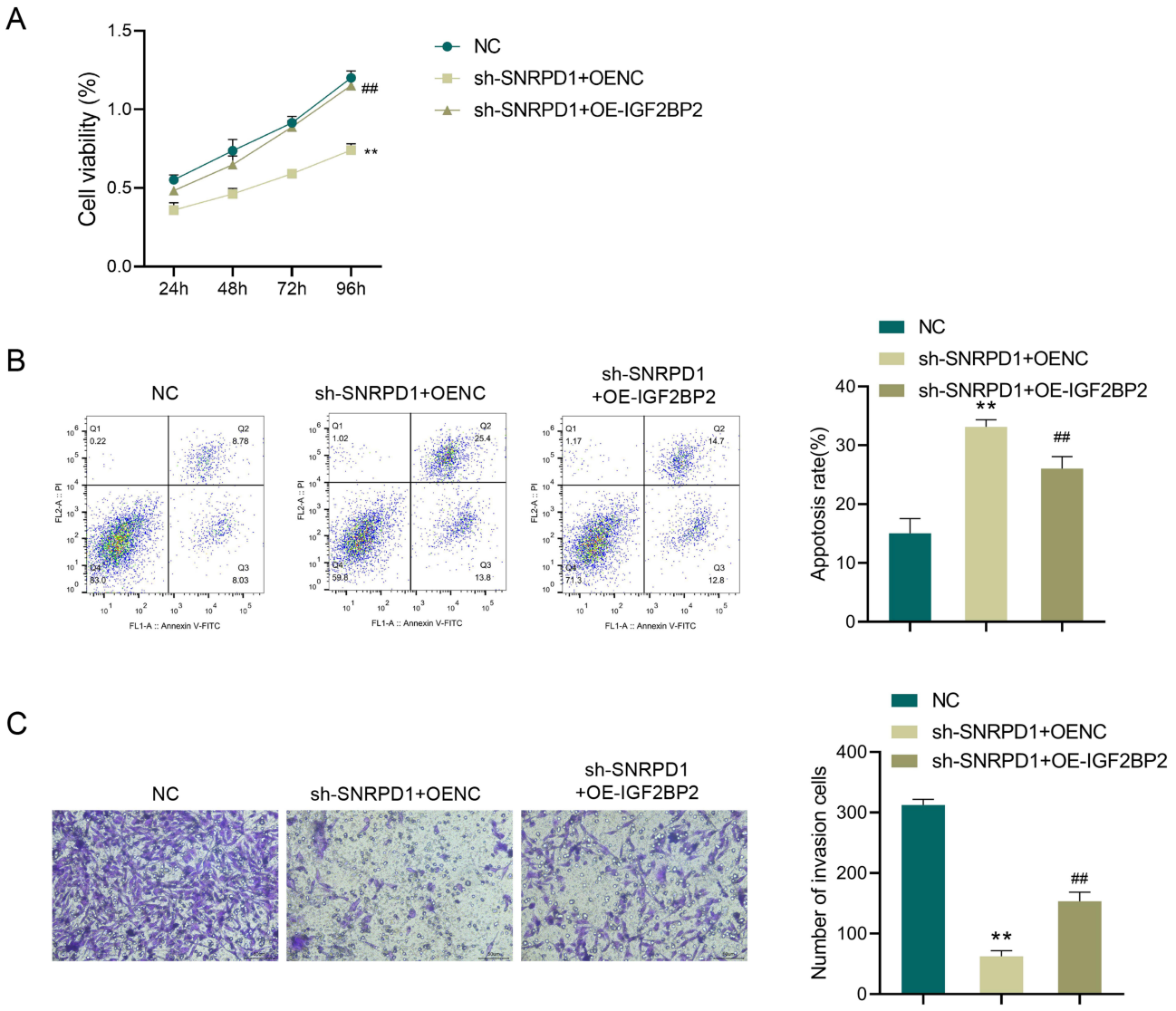

Figure 3 Overexpression of IGF2BP2 reverses the antitumor effects of SNRPD1 knockdown. (**A**), MTT assay was utilized to assess the viability of MDA-MB-231 cells in the NC, sh-SNRPD1+OENC, and sh-SNRPD1+OE-IGF2BP2 groups; (**B**), Flow cytometry to detect the apoptosis levels of MDA-MB-231 cells in the NC, sh-SNRPD1+OENC, and sh-SNRPD1+OE-IGF2BP2 groups; (**C**), Transwell assay to measure the invasiveness of MDA-MB-231 cells in the NC, sh-SNRPD1+OENC, and sh-SNRPD1+OE-IGF2BP2 groups. Data were presented as mean ± SD; Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test; $^{**}P < 0.01$ vs NC group; $^{\#\#}P < 0.01$ vs sh-SNRPD1+OENC group.

Abbreviations: IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2; SNRPD1, small nuclear ribonucleoprotein D1 polypeptide.

Discussion

In this study, we explored the role of IGF2BP2 in regulating SNRPD1 mRNA stability via m6A modification and its implications for TNBC. Our findings revealed that both SNRPD1 and IGF2BP2 were significantly up-regulated in TNBC MDA-MB-231 cells, contributing to increased cell viability, invasiveness, and inhibition of apoptosis. Notably, IGF2BP2 enhanced the stability of SNRPD1 mRNA through m6A modification, and overexpression of IGF2BP2 effectively reversed the antitumor effects of SNRPD1 knockdown, underscoring a potential regulatory axis in TNBC.

The role of alternative splicing in genetic diversity and its impact on cellular processes such as proliferation, survival, and differentiation is well-established.[16] However, abnormal expression or genetic mutations in spliceosomal components can disrupt these processes, promoting tumor development and progression.[17] SNRPD1, a key splicing factor, has been identified as abnormally expressed in various cancers, suggesting its potential as a clinical prognostic marker.[18] For instance, Yi et al[19] proposed that SNRPD1 was significantly overexpressed in lung adenocarcinoma and could serve as

a clinical prognostic biomarker through the use of weighted gene co-expression network analysis. Gupta et al[20] found that SNRPD1 was abnormally up-regulated in acute lymphoblastic leukemia through genomic and proteomic approaches. Our results aligned with previous studies, demonstrating that SNRPD1 was significantly up-regulated in TNBC cells. Silencing SNRPD1 led to decreased cell viability and invasiveness, inducing apoptosis, indicating that SNRPD1 its role in the progression of TNBC. These finding were consistent with the results of Dai et al,[15] suggesting that SNRPD1 may serve as a potential marker for TNBC.

IGF2BP2 has been demonstrated to play a significant role in the progression of various cancers.[21] Also, the role of IGF2BP2 in the rapid proliferation of multiple tumors, including colorectal cancer, pancreatic cancer, thyroid cancer, gastric cancer, and prostate cancer, has been well documented.[9,22–24] Specifically, Liu et al[25] discovered a relationship between IGF2BP2 and poor prognosis in breast cancer by analyzing genomic DNA from the peripheral blood of women. As an RNA-binding protein, IGF2BP2 has long been recognized for its ability to enhance the stability of target mRNAs.[22,26] Huang et al[8] first proposed that the IGF2BP family could recognize and bind to m6A sites on mRNA through its KH3-4 domain, thereby enhancing the stability and translation of mRNA in an m6A-dependent manner. In addition, a recent study revealed that IGF2BP2 is significantly over-expressed in TNBC cells, and the IGF2BP2 methylation in TNBC patients is much lower than in non-TNBC patients. Mechanically, IGF2BP2 can promote TNBC proliferation and cell cycle transition by directly regulating the stability of cyclin-dependent kinase 6 in an m6A-dependent manner.[11] Our study further supported these findings, showing that IGF2BP2 was overexpressed in TNBC cells and can interact with SNRPD1 to regulate its stability through m6A modification. RIP assays confirmed this interaction, and stability assays demonstrated that IGF2BP2 overexpression enhanced the stability of SNRPD1 in cells, which was consistent with the findings of Huang et al.[8] Thus, IGF2BP2 might promote the progression of TNBC by stabilizing of SNRPD1 mRNA, contributing to the aggressive nature of these cancer cells.

Despite the significant discoveries made in this study, several limitations must be acknowledged. First, our research predominantly relied on in vitro experiments using the MDA-MB-231 TNBC cell line, which limited the generalizability of the findings. TNBC is a heterogeneous disease, and different cell lines may exhibit varying responses to the manipulation of IGF2BP2 and SNRPD1. Including additional TNBC cell lines in future studies will help verify the consistency of the results and present a broader standpoint on these proteins' roles in TNBC. In addition, while the in vitro models provided important insights into the role of IGF2BP2 and SNRPD1, they did not fully capture the complexity of tumor microenvironments in vivo. Consequently, the interactions and effects observed may differ in a living organism, and future research should encompass i*n vivo* models to confirm the above findings and elucidate the mechanisms involved more thoroughly. Furthermore, while we disclosed an association between IGF2BP2 and SNRPD1 expression and TNBC progression, the causal relationship and underlying molecular mechanisms need further exploration. Specifically, the exact pathways through which IGF2BP2 stabilizes SNRPD1 mRNA via m6A modification remain to be elucidated. Detailed mechanistic studies are necessary to uncover these pathways, particularly focusing on how IGF2BP2 may concurrently influence mRNA stability and post-translational regulation of SNRPD1.

In conclusion, these findings not only elucidate the relationship between IGF2BP2 and SNRPD1 in TNBC but also suggest that targeting this regulatory axis could provide new therapeutic strategies for managing TNBC.

Conclusion

To sum up, this study revealed that IGF2BP2 was highly expressed in TNBC and plays a crucial role in increasing the stability and translation of SNRPD1 in an m6A-dependent manner, thereby promoting the progression of TNBC (Figure 4). Importantly, our data demonstrated that overexpression of IGF2BP2 could reverse the antitumor effects observed following SNRPD1 knockdown, as evidenced by restored cell viability, increased invasiveness, and reduced apoptosis in MDA-MB-231 cells. These findings not only provide potential therapeutic targets and new strategies for the diagnosis and treatment of TNBC but also offer strong evidence that IGF2BP2, acting as an m6A reader, plays a positive role in initiating translation and driving tumorigenesis.

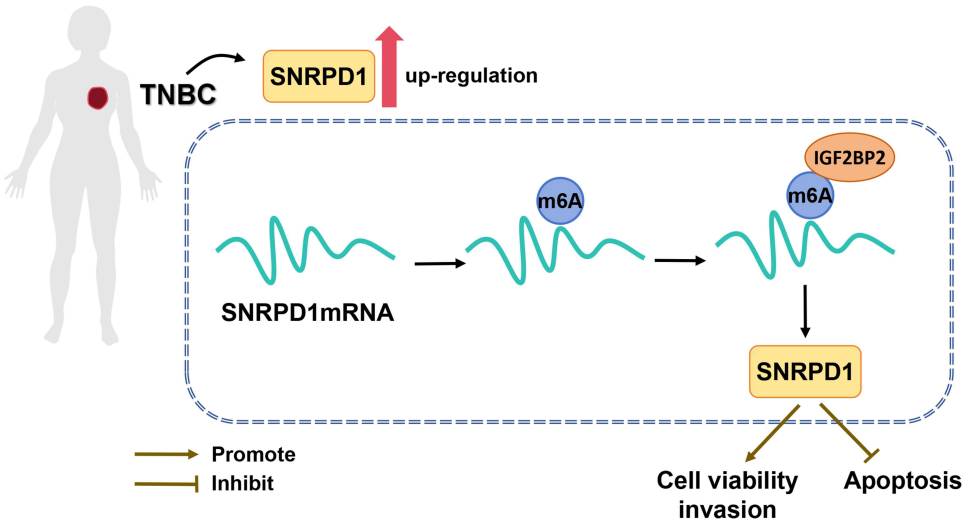

Figure 4 Potential interaction and mechanism between IGF2BP2 and SNRPD1 in Triple-Negative Breast Cancer (TNBC).

Abbreviations

TNBC, triple-negative breast cancer; qRT-PCR, Quantitative real-time polymerase chain reaction; mRNA, messenger RNA; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; RBPs, RNA-binding proteins; IGF2BPs, IMPs, insulin-like growth factor 2 mRNA-binding proteins; SNRPB, small nuclear ribonucleoprotein polypeptide B; SNRPD1, small nuclear ribonucleoprotein D1 polypeptide; SNRPD2, small nuclear ribonucleoprotein D2 polypeptide; SNRPD3, small nuclear ribonucleoprotein D3 polypeptide; FBS, fetal bovine serum; sh, short hairpin; shNC, short hairpin RNA negative control; OENC, overexpression negative control; MeRIP, methylated RNA immunoprecipitation; RIP, RNA immunoprecipitation; SD, standard deviation.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author.

Consent for Publication

All authors have consented to the publication of the manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have declared that no competing financial interests or personal relationships exists in this paper.

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