Original Article

The synergistic effect of *CDKN2B-AS1* and *SPC25* on triple-negative breast cancer

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Background: Accumulating evidence suggests that long non-coding ribonucleic acid (RNA) cyclin-dependent kinase inhibitor 2B antisense RNA 1 (*CDKN2B-AS1*) and messenger RNA (mRNA) spindle component 25 (*SPC25*) contribute to tumorigenesis and progression in various cancers. However, the synergistic effect between *CDKN2B-AS1* and *SPC25* has not yet been fully elucidated in triple-negative breast cancer (TNBC). This study sought to examine the synergistic effect of *CDKN2B-AS1* and *SPC25* and uncover a novel mechanism for the progression of TNBC.

Methods: The transcriptome profiles of TNBC in The Cancer Genome Atlas (TCGA) were calculated for differentially expressed genes (DEGs). Gene co-expression networks were constructed via a weighted correlation network analysis. We validated the relationship between *CDKN2B-AS1* and *SPC25* by bioinformatics and *in-vitro* studies (including Cell Counting Kit-8, transwell assays, and quantitative real-time polymerase chain reaction).

Results: *CDKN2B-AS1* was found to be carcinogenic and was significantly upregulated and co-expressed with elevated *SPC25* expression levels in the TNBC cells and sequencing profiles. Notably, the *SPC25* mRNA levels were associated with poor clinical outcomes in TNBC patients. Specifically, the knockdown of *CDKN2B-AS1* significantly inhibited TNBC cell proliferation and migration.

Conclusions: We identified a novel cancer-promoting regulation axis. The co-expression of *CDKN2B-AS1* and *SPC25* is expected to serve as a powerful candidate biomarker for diagnostic and prognostic purposes in TNBC.

Keywords: Triple-negative breast cancer (TNBC); cyclin-dependent kinase inhibitor 2B antisense RNA 1 (*CDKN2B-AS1*); spindle component 25 (*SPC25*); proliferation

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Introduction

In 2020, breast cancer (BC) was one of the most common cancers and the primary cause of cancer-related mortality in women worldwide (1). Despite crucial advances in the accurate diagnosis and early intervention of BC, the survival times of some patients remain short. BC is a heterogeneous disease that manifests in different molecular and clinicopathologic features (2). Triple-negative breast

cancer (TNBC) is the most aggressive and fatal subtype of BC and accounts for 10–20% of all BC cases (3). Thus, novel therapeutic targets for TNBC desperately need to be identified.

Spindle component 25 (*SPC25*) is a crucial portion of the NDC80 complex that participates in spindle checkpoint activity and kinetochore-microtubule clustering (4). Recent research has shown that upregulated *SPC25* accelerates the proliferation of hepatocellular carcinoma (HCC) and is related to a poor prognosis (5-7). Additionally, previous studies have also reported that *SPC25* is an important tumor promoter for cell cycle and malignant progression, and stem cell proliferation in prostate cancer (8). Further, *SPC25* has been shown to regulate the stemness of lung cancer cells significantly (9).

Long non-coding ribonucleic acids (lncRNAs) belong to a kind of non-coding RNA that modulates gene transcription or protein translation by multiple distinct mechanisms (10). Alterations in lncRNA expression may exhibit anti-oncogenic or oncogenic activities. The lncRNA cyclin-dependent kinase inhibitor 2B antisense RNA 1 (*CDKN2B-AS1*) is located at chromosome 9p21, and increases the malignancy of renal cancer (11). Additionally, *CDKN2B-AS1* has been shown to induce the proliferation and migration of colorectal cancer (12).

In this study, we found that upregulated *SPC25* had an oncogenic function and predicted a poor overall survival (OS) time. Notably, *CDKN2B-AS1*-activated *SPC25* enhanced the proliferation of TNBC cells. Consequently, our research findings may enable the exploration of novel targets for TNBC treatment and prognostic evaluation. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2900/rc).

Methods

Retrieving data

We downloaded the transcriptome profiles and survival information of TNBC from The Cancer Genome Atlas Program (https://portal.gdc.cancer.gov/). GSE45827 dataset was obtained from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database. We further investigated the pan-cancer expression profiles based on the Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) (13). A Clinical Proteomic Tumor Analysis Consortium (CPTAC, https://cptac-data-portal.georgetown.edu/) was conducted to confirm the protein expression analysis. The Human Protein Atlas (https://www.proteinatlas.org/) was used to verify protein expression levels in diverse tissues and the subcellular localization of the SPC25 protein. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of DEGs and the weighted gene co-expression network analysis (WGCNA)

R software DESeq2 (14) and limma (15) packages were used to identify the differentially expressed genes (DEGs) of the transcriptome data. A WGCNA algorithm (16) was conducted to identify the gene modules that were highly correlated with TNBC. Gene significance (GS) and module membership (MM) were applied to quantify the features and configurations of the modules. The protein–protein interaction network was constructed by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) with minimum required interaction score >0.4 and PPI enrichment P value $<1.0\times10^{-16}$. Hub genes of TNBC were identified and visualized by the Molecular Complex Detection (MCODE) plugins in Cytoscape. LncSEA as a comprehensive lncRNA sets resource and enrichment analysis platform was performed to analyze functions of lncRNAs.

Functional enrichment analysis

A gene set enrichment analysis (GSEA) was conducted to explore the key module genes and detect some crucial biological functions, such as cell cycle and cellular signaling pathway. GSEA software was used for the GSEA with the following parameters: number of permutations =1,000; Normalized Enrichment Score (NES) >2; nominal (NOM) P value <0.05; and false discovery rate <0.05. The STRING (https://cn.string-db.org/) database was used to identify the proteins co-expressed with *SPC25*. Subsequently, a Gene Ontology (GO) pathway enrichment analysis was conducted using the clusterProfiler (17) packages in R software.

Single-cell analysis and survival analysis

CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/) was applied to reveal the potential function of *SPC25* in BC (18). A survival analysis was performed and visualized using the survival and survminer packages in R software.

Cell culture and transfection

Normal human breast cell lines (MCF10A) and TNBC cell lines (i.e., BT549, MDA-MB-231, and MDA-MB-468) were obtained from American Type Culture Collection (ATCC). All the cell lines were authenticated by short tandem repeat deoxyribonucleic acid (DNA) profiling and tested for mycoplasma (GENEWIZ Co., Ltd., Suzhou, China) within 6 months. Lipofectamine 2,000 (Invitrogen, USA) was used for transfection. The GeneCreate Biological Engineering Company (Wuhan, China) synthesized small-interfering RNA specifically targeting *CDKN2B-AS1* (*si-CDKN2B-AS1*) and corresponding non-specific siRNA (si-NC). The siRNA sequences used in our research were: 5'-CCACAUCCCUUGGAGUAAUTT and AUUACUCCAAGGGAUGUGGTT-3'.

RNA extraction and qRT-PCR

We applied the Trizol reagent (Introvigen, USA) to extract total RNA from the cell lines. Subsequently, the RNAs were reverse transcribed into complementary DNA and quantitative real-time polymerase chain reaction (qRT-PCR) assays were conducted. The $2^{-\Delta\Delta Ct}$ method was used to calculate the expression level of *CDKN2B-AS1* and *SPC25*. The primers were purchased from The GeneCreate Biological Engineering Company (Wuhan, China). The primer sequences of the RNAs are shown in Table S1.

Cell proliferation

Cell counting kit-8 (CCK-8) and transwell assays were used to assess cell proliferation viability and migration. First, the MDA-MB-231 cells were transfected with *si-CDKN2B-AS1* or si-NC. Second, the CCK-8 solution (Beyotime, China) was supplemented, and the absorbance value at 450 nm was measured at different time points (i.e., 0, 24, 48, and 72 h).

Cell migration experiments

The transfected cells (2×10^4/well) were seeded into the upper compartment of a 24-well transwell chamber (Corning Costar, USA). The lower chambers contained 10% fetal bovine serum. After incubation for 48 h, the non-migrated cells were carefully removed from the upper chamber with cotton swabs. We randomly selected the average confluence of the migrated cells from 3 visual fields (captured by a 100× microscope) to count the number of cells.

Cell apoptosis experiments

Cells were harvested and washed twice with PBS buffer, 5 μL of Annexin V-FITC and 5 μL of 7-AAD were added to the cells and incubated for 30 min in the dark. Flow cytometer (BD Accuri TM C6) and BD Accuri TM C6 software used to analyze

All of the above experiments was replicated 3 times in laboratory.

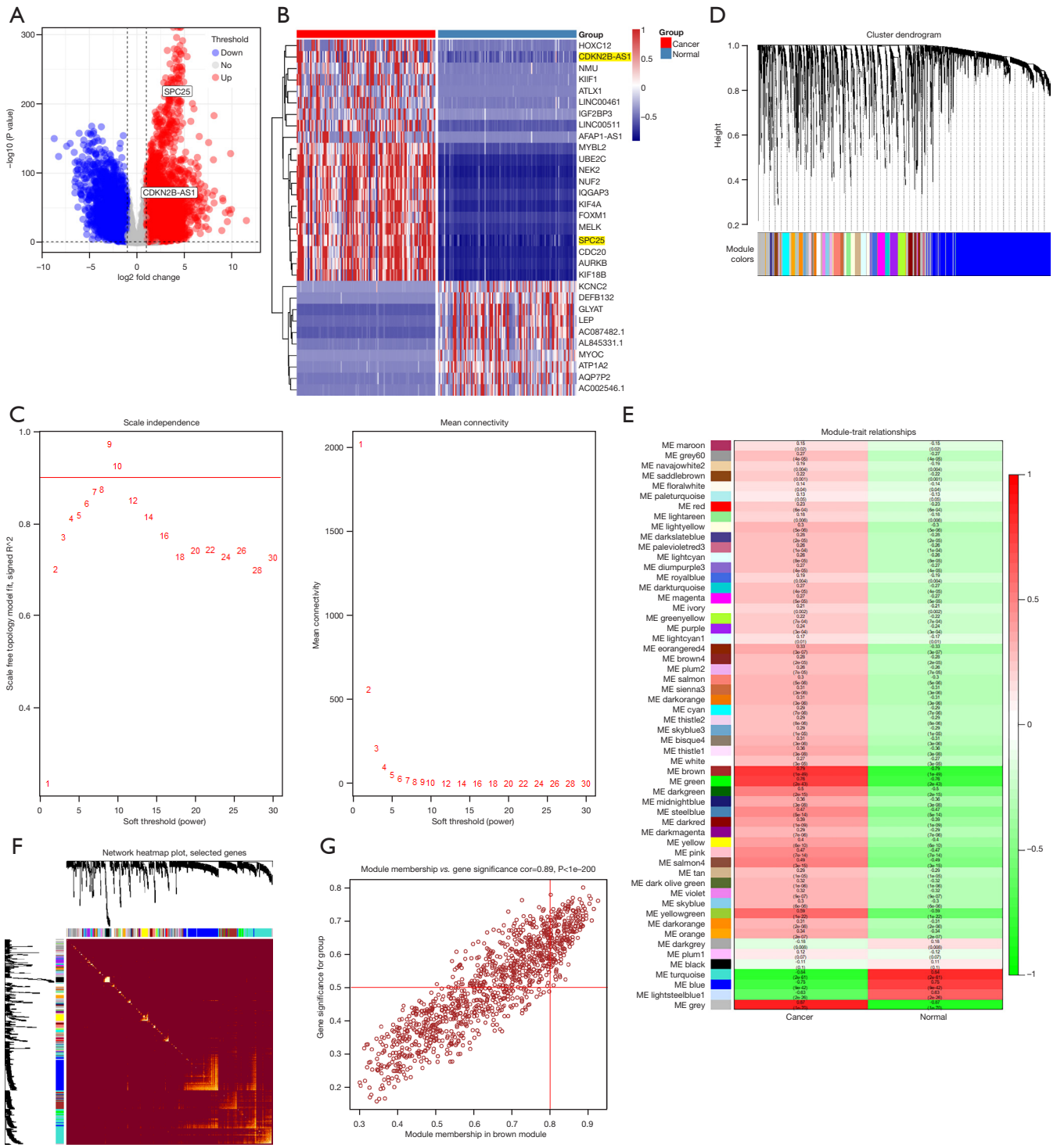
Statistical methods

The statistical analysis was conducted using R (v.4.0.3) software. The DESeq2 package was used to analyze the DEGs of the transcriptome data. The WGCNA package was used to construct the co-expression networks. The limma package was used to calculate the DEGs of the microarray data. The gene expression correlation analyses were conducted using Pearson's coefficients. GSEA software and the clusterProfiler package was used for the pathway enrichment analysis. The survival package was used for the survival analysis. The mean ± standard deviation were calculated, and the statistical difference was examined by the Student's *t*-test. A P value <0.05 was considered statistically significant.

Results

WGCNA and GSEA

A total of 12,993 DEGs, including lncRNAs, messenger RNAs (mRNAs), and small-nucleolar RNAs, were identified based on the transcriptome data in TCGA (see *Figure 1A*), and some representative genes were selected to illustrate this different expression patterns (see *Figure 1B*). The WGCNA were used to identify the gene modules in the para-cancerous tissues and TNBC. To satisfy the scale-free topology, the soft threshold β value was set at 7 (see *Figure 1C*). In total, 56 gene modules (which were not merged) were constructed by average-linkage hierarchical clustering (see *Figure 1D*). Additionally, a network heatmap of 600 randomly selected genes revealed a high level of independence across the co-expression clusters (see *Figure 1E*). The module-trait relationships of different co-expression modules revealed that the brown module showed the highest positive correlation with TNBC (see *Figure 1F*).



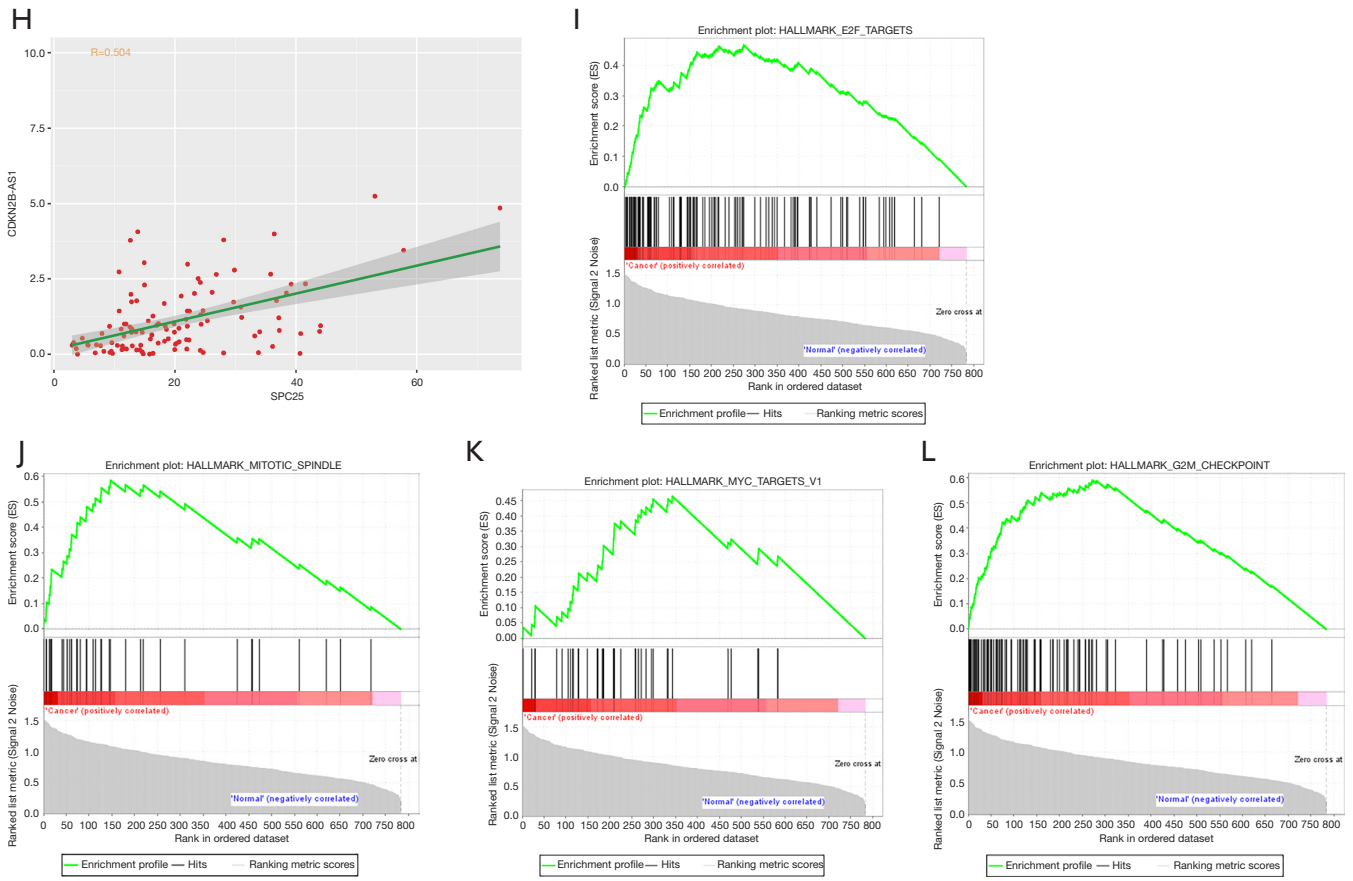


Figure 1 Identification of hub genes in WGCNA. (A,B) *CDKN2B-AS1* and *SPC25* expression was significantly higher in the TNBC tissues than the adjacent normal tissues. (C) The approximate soft threshold (power =7) was selected. (D) A genetic clustering tree-map was generated based on 56 gene modules using average-linkage hierarchical clustering. The modules were identified using the dynamic cut-tree method and named using various colors. (E) The heatmap analysis showed that the brown module exhibited the highest positive correlation with TNBC. (F) The interacting-relationship analysis of the co-expressed genes. The different colors in the vertical and horizontal axes represent different modules. The connection degree among different modules is indicated by the yellow intensity. (G) Scatter plot of module eigengenes in the brown module. (H) The Spearman correlation analysis showed that *CDKN2B-AS1* and *SPC25* were highly and positively correlated. (I-L) The GSEA analysis of genes in the brown module. WGCNA, weighted gene co-expression network analysis; *CDKN2B-AS1*, cyclin-dependent kinase inhibitor 2B antisense RNA 1; *SPC25*, spindle component 25; TNBC, triple-negative breast cancer; GSEA, gene set enrichment analysis.

In total, 1,155 hub genes were confirmed to have a MM >0.8 and a GS >0.5 in the brown module (available online: https://cdn.amegroups.cn/static/public/atm-22-2900-1.xlsx and *Figure 1G*). Additionally, in the brown module, *CDKN2B-AS1* and *SPC25* were more highly expressed in the TNBC tissues than the corresponding normal tissues (see Figure S1A,S1B). The Spearman correlation analysis also revealed that *CDKN2B-AS1* and *SPC25* were highly and positively correlated (see *Figure 1H*). STRING database was utilized to enrich the PPI network of DEGs of TNBC. *SPC25* as one of hub genes was identified by the MCODE plugin in Cytoscape (see Figure S2A). *CDKN2B-AS1* enrichment analysis on LncSEA indicated to be associated with a variety of tumors, including TNBC (see Figure S2B). According to the GSEA, the mRNAs in the brown module were predominantly enriched in the transcription factor E2F and MYC targets, mitotic spindle, and the G2M checkpoint, which are closely associated with cell-cycle regulation (see *Figure 1I-1L*).

Expression validation, survival analysis, and protein-protein interactions

The expression levels of *CDKN2B-AS1* and *SPC25* in more than 30 cancer types from TCGA were extracted from GEPIA database. Notably, *CDKN2B-AS1* and *SPC25* were upregulated in most cancer types (see *Figure 2A,2B*). Further, the microarray data of GSE45827, comprising 41 basal samples, 14 cell line samples, 30 human epidermal growth factor receptor 2 (Her2+) samples, 29 Luminal A samples, 30 Luminal B samples, and 11 normal samples, were used to validate the upregulation of mRNA *SPC25* between the basal samples and normal samples (see *Figure 2C*). Additionally, the TCGA Cancer Proteome Study of Breast Tissue of The Clinical Proteomic Tumor Analysis Consortium (CPTAC) revealed that the SPC25 protein was highly expressed (see *Figure 2D*). Subsequently, the *SPC25* expression confirmed by the Human Protein Atlas was higher in tumors compared to normal tissue (see *Figure 2E,2F*). The subcellular location of *SPC25* is in the cytoplasm and *SPC25* co-locates with microtubule proteins, which play an important role in cell division (see *Figure 2G*).

Based on the OS times of TNBC patients in TCGA, a survival analysis of *SPC25* was performed to evaluate the difference between the high and low expression groups. Notably, the OS time was lower in patients who had a higher *SPC25* expression (see *Figure 2H*). The protein-protein interaction network of the *SPC25* protein was established via the STRING database. The proteins co-expressed with SPC25 were retrieved, and included SPC24, CDK1, ZWINT, MIS12, TOP2A, NUF2, NDC80, DSN1, BUB1, and CASC5 (see *Figure 2I*). The GO annotation of the co-expressed genes revealed their relevant biological processes (see *Figure 2J*), which included chromosome segregation, mitotic cell-cycle checkpoint, and nuclear division.

Functions of co-expressed SPC25 and CDKN2B-AS1

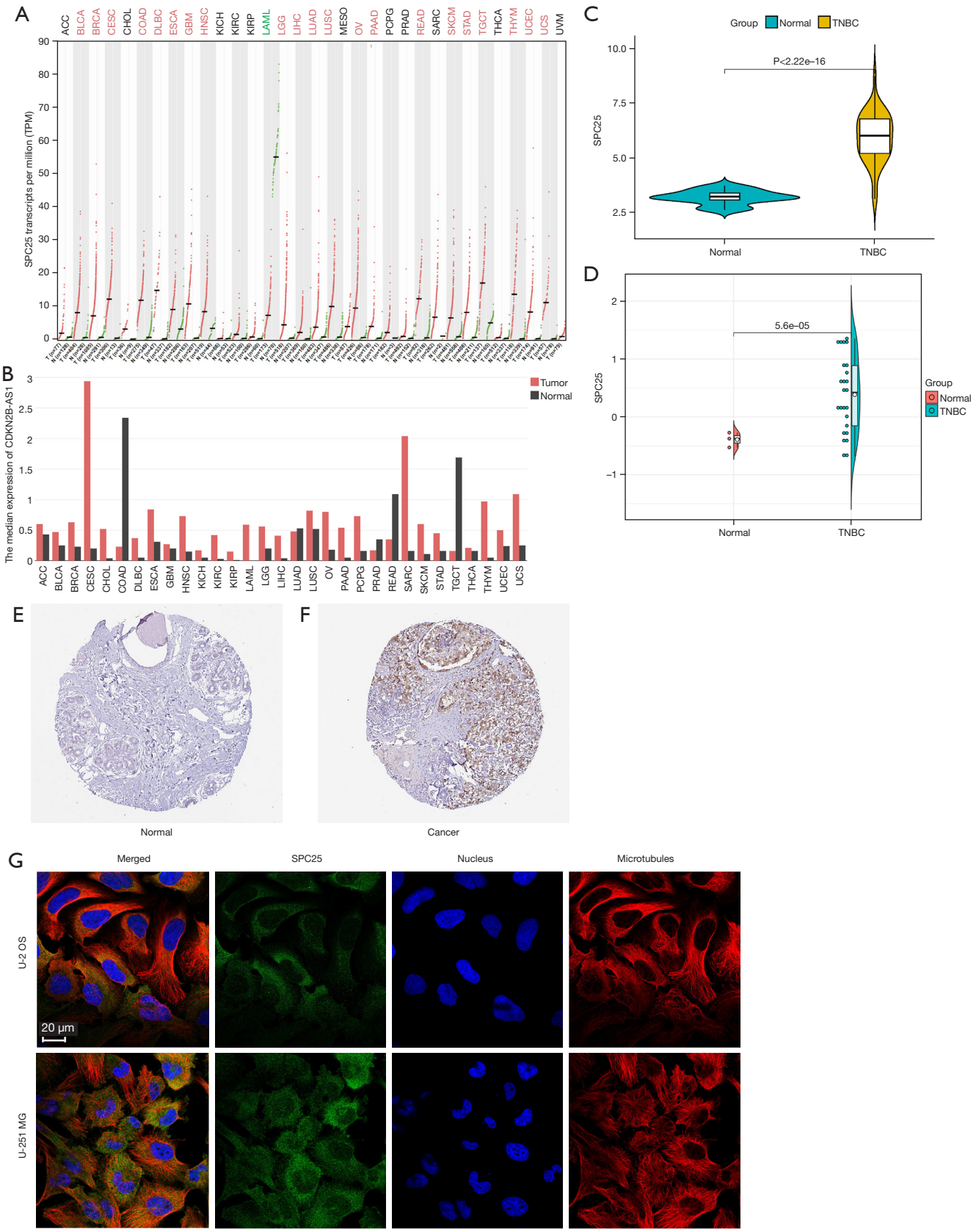
Based on the data of the Braune EB (number cells: 369) in CancerSEA, a single-cell analysis was conducted to examine the functions of *SPC25*, which showed that *SPC25* might participate in the regulation of the cell cycle in BC (see *Figure 3A,3B*). Additionally, the expression of *SPC25* was positively correlated with the above-mentioned biological processes (see *Figure 3C-3F*). The correlation analysis revealed that *SPC25* was highly correlated with the cell cycle–related genes (i.e., *CDC25A*, *CCNE1*, *CCNA2*, *CCNB1*, *MKI67*, and *CDK1*) (see *Figure 3G*).

In-vitro studies were used to explore the correlation of *SPC25* expression with dysregulated lncRNA *CDKN2B-AS1*. The validation of the differentially expressed *CDKN2B-AS1–SPC25* pair was performed by qRT-PCR. We found that *CDKN2B-AS1* and *SPC25* were more upregulated in the BT549, MDA-MB-231, and MDA-MB-468 cell lines than the MCF10A cell lines (see *Figure 3H,3I*). MDA-MB-231 was selected for further interfering assays. As expected, the knockdown of *CDKN2B-AS1* inhibited *SPC25* expression (see *Figure 3J*). Further, the cell number monitoring assays revealed that the cell proliferation varied (see *Figure 3K*) and the invasion ability was restrained (see *Figure 3L-3N*) after the knockdown of *CDKN2B-AS1*. In addition, flow cytometry cell apoptosis analysis showed that knockdown of *CDKN2B-AS1* significantly increased the percentage of apoptotic cells (see *Figure 3O*).

Discussion

In our study, a WGCNA was conducted to find the co-expression gene modules, and we identified the key gene modules associated with tumorigenesis in the network. The WGCNA package uses bioinformatics approaches to describe clusters of highly correlated genes (19). We discovered that *CDKN2B-AS1* and mRNA *SPC25* in the brown module were significantly overexpressed in TNBC. We further characterized the synergistic effect of *CDKN2B-AS1* and *SPC25* and found that they accelerated TNBC. To examine the effects of the upregulation of *CDKN2B-AS1* on TNBC proliferation and migration, CCK-8 and transwell assays were conducted, and revealed that the overexpression of *SPC25* was positively correlated with the poor OS of TNBC patients. Further, our findings revealed that *CDKN2B-AS1* may promote TNBC proliferation by upregulating *SPC25* and accelerating the cell cycle.

Recently, studies have revealed that lncRNAs are aberrantly expressed in breast cancer and closely associated with the development and progression of breast cancer (20). LncRNAs can regulate mRNA expression through multiple mechanisms: epigenetic regulation, transcriptional regulation, post-transcriptional regulation, acting as ceRNA and other molecules mechanisms (21). However, the specific mechanism is still unclear and it is a challenge to use lncRNAs as biomarkers for diagnosis or therapeutic targets. We found that *CDKN2B-AS1* and mRNA *SPC25*



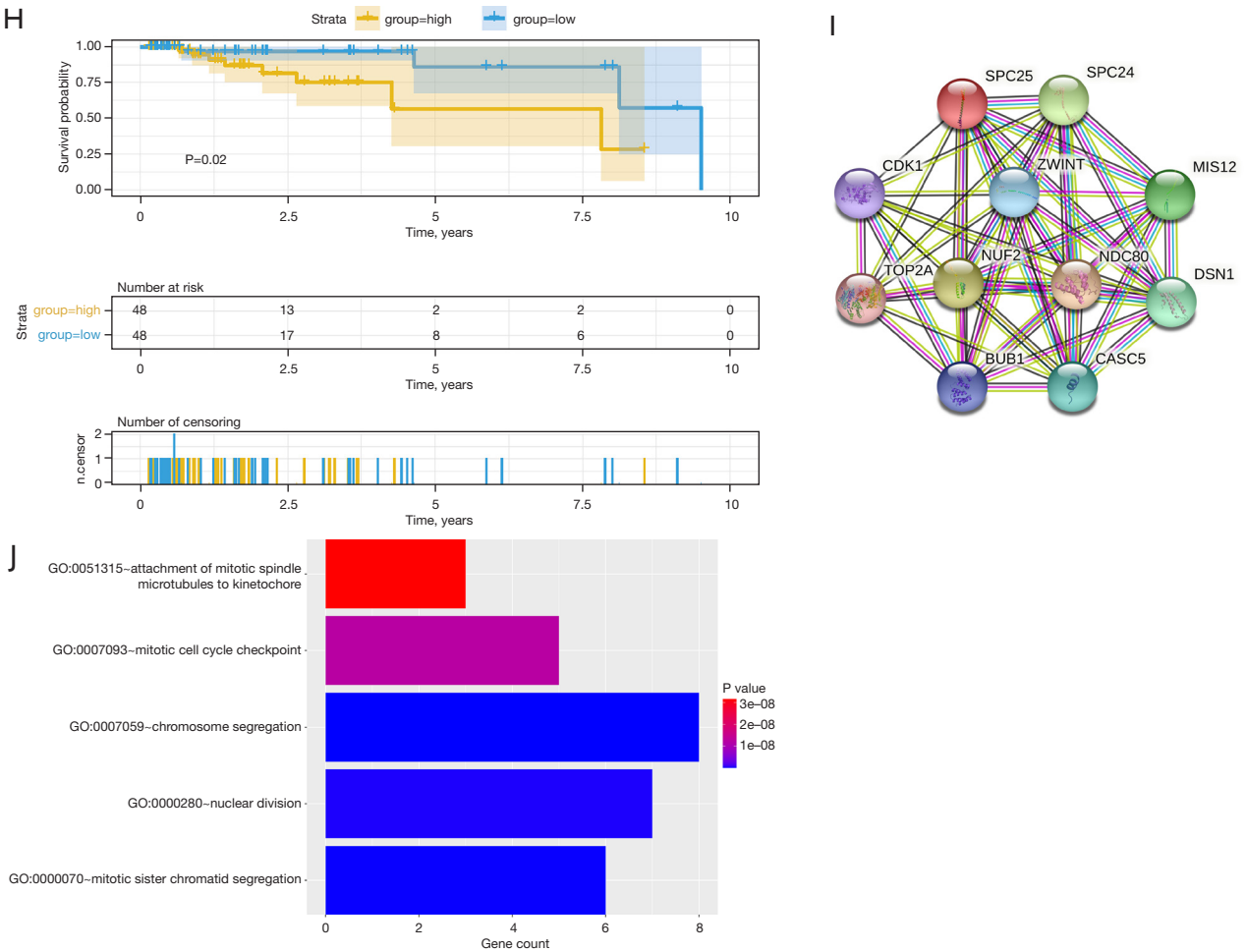
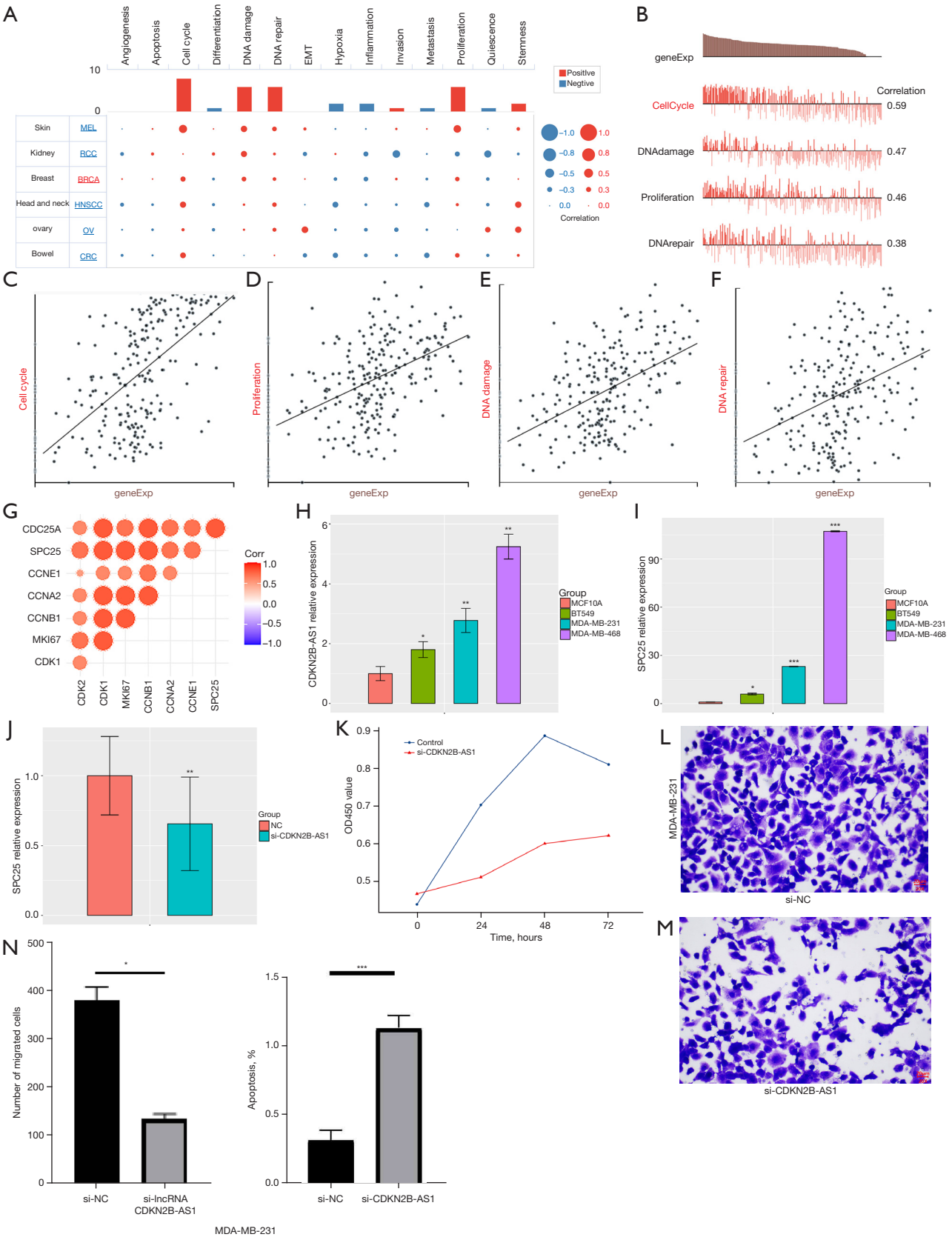


Figure 2 Validation of *SPC25* and functional annotation. (A,B) The mRNA expression of *SPC25* and *CDKN2B-AS1* is notably increased in most human cancer tissues compared to corresponding normal tissues. Red dots represent *SPC25* expression in tumor samples. Green dots represent *SPC25* expression in paired normal samples. (C) The upregulation of mRNA *SPC25* was validated in GSE45827. (D) The protein expression of *SPC25* in the CPTAC database was significantly higher in the TNBC samples than the normal samples. (E,F) *SPC25* expression in normal breast tissues and BC tissues by immunohistochemical stain. Magnification, ×100. (G) According to the Human Protein Atlas database, *SPC25* was localized in the cytoplasm of multiple human cells, such as U-2 OS and U-251 MG by immunofluorescence staining and photographed by confocal microscopy. Scale bar, 20 μm. (H) High *SPC25* expression displayed a significant correlation with poor OS in TNBC patients. (I) *SPC25*-interaction proteins in TNBC. (J) A GO enrichment analysis was used to identify relevant the biological processes of the co-expressed genes. *SPC25*, spindle component 25; *CDKN2B-AS1*, cyclin-dependent kinase inhibitor 2B antisense RNA 1; CPTAC, Clinical Proteomic Tumor Analysis Consortium; OS, overall survival; TNBC, triple-negative breast cancer; BC, breast cancer; U-2 OS, human osteosarcoma cell line; U-251 MG, human glioblastoma cell line; GO, Gene Ontology.

were significantly overexpressed in TNBC. LncRNA *CDKN2B-AS1* participates in a variety of regulatory roles, including the cell cycle of tumors (11,22-24). The aberrant expression of *CDKN2B-AS1* has been reported in multiple cancers, and it might accelerate proliferation and inhibit the apoptosis of tumor cells (25). Studies have revealed that the mRNA level of *CDKN2B-AS1* is increased and exerts carcinogenic effects in various tumors (11,26,27). Research has shown that *CDKN2B-AS1* is overexpressed in gliomas and enhances cell proliferation, invasion, and migration, and inhibits the apoptosis of glioma cells (28). *CDKN2B-AS1* has also been shown to be significantly overexpressed in renal carcinoma cells, while the downregulation of *CDKN2B-AS1* has been shown to suppress the tumor growth involved in



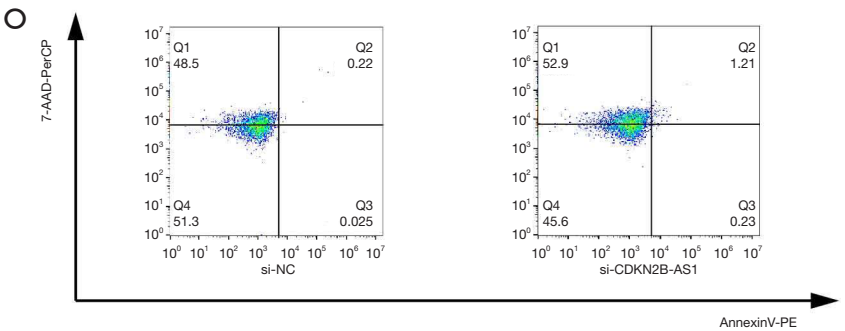


Figure 3 The function of *SPC25* in BC cells. (A,B) A single-cell analysis showed that *SPC25* affected numerous cellular events, including cell-cycle regulation and DNA damage responses in BC. (C-F) *SPC25* expression was positively correlated with above-mentioned biological processes. (G) Correlation between *SPC25* and cell cycle–related genes. (H,I) RT-PCR validation of the relative expression of *CDKN2B-AS1* and *SPC25* in cell lines. (J) The relative expression of *SPC25* was downregulated in si-*CDKN2B-AS1*–transfected MDA-MB-231 cells. (K) CCK-8 were performed to show that si-*CDKN2B-AS1* inhibits the proliferation of MDA-MB-231. (L-N) Transwell were performed to detect the proliferation of MDA-MB-231 by crystal violet assay. (O) The flow cytometry assay showed that the apoptosis rate was significantly increased in *CDKN2B-AS1* knockdown cells. *P<0.05, **P<0.01, ***P<0.001. ×20. *SPC25*, spindle component 25; BC, breast cancer; *CDKN2B-AS1*, cyclin-dependent kinase inhibitor 2B antisense RNA 1; CCK-8, cell counting kit-8.

proliferation, migration, and invasion (29).

As a part of the Ndc80 complex, the *SPC25* homolog plays an important role in the mitosis of cell-cycle processes, including spindle formation and chromosome alignment (30-32). *SPC25* and *CDKN2B-AS1* expression levels are upregulated in most of the cancers identified by the GEPIA database, and the GEO analysis confirmed that the expression of *SPC25* was significantly more elevated in TNBC tissues than normal tissues. *SPC25* has been shown to be upregulated and involved in cell proliferation in lung cancer (33). We found that the expression of *SPC25* was downregulated by siRNA-mediated *CDKN2B-AS1* knockdown. Additionally, the Spearman correlation analysis revealed that the expression levels of *SPC25* and *CDKN2B-AS1* were highly and positively correlated. The CCK-8 and transwell assays indicated that proliferation and migration were strongly inhibited in the si-*CDKN2B-AS1* cell line. We confirmed the synergistic effect of *CDKN2B-AS1* and that *SPC25* enhances the proliferation and migration capacity of TNBC. Similarly, previous studies have demonstrated that the downregulation of *SPC25* significantly reduces the proliferation of prostate cancer (34). Additionally, *SPC25* has been shown to interact with checkpoint-related proteins and increase proliferation in lung cancer (33). *SPC25* facilitates tumor growth via the tumor protein P53 pathway and may be a novel tumor-promoting factor and a therapeutic target in HCC (7). The downregulation of *SPC25* also leads to aberrant spindle organization with misaligned chromosomes (31). Thus, we hypothesize that *SPC25* overexpression is involved in genetic alterations in the cell cycle caused by incorrect chromosome segregation, in which motility plays a crucial role, and ultimately leads to tumorigenesis. We also observed that *SPC25* was colocalized with microtubule proteins. Previous research has shown that tubulin plays a vital role in cell proliferation (35). Thus, we hypothesize that *SPC25* binds to microtubule proteins and then accelerates TNBC tumorigenesis and progression.

Next, the survival analysis confirmed that the *SPC25* level was an independent prognostic factor of TNBC. High levels of *SPC25* were related to poor OS and recurrence-free survival in lung adenocarcinoma patients (9). Research has indicated that increased *SPC25* expression levels are correlated with poor OS in BC patients (36). The high expression of *SPC25* has also been shown to be correlated with short survival time and to enhance the proliferation of HCC cells (5). The patient with higher *SPC25* expression had higher recurrence rates, which was associated with shorter OS in BC patients (37). Our study was the first to reveal that *SPC25* could have potential clinical value in TNBC.

In this study, the GO annotation of the co-expressed genes identified the relevant biological processes, including chromosome segregation, mitotic cell-cycle checkpoint, and nuclear division. We performed a GSEA to evaluate the underlying function of the brown module. WGCNA

and pathway analyses have shown that *SPC25* is a potential marker of HCC and is associated with the mitotic cell cycle (38,39). Genes in the brown module were primarily related to cell-cycle regulation, which is a pervasive and complex sequence of events (40). Aberrant cell cycles, which allow cells to divide indefinitely, are hallmarks of cancer. Thus, therapies targeting cell cycles have long been regarded as promising anti-cancer strategies. Genes in the brown module may potentially promote tumor development via the transcription of E2F and MYC to accelerate cell cycles. In the future, the synergistic effect of *CDKN2B-AS1* and *SPC25* might be important for improving the optimal development and application of cell-cycle targeting drugs in clinical practice.

The single-cell analysis demonstrated that *SPC25* may contribute to the progression of BC by accelerating the cell cycle. The downregulation of *SPC25* expression is associated with cell-cycle at M-phase arrest (32). The correlation analysis revealed that *SPC25* may increase the cell cycle through co-expression with *CDC25A*, *CCNE1*, *CCNA2*, *CCNB1*, *MKI67*, and *CDK1*. Further experiments need be conducted to verify the interaction of *SPC25* with these cell cycle-related genes.

In summary, our research shows that the synergistic effect of *CDKN2B-AS1* and *SPC25* on the occurrence and development of TNBC. *CDKN2B-AS1* may play a significant role in the diagnosis, prognosis, and specific therapeutic target in TNBC. Our results provided new insights into the potential biomarkers and mechanisms of TNBC oncogenesis and also suggest a potential application of treatment in the future.

This study had a number of limitations. First, while *CDKN2B-AS1* and *SPC25* are potential markers, large-scale validations studies urgently need to be conducted to apply these findings in a clinical setting. Second, the WGCNA method has limitations, as its selection criteria and thresholds may affect the ultimate findings.

Conclusions

In brief, we used the WGCNA method to identify the synergistic effect of lncRNAs and mRNAs on TNBC progression. We also verified the functional roles and molecular process of *CDKN2B-AS1* as a tumor-promoting factor that upregulates *SPC25* expression in tumor tissues. Collectively, we identified a number of TNBC co-expression modules and hub genes. Our findings provide a theoretical foundation for future research. The candidate factors may be applied as diagnostic and prognostic biomarkers or clinical treatment targets in the future.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2900/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2900/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2900/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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