Original Article

# Combining Bulk RNA-seq and scRNA-seq data to identify RNA m5C methyltransferases *NSUN1*: a rising star as a biomarker for cancer diagnosis, prognosis and therapy

**Zhen Fang[1], Peijuan Li[2], Fei Li[1]**

[1]Department of General Surgery, Xuanwu Hospital, Capital Medical University, Beijing, China; [2]The First Affiliated Hospital of Dalian Medical University, Dalian Medical University, Dalian, China

*Contributions:* (I) Conception and design: All authors; (II) Administrative support: F Li; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: Z Fang, P Li; (V) Data analysis and interpretation: Z Fang, P Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*Correspondence to:* Fei Li, PhD. Department of General Surgery, Xuanwu Hospital, Capital Medical University, 45 Changchun Rd., Beijing 100053, China. Email: lfei21@126.com; Peijuan Li, PhD. The First Affiliated Hospital of Dalian Medical University, Dalian Medical University, 222 Zhongshan Rd., Xigang District, Dalian 116011, China. Email: huajuan0208@126.com.

**Background:** RNA 5-methylcytosine (m5C) methyltransferases *NSUN1* is a member of the NOP2/SUN (NSUN) RNA methyltransferase family. Studies have found that the expression of *NSUN1* is elevated in breast and colon cancer and can predict poor prognosis. However, the *NSUN1* gene has only been studied in a few tumors.

**Methods:** Single-cell RNA sequencing (scRNA-seq) and Bulk RNA-seq data were used for comprehensive analysis of *NSUN1* in cancers. The Human Protein Atlas (HPA) database was used to identify the gene location. Immunofluorescence staining was used to detect *NSUN1* subcellular distribution within the nucleus, endoplasmic reticulum (ER), and microtubules of A-431, U-2, U-251 cells. The cBioPortal tool was used to analyze the alteration frequency and mutation type. The epigenetic profile of *NSUN1* also was analyzed by using the University of Alabama at Birmingham CANcer data analysis Portal (UCLCAN). Tumor mutation burden (TMB), microsatellite instability (MSI), and immune checkpoint expression in cancers were analyzed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to perform enrichment and visualization. The study was based on online resources and public databases.

**Results:** Elevated *NSUN1* expression had been observed in most human cancers. Analysis of scRNA-seq data showed that *NSUN1* was highly expressed in immune cells such as T cells, B cells, and dendritic (DC) cells. High *NSUN1* expression indicated poor overall survival (OS) and disease-free survival (DFS). The characteristics of genetic alteration, methylation and phosphorylation of *NSUN1* were analyzed and higher levels of phosphorylation in tumor tissues were found. In addition, the expression of *NSUN1* was closely related to tumor-infiltrating immune cells. At the same time, the expression of *NSUN1* was positively correlated with the expression of multiple immune checkpoints.

**Conclusions:** The gene expression profile, survival status, genetic alteration, methylation, phosphorylation and infiltrating immune cells of *NSUN1* in human cancers were comprehensively analyzed. The results herein implied that *NSUN1* may be an effective biomarker for early cancer diagnosis, prognosis and therapy.

**Keywords:** *NSUN1*; cancer; tumor microenvironment; biomarker

Submitted Jan 13, 2023. Accepted for publication Jul 14, 2023. Published online Aug 28, 2023.
doi: 10.21037/tcr-23-66
**View this article at:** https://dx.doi.org/10.21037/tcr-23-66

## Introduction

RNA 5-methylcytosine (m5C) methyltransferases *NSUN1*, also known as NOP2 and P120, is a member of the NOP2/SUN (NSUN) RNA methyltransferase family, which includes seven members: NSUN1-7 (1-4). *NSUN1* is a nucleolar RNA-binding protein located on the chromosome 12p13.31 (5). Studies have found that *NSUN1* is involved in the positive regulation of cell population proliferation, the regulation of signal transduction by p53 class mediators, and the assembly of large ribosomal subunits (6,7).

It is noteworthy that *NSUN1* has been reported in six human cancers. The expression of *NSUN1* was significantly higher in cancer tissues than in the corresponding tumor adjacent normal tissues, as in the case of breast cancer (BRCA) (8), colon adenocarcinoma (COAD) (9), stomach adenocarcinoma (STAD) (10), prostate adenocarcinoma (PRAD) (11,12), rectum adenocarcinoma (READ) (13), and hepatocellular cancer (HCC) (14). It can promote the proliferation, invasion, migration of cancer cells *in vitro* and promote tumor growth and metastasis *in vivo* (10,14). In addition, it was found that long noncoding RNA (lncRNA)-hPVT1 promoted HCC cell proliferation, cell cycle and acquired stem cell-like properties by stabilizing NSUN1 protein. The regulation of lncRNA-hPVT1/NSUN1 pathway may have a positive effect on the treatment of HCC (14). The study also found that *NSUN1* gene expression was significantly associated with microsatellite instability (MSI), tumor mutation burden (TMB) and immunity in renal cancer, and elevated expression was associated with poor overall survival (OS) (13). These results suggest that *NSUN1* may be a potential prognostic factor for OS and a molecular marker for immunotherapy.

### Highlight box

**Key findings**

- *NSUN1* may be an effective biomarker for early cancer diagnosis, prognosis and therapy.

**What is known and what is new?**

- The expression of *NSUN1* is elevated in breast and colon cancer and can predict poor prognosis.
- Elevated *NSUN1* expression have been observed in most human cancers. High *NSUN1* expression indicates poor overall survival and disease-free survival.

**What is the implication, and what should change now?**

- These results herein imply that *NSUN1* may be an effective biomarker for early cancer diagnosis, prognosis and therapy.

To gain a more complete and comprehensive understanding of the *NSUN1* molecule and its role in various cancers, the expression, localization, variation, epigenetic modification and biological function of *NSUN1* in human cancers were analyzed herein. This study also characterized the expression of *NSUN1* in different immune cell types in the tumor immune microenvironment using single-cell RNA sequencing (scRNA-seq) data from public databases. The analysis herein provided evidence that *NSUN1* could be used as a potential diagnostic, prognostic, and therapeutic biomarker for cancer. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-66/rc).

## Methods

### *Gene expression analysis using Bulk RNA-seq and scRNA-seq data*

Gene expression in different tumor and normal tissues was analyzed using Tumor Immune Estimation Resource (TIMER2; http://timer.cistrome.org/). The Cancer Genome Atlas (TCGA) provided the data source of the public dataset. Receiver operating characteristic (ROC) curve (https://www.xiantao.love/) was drawn by using Xiantao tools. scRNA-seq and immunohistochemistry data were fetched from The Human Protein Atlas (HPA; https://www.proteinatlas.org/) website, and mapping was performed to demonstrate the expression of *NSUN1* in different cell types.

### *TMB, MSI and immune checkpoints expression analysis*

Studying the relationship between the expression of *NSUN1* and immune checkpoints [programmed death-1 (PD1), programmed cell death-ligand 1 (PDL1), cytotoxic T-lymphocyte antigen 4 (CTLA4), etc.] using Assistant for Clinical Bioinformatics (ACLBI; https://www.aclbi.com). Using the ACLBI was applied to study the relationship between *NSUN1* expression and TMB/MSI in various cancers. "Immune-related" and "Mutation Analysis" function were used in ACLBI tool. The parameter filled in "sample" was "all tumors".

### *Gene location and alteration analysis*

The HPA was used to identify the gene location. "SUBCELLULAR" and "HUMAN CELLS" function were used in HPA database. Immunofluorescence staining

was used to observe the localization of NSUN1 protein in A-431, U-2 OS and U251 MG cells. Immunohistochemical staining images were also obtained from the HPA database. The cBioPortal tool (http://www.cbioportal.org/) was used to analyze the alteration frequency and mutation type (15,16). "TCGA PanCancer Atlas Studies" was selected to analysis by "Query By Gene". The parameter filled in "Enter Genes" was "NOP2".

### *Gene methylation and phosphorylation characteristics analysis*

The University of ALabama at Birmingham CANcer data analysis Portal (UCLCAN; http://ualcan.path.uab.edu/index.html) was used to analyze the epigenetic profile of *NSUN1*. Genomic and proteomic data were obtained from the TCGA and Clinical Proteomic Tumor Analysis Consortium (CPTAC; https://cptac-data-portal.georgetown.edu/cptacPublic/).

### *Survival analysis*

The relationship between *NSUN1* expression and OS and disease-free survival (DFS) was analyzed using Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (17). High and low expression groups were split using the median as the expression threshold. Survival plots use Cox proportional hazard ratio and 95% confidence interval information. Hypothesis testing was performed using the log-rank test.

### *Enrichment analysis*

The top100 genes significantly correlated with *NSUN1* expression were found by GEPIA2 analysis. The R package of "clusterProfiler", "enrichplot" and "ggplot2" were used to perform the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment and visualization.

### *Statistical analysis*

Data and figures for the article was obtained from public databases (Table S1). GO and KEGG pathway enrichment analyses were performed using R software (Version 4.0.2). This study was conducted in compliance with the Helsinki Declaration (as revised in 2013).

## Results

### *NSUN1 is highly expressed in cancer*

We analyzed the expression of *NSUN1* in various tumors using Bulk RNA-seq data, and found that the expression of *NSUN1* increased significantly in most tumors, and it was more significant in BRCA, cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC) (*Figure 1A*, Figure S1). The increased *NSUN1* expression was also confirmed at protein level (*Figure 1B*, Figure S2A). Furthermore, the ROC curve was used to evaluate the sensitivity and specificity of *NSUN1* in predicting cancer, and the results confirmed the important value of *NSUN1* in predicting cancer (Figure S2B). This finding has important clinical value in the early diagnosis of cancer. *NSUN1* may be a promising biomarker for the early and accurate diagnosis.

scRNA-seq is widely used in cancer research, and its ability to analyze specific molecular signatures of different cell groups is of great value in revealing tumor heterogeneity. Understanding the infiltration characteristics of immune cells in the tumor immune microenvironment is of great significance for exploring new immune checkpoints and guiding the application of immunotherapy drugs. Using scRNA-seq data from public databases, we found that *NSUN1* expression was significantly high in immune cells than in other cell types in breast, liver, lung, stomach, lymph node, endometrium, and bone marrow (*Figure 2A-2G*). For example, in breast, the expression of *NSUN1* was higher in T cells and dendritic cells (DCs) (*Figure 2A*). In liver, stomach and lymph nodes, *NSUN1* expression was higher in B cells and T cells (*Figure 2B,2F,2G*). These findings suggest that *NSUN1* may be more involved in immune regulation in these organs. In cancers derived from these organs, *NSUN1* may play a more important role in immune regulation.

### *The expression of NSUN1 was highly correlated with the expression of TMB, MSI and immune checkpoints*

TMB and MSI status were closely related to immunotherapy efficacy. Microsatellite-stable (MSS) tumors with high tumor mutational burden (TMB-H) benefit from immunotherapy. We analyzed TMB, MSI and immune checkpoints expression in cancers. *NSUN1* expression was positively correlated with MSI in most

 

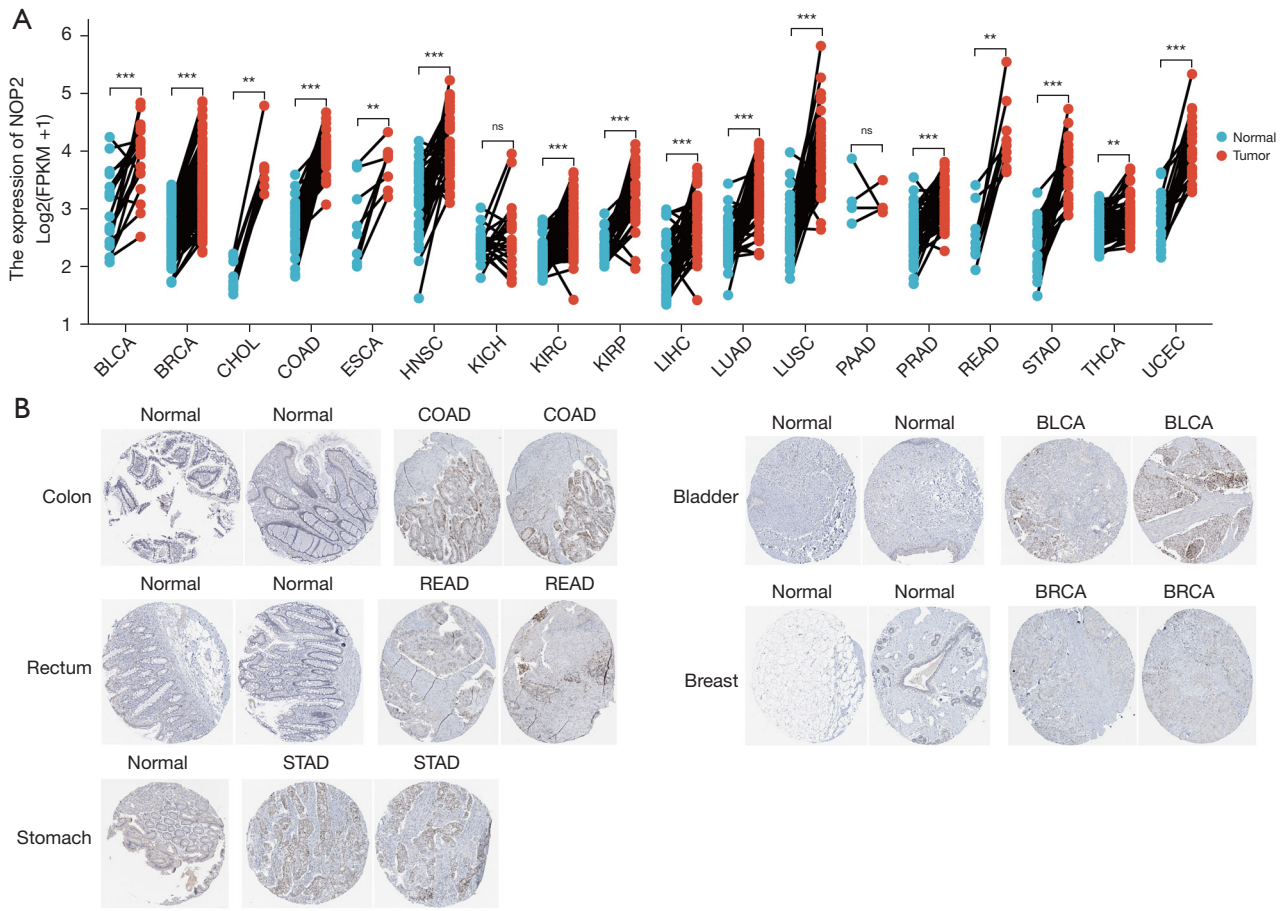


**Figure 1** The expression of NSUN1 in different tumors. (A) Expression levels of NSUN1 in tumor and normal tissues (TCGA database). (B) NSUN1 immunohistochemistry in colon, rectum, stomach, bladder, and breast (×40) (https://www.proteinatlas.org/ENSG00000111641-NOP2/pathology) (HPA database). ns, no statistical significance; **, P<0.01; ***, P<0.001. FPKM, Fragments per Kilobase Million; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; TCGA, The Cancer Genome Atlas; HPA, Human Protein Atlas.

cancers, except for diffuse large B-cell lymphoma (DLBC), CHOL, uterine carcinosarcoma (UCS), acute myeloid leukemia (LAML) and head and neck squamous cell carcinoma (HNSC) (*Figure 3A*). Moreover, we also observed a positive correlation between *NSUN1* expression and TMB in most cancers, except for thymoma (THYM), UCS, LAML, CHOL, uveal melanoma (UVM), ESCA and kidney renal papillary cell carcinoma (KIRP) (*Figure 3B*). Interestingly, we found that expression of immune checkpoints-related genes was associated with *NSUN1* expression, especially in skin cutaneous melanoma (SKCM), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), UCS, LIHC and kidney renal clear cell carcinoma (KIRC) (*Figure 3C*). It remains to be studied whether *NSUN1* expression may affect the efficacy of immunotherapy. In addition, we researched the relationship between *NSUN1* expression and immune cells in the tumor microenvironment. We found that *NSUN1* expression was negatively associated with the number of infiltrating immune cells in some cancers, such as COAD, lung adenocarcinoma (LUAD), LUSC, SKCM, STAD and TGCT, suggesting that *NSUN1* might play a role in immune evasion (Figure S3).

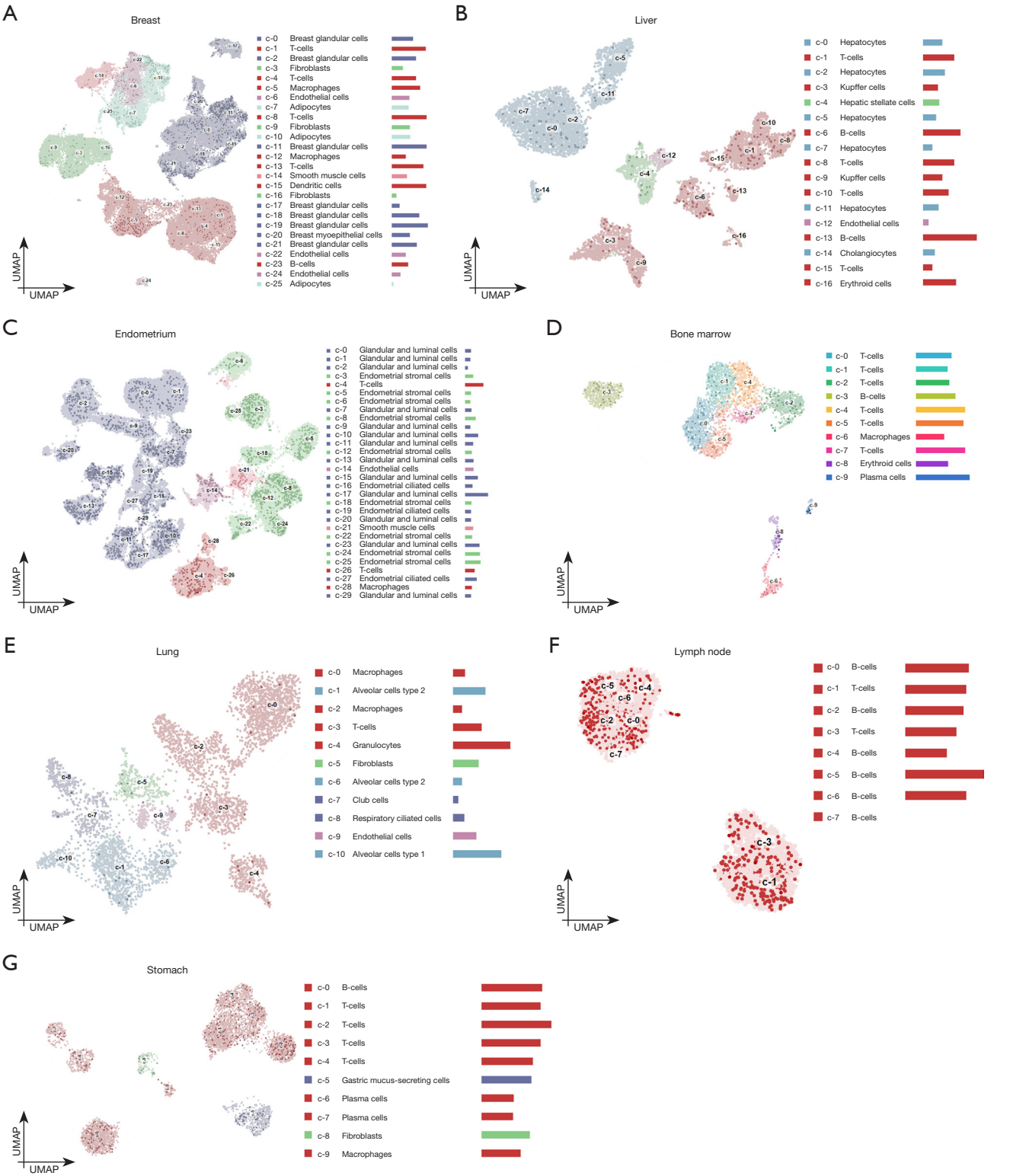


**Figure 2** scRNA-seq data analysis was performed to show the expression of NSUN1 in different cell types in breast (A), liver (B), endometrium (C), bone marrow (D), lung (E), lymph node (F), and stomach (G) (https://www.proteinatlas.org/ENSG00000111641-NOP2/single+cell+type) (HPA database). UMAP, uniform manifold approximation and projection; HPA, Human Protein Atlas.

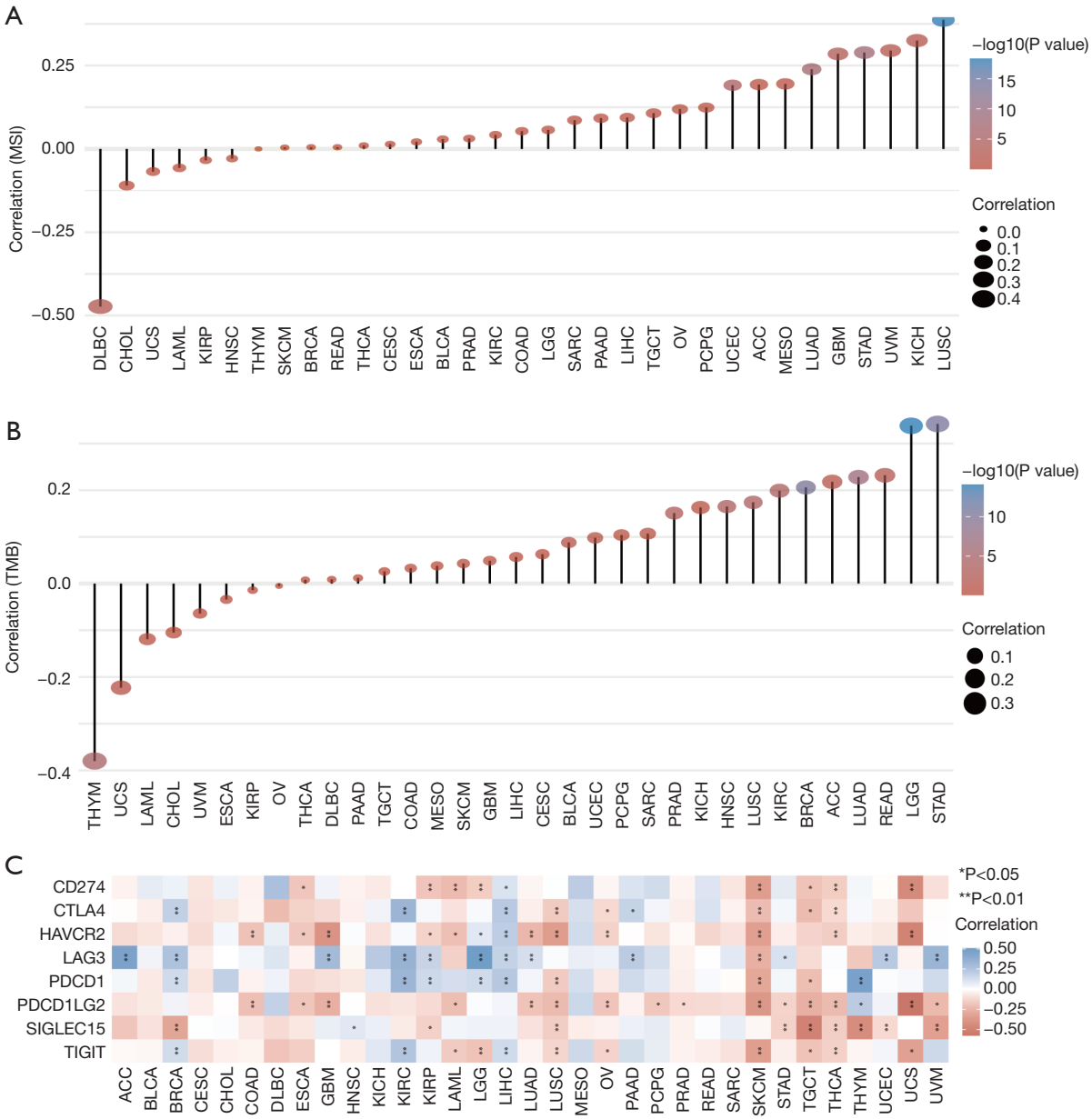


**Figure 3** The relationship between MSI, TMB, immune checkpoints expression and *NSUN1* expression. (A) MSI. (B) TMB. (C) Immune checkpoints (TCGA database). *, P<0.05; **, P<0.01. MSI, microsatellite instability; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; CHOL, cholangiocarcinoma; UCS, uterine carcinosarcoma; LAML, acute myeloid leukemia; KIRP, kidney renal papillary cell carcinoma; HNSC, head and neck squamous cell carcinoma; THYM, thymoma; SKCM, skin cutaneous melanoma; BRCA, breast invasive carcinoma; READ, rectum adenocarcinoma; THCA, thyroid carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; ESCA, esophageal carcinoma; BLCA, bladder urothelial carcinoma; PRAD, prostate adenocarcinoma; KIRC, kidney renal clear cell carcinoma; COAD, colon adenocarcinoma; LGG, brain lower grade glioma; SARC, sarcoma; PAAD, pancreatic adenocarcinoma; LIHC, liver hepatocellular carcinoma; TGCT, testicular germ cell tumors; OV, ovarian serous cystadenocarcinoma; PCPG, pheochromocytoma and paraganglioma; UCEC, uterine corpus endometrial carcinoma; ACC, adrenocortical carcinoma; MESO, mesothelioma; LUAD, lung adenocarcinoma; GBM, glioblastoma multiforme; STAD, stomach adenocarcinoma; UVM, uveal melanoma; KICH, kidney chromophobe; LUSC, lung squamous cell carcinoma; TMB, tumor mutational burden; TCGA, The Cancer Genome Atlas.

### *Location, variation, methylation, and phosphorylation characteristics of NSUN1*

Immunofluorescence staining was used to detect *NSUN1* subcellular distribution within the nucleus, endoplasmic reticulum (ER), and microtubules of A-431, U-2, U-251 cells. The result showed that the *NSUN1* was located in the nucleus (*Figure 4*). The DNA alteration of *NSUN1* was mainly amplification and mutation in TCGA pan-cancer. The frequency of *NSUN1* alteration (>6%) was the highest in ovarian serous cystadenocarcinoma (OV) with "amplification" as the primary type (*Figure 5A*). In the log-rank test, patients in altered group did significantly worse in OS (P=0.0151), progression-free survival (PFS; P<0.0001) and disease-specific survival (DSS; P=0.0127) (*Figure 5B*). These data suggested that genetic variation of *NSUN1* may be important prognostic factors for cancer patients.

Phosphorylation is a critical signaling process for cell biological behavior. Interestingly, the present study found that phosphorylation of NSUN1 protein was significantly increased in tumor tissues compared with normal samples. Results from different cancers and different phosphorylation sites were reproducible and consistent (*Figure 6*, Figure S4). The results suggest that the phosphorylation modification of *NSUN1* may be an important factor in the regulation of gene function. To clarify epigenetic characteristics of *NSUN1*, five probes in promoter were used for detecting DNA methylation level of *NSUN1* (*Figure 7A*). This result suggests that the DNA methylation levels increased in BRCA, COAD, THCA etc., while decreased in KIRC, LIHC etc. (*Figure 7B*,*7C*). Our analysis shows that, across different cancers, promoter methylation levels are inconsistent with *NSUN1* expression.

### *High expression of NSUN1 predicts poor prognosis*

We analyzed the impact of *NSUN1* on clinical prognosis. It was found that *NSUN1* has good predictive value for OS and DFS in cancer patients. In adrenocortical carcinoma (ACC), kidney chromophobe (KICH), KIRC, KIRP, LAML, brain lower grade glioma (LGG), mesothelioma (MESO), SKCM and UVM, patients with high *NSUN1* expression had worse OS. In ACC, KIRP, LGG, LIHC, sarcoma (SARC), SKCM and UVM, patients with high *NSUN1* expression had worse DFS (*Figure 8*). These results suggest that *NSUN1* may be an effective biomarker for prognosis.

### *GO and KEGG enrichment*

Finally, we used the TCGA dataset to obtain 100 genes that were significantly correlated with *NSUN1* expression through correlation analysis, and performed enrichment analysis. GO enrichment analysis showed that *NSUN1*-related genes were significantly related to ribosome biogenesis, DNA replication, rRNA processing and other biological processes (*Figure 9A*,*9B*). The locations of action were mainly chromosomes and ribosomes (*Figure 9C*,*9D*). *NSUN1* can bind to DNA/RNA to exert biological functions such as helicase and catalytic activity (*Figure 9E*,*9F*). In addition, KEGG enrichment analysis found that *NSUN1* may also be related to biological behaviors such as cell cycle and mismatch repair (*Figure 9G*).

## Discussion

*NSUN1* is an RNA m5C methylase that mainly affects the expression or function of downstream molecules through epigenetic modifications. Studies have found that *NSUN1* can regulate biological behaviors such as tumor cell growth and migration (12,13,18,19). At present, *NSUN1* has been found to play a role in promoting cancer in 7 kinds of tumors, and high expression of *NSUN1* predicts poor prognosis (8-13,20). So far, the research on *NSUN1* is still in its infancy, with only few examples reported. Therefore, we combined scRNA-seq and Bulk RNA-seq data to conduct a comprehensive analysis of the molecular biological characteristics of *NSUN1* based on the TCGA, Gene Expression Omnibus (GEO), and CPTAC databases, with the aim of revealing the role of *NSUN1* in human cancers.

This study found that *NSUN1* was mainly localized in the nucleus and was highly expressed in most tumor tissues. This study showed that *NSUN1* was highly expressed in bladder urothelial carcinoma (BLCA), BRCA, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), CHOL, COAD, ESCA, glioblastoma multiforme (GBM), HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, uterine corpus endometrial carcinoma (UCEC). Furthermore, we found that *NSUN1* overexpression generally predicted poor OS and/or DFS in patients with ACC, KICH, KIRC, KIRP, LAML, LGG, LIHC, MESO, SARC, SKCM, and UVM. These findings suggest that *NSUN1* may be a valuable marker for early diagnosis and prognosis.

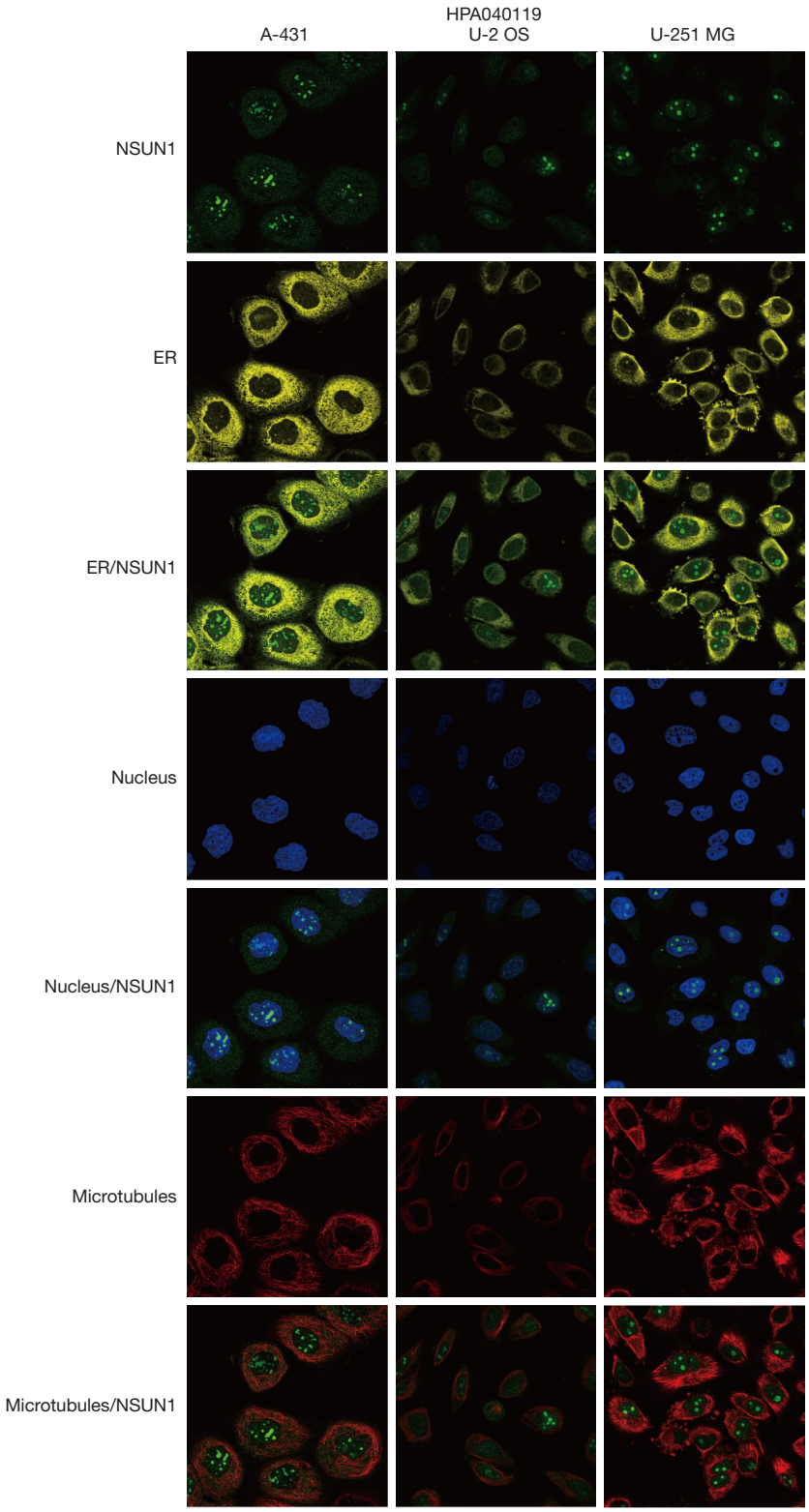


**Figure 4** Immunofluorescence staining. Detect *NSUN1* subcellular distribution within the nucleus, ER, and microtubules of A-431, U-2, U-251 cells (×1,000) (https://www.proteinatlas.org/ENSG00000111641-NOP2/subcellular) (HPA database). ER, endoplasmic reticulum; HPA, Human Protein Atlas.

 

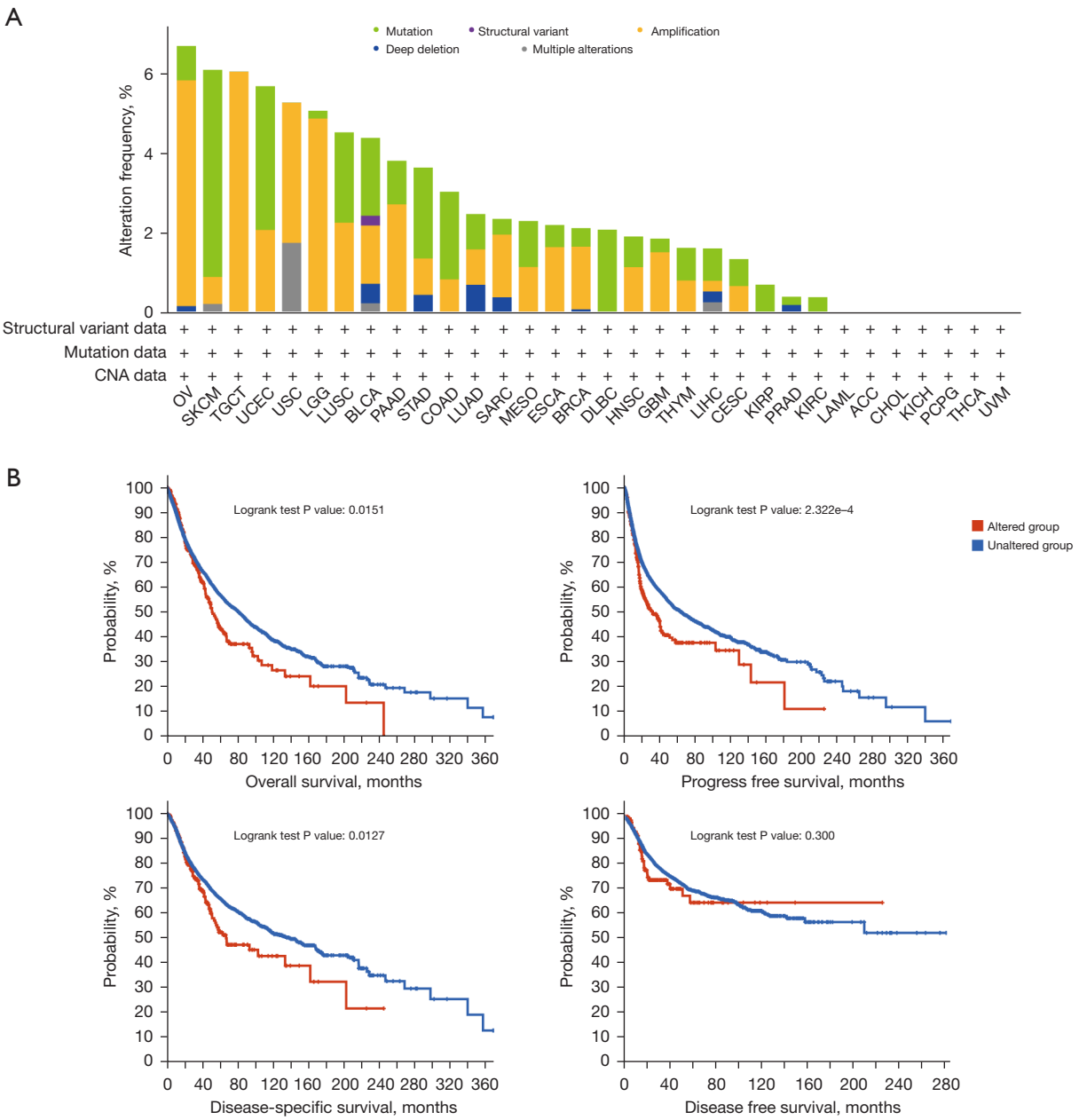


**Figure 5** Mutation feature of *NSUN1* in human tumors. (A) The alteration frequency in different cancers. (B) Impact of *NSUN1* altered variant on OS, PFS, DSS and DFS (cBioPortal database). CNA, copy number alterations; OV, ovarian serous cystadenocarcinoma; SKCM, skin cutaneous melanoma; TGCT, testicular germ cell tumors; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; LGG, brain lower grade glioma; LUSC, lung squamous cell carcinoma; BLCA, bladder urothelial carcinoma; PAAD, pancreatic adenocarcinoma; STAD, stomach adenocarcinoma; COAD, colon adenocarcinoma; LUAD, lung adenocarcinoma; SARC, sarcoma; MESO, mesothelioma; ESCA, esophageal carcinoma; BRCA, breast invasive carcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; HNSC, head and neck squamous cell carcinoma; GBM, glioblastoma multiforme; THYM, thymoma; LIHC, liver hepatocellular carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; KIRP, kidney renal papillary cell carcinoma; PRAD, prostate adenocarcinoma; KIRC, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; ACC, adrenocortical carcinoma; CHOL, cholangiocarcinoma; KICH, kidney chromophobe; PCPG, pheochromocytoma and paraganglioma; THCA, thyroid carcinoma; UVM, uveal melanoma; OS, overall survival; PFS, progression-free survival; DSS, disease-specific survival; DFS, disease-free survival.

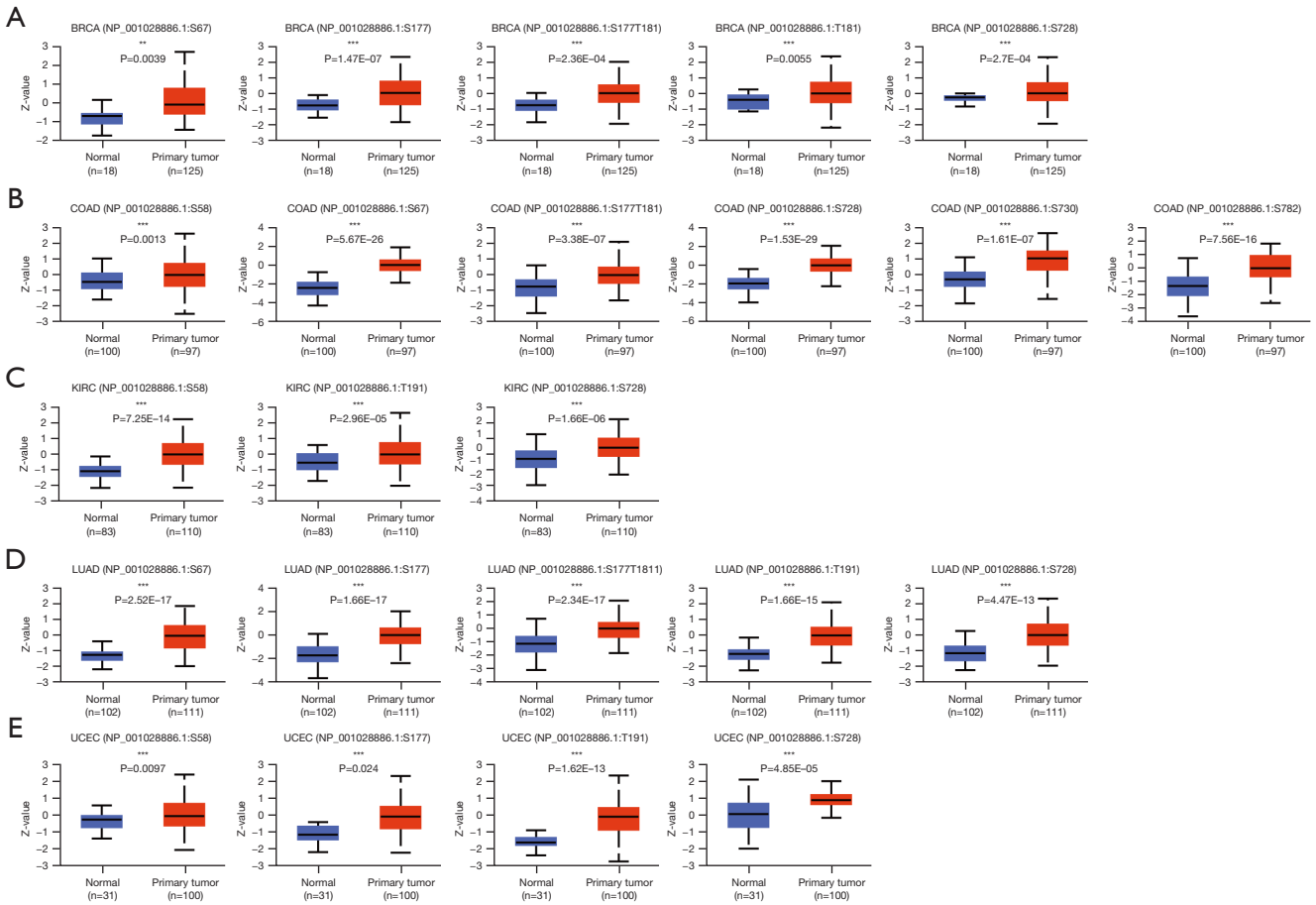


**Figure 6** Protein phosphorylation of NSUN1 in different tumors. (A) BRCA. (B) COAD. (C) KIRC. (D)LUAD. (E) UCEC (UALCAN database). **, P<0.01; ***, P<0.001. BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; UALCAN, The University of ALabama at Birmingham CANcer data analysis Portal.

Predictive biomarkers in cancer patients are almost always based on the analysis of the entire biopsy sample, and their specific molecular mechanisms are still unclear, which affects the clinical application. The application of scRNA-seq technology has solved this problem to a certain extent. In this study, by analyzing scRNA-seq data, we found that in breast, liver, and stomach, the expression of *NSUN1* was significantly higher in T cells, B cells, and DC cells than in other types of cells. This result suggests that *NSUN1* may participate in various biological processes mainly by affecting the function of immune cells. In addition, this suggests that if *NSUN1* becomes a molecular target for tumor therapy in the future, its main regulatory mechanism is to affect the functions of T cells and B cells to participate in molecular therapy.

The immune-related research results of this study found that *NSUN1* negatively regulated the infiltration of immune cells in most tumors and participated in the regulation of the tumor immune microenvironment. *NSUN1* inhibited the infiltration of DC cells, monocytes/macrophages and $CD8^+$ T cells, especially in SKCM, STAD, TGCT. This result preliminarily confirmed our hypothesis: *NSUN1* promotes the proliferation, invasion and metastasis of cancer cells by inhibiting the infiltration of immune cells in tumor tissues.

Phosphorylation is one of the most common post-translational modifications in proteins and is involved in a wide range of cellular activities including cell growth, differentiation and apoptosis (21,22). In cancer, the balance of activation and inactivation of many key kinases is delicately maintained through phosphorylation, and

 

A

| Illumina ID | RefGene_group |
|---|---|
| cg22225892 | TSS200 |
| cg17975492 | TSS200 |
| cg0686393 | TSS200 |
| cg12806521 | TSS200 |
| cg00383178 | TSS200 |

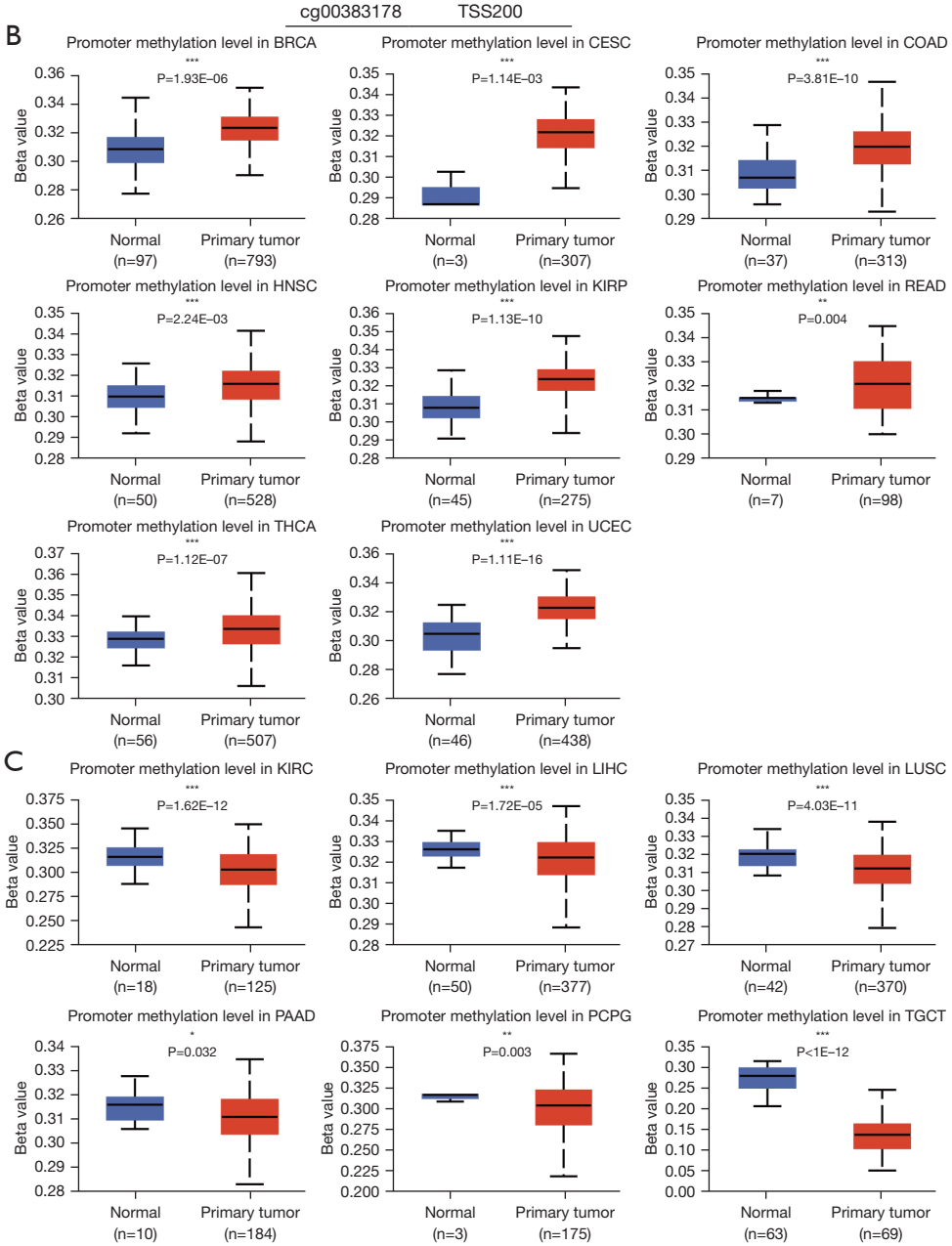


**Figure 7** The DNA methylation level in different tumors. (A) Illumina probe ID. (B) Promoter methylation level upregulated tumors. (C) Promoter methylation level downregulated tumors (UALCAN database). *, P<0.05; **, P<0.01; ***, P<0.001. BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD, colon adenocarcinoma; HNSC, head and neck squamous cell carcinoma; KIRP, kidney renal papillary cell carcinoma; READ, rectum adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; TGCT, testicular germ cell tumors; UALCAN, The University of ALabama at Birmingham CANcer data analysis Portal.

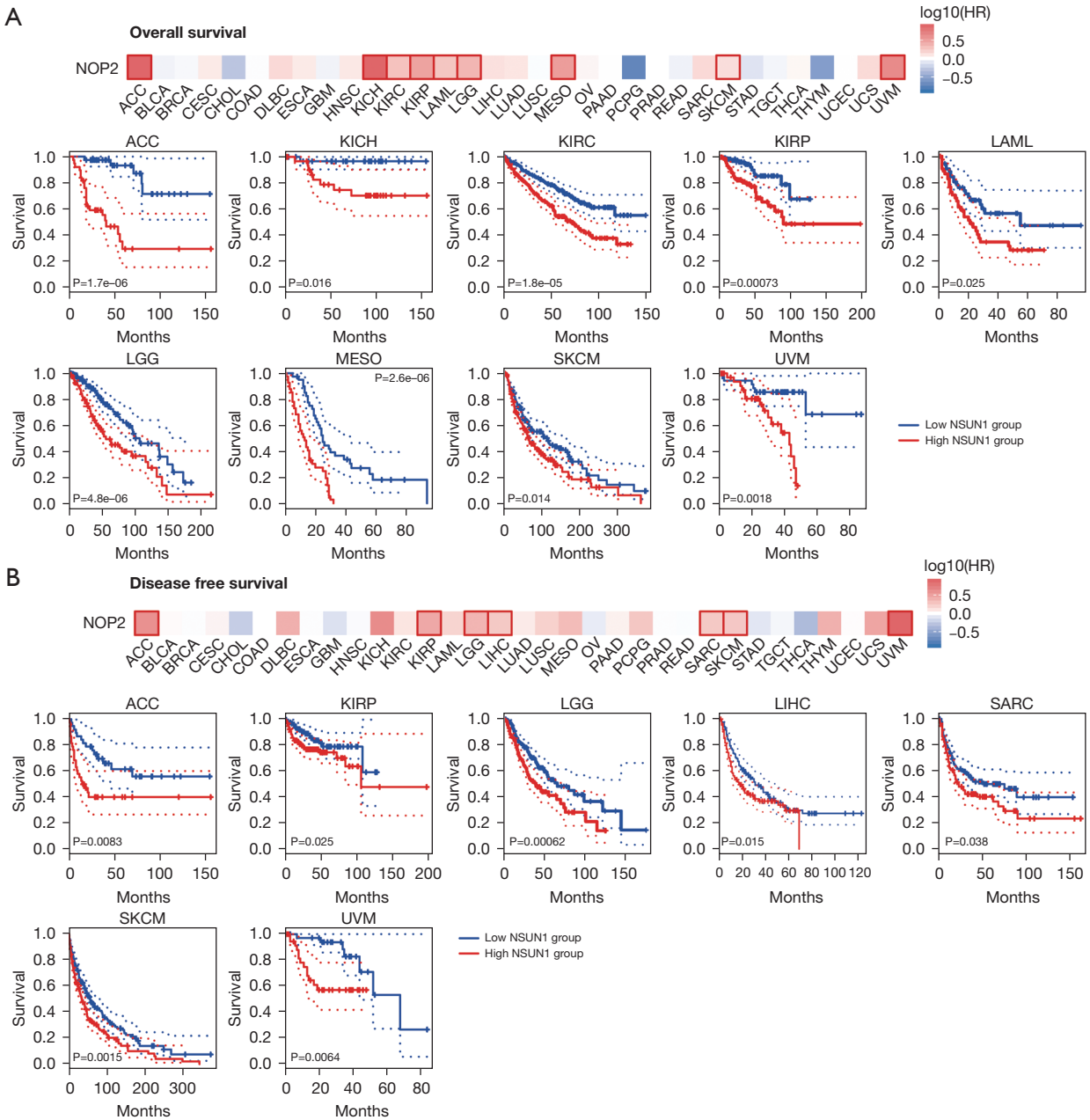


**Figure 8** Relationship between *NSUN1* expression level and survival. (A) Overall survival. (B) Disease-free survival. The positive results of survival map and Kaplan-Meier curves are listed (GEPIA2 database). HR, hazard ratio; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; GEPIA2, gene expression profiling interactive analysis.

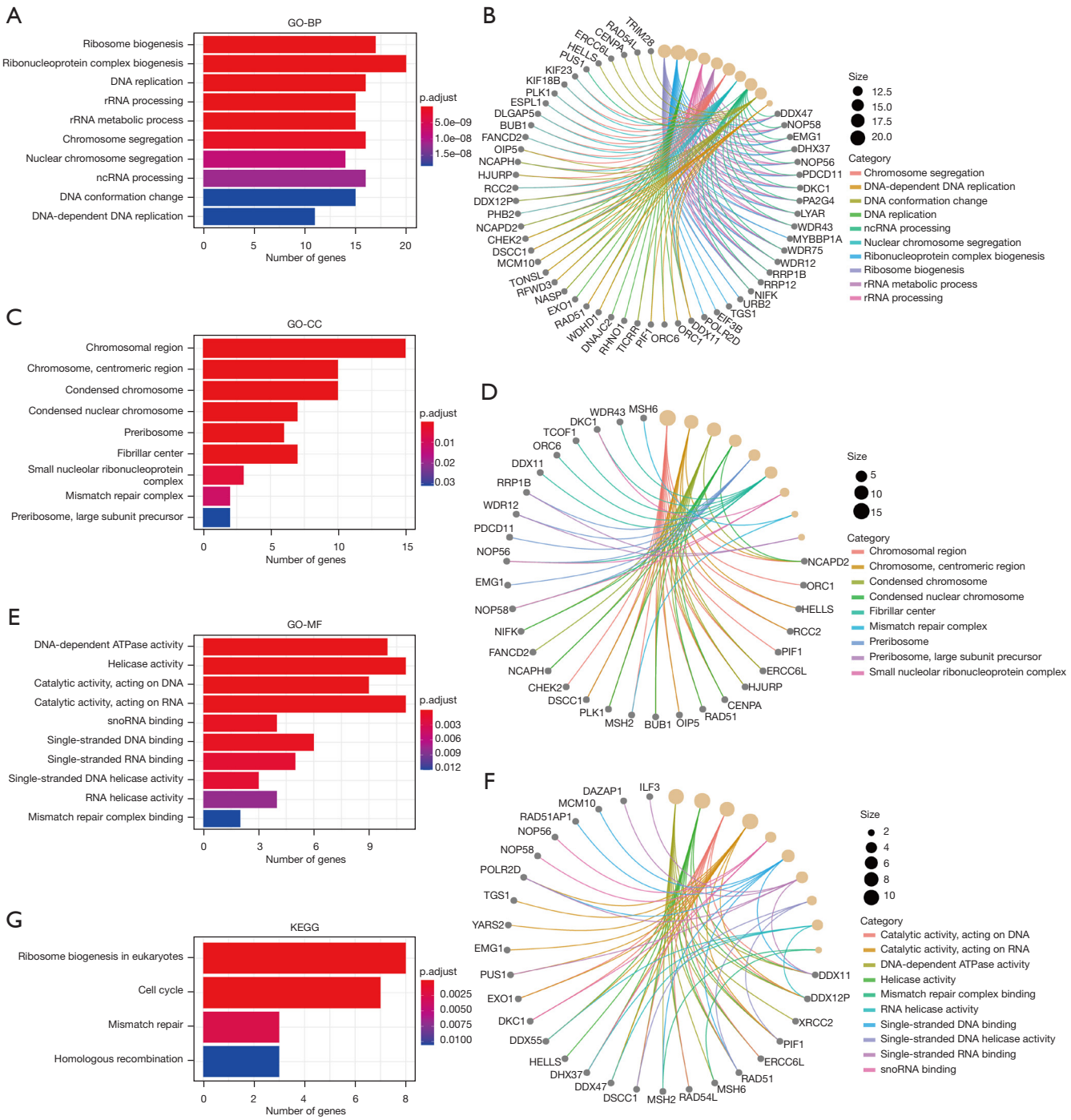


**Figure 9** *NSUN1*-related genes enrichment. (A) Barplot and (B) Cnetplot of GO-BP analysis. (C) Barplot and (D) cnetplot of GO-CC analysis. (E) Barplot and (F) cnetplot of GO-MF analysis. (G) KEGG pathway analysis. GO, Gene Ontology; BP, biological process; CC, cell component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

dysregulation of these processes results in disruption of signal transduction and metabolism. In this study, we found that the phosphorylation sites of *NSUN1* were different in different tumors, but the phosphorylation level in tumors was higher than that in the corresponding normal tissues. This finding indicates that the phosphorylation of *NSUN1* may be involved in the biological process of tumors as an important regulatory way. By targeting and regulating the phosphorylation of *NSUN1*, it may become a new approach for cancer treatment in the future. A limitation of this study is that all research findings are based on data analysis. The specific molecular mechanism still needs to be explored and discovered through basic research. The significance of this study is that it provides a new direction for future basic research.

## Conclusions

Four results of this study are very important. First, among all tumors with differential *NSUN1* expression, *NSUN1* expression was higher in tumor tissues. Second, in survival analysis, high *NSUN1* expression predicted poor prognosis. Third, in different tumors, the phosphorylation modification level of tumor tissue was higher than that of normal tissue. Fourth, *NSUN1* is associated with immune cell infiltration and may be involved in regulating the immune microenvironment. These findings indicate that *NSUN1* plays a key role in the occurrence and development of tumors, and reflects its important value in the early diagnosis and prognosis of tumors.

## Acknowledgments

We would like to thank Elvira Elizabeth (Shandong Foreign Language Vocational and Technical University) for her help in polishing our paper.
*Funding:* This work was supported by the Beijing Municipal Science & Technology Commission (No. Z171100001017077).

## Footnote

*Reporting Checklist:* The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-66/rc

*Peer Review File:* Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-66/prf

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-66/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. This study was conducted in compliance with the Helsinki Declaration (as revised in 2013).



## References

1. Delaunay S, Pascual G, Feng B, et al. Mitochondrial RNA modifications shape metabolic plasticity in metastasis. Nature 2022;607:593-603.
2. Liao H, Gaur A, McConie H, et al. Human NOP2/NSUN1 regulates ribosome biogenesis through non-catalytic complex formation with box C/D snoRNPs. Nucleic Acids Res 2022;50:10695-716.
3. Blanco S, Frye M. Role of RNA methyltransferases in tissue renewal and pathology. Curr Opin Cell Biol 2014;31:1-7.
4. Kong W, Biswas A, Zhou D, et al. Nucleolar protein NOP2/NSUN1 suppresses HIV-1 transcription and promotes viral latency by competing with Tat for TAR binding and methylation. PloS Pathog 2020;16:e1008430.
5. Yu J, Deshmukh H, Payton JE, et al. Array-based comparative genomic hybridization identifies CDK4 and FOXM1 alterations as independent predictors of survival in malignant peripheral nerve sheath tumor. Clin Cancer Res 2011;17:1924-34.
6. Sloan KE, Bohnsack MT, Watkins NJ. The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress. Cell Rep 2013;5:237-47.
7. Heissenberger C, Rollins JA, Krammer TL, et al. The ribosomal RNA m(5)C methyltransferase NSUN-1

 

modulates healthspan and oogenesis in Caenorhabditis elegans. Elife 2020;9:e56205.
8. Fonagy A, Swiderski C, Ostrovsky AM, et al. Effect of nucleolar P120 expression level on the proliferation capacity of breast cancer cells. Cancer Res 1994;54:1859-64.
9. Bi J, Huang Y, Liu Y. Effect of NOP2 knockdown on colon cancer cell proliferation, migration, and invasion. Transl Cancer Res 2019;8:2274-83.
10. Feng J, Zhang J, Li Y, et al. Upregulated expression of NOP2 predicts worse prognosis of gastric adenocarcinoma by promoting tumor growth. Saudi J Gastroenterol 2022;28:369-77.
11. Sun F, Wu K, Yao Z, et al. Long Noncoding RNA PVT1 Promotes Prostate Cancer Metastasis by Increasing NOP2 Expression via Targeting Tumor Suppressor MicroRNAs. Onco Targets Ther 2020;13:6755-65.
12. Sun F, Wu K, Yao Z, et al. Long noncoding RNA LINC00963 induces NOP2 expression by sponging tumor suppressor miR-542-3p to promote metastasis in prostate cancer. Aging (Albany NY) 2020;12:11500-16.
13. Wang G, Qu F, Liu S, et al. Nucleolar protein NOP2 could serve as a potential prognostic predictor for clear cell renal cell carcinoma. Bioengineered 2021;12:4841-55.
14. Wang F, Yuan JH, Wang SB, et al. Oncofetal long noncoding RNA PVT1 promotes proliferation and stem cell-like property of hepatocellular carcinoma cells by stabilizing NOP2. Hepatology 2014;60:1278-90.
15. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.
16. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
17. Tang Z, Kang B, Li C, et al. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019;47:W556-60.
18. Saijo Y, Sato G, Usui K, et al. Expression of nucleolar protein p120 predicts poor prognosis in patients with stage I lung adenocarcinoma. Ann Oncol 2001;12:1121-5.
19. Saijo Y, Perlaky L, Valdez BC, et al. The effect of antisense p120 construct on p120 expression and cell proliferation in human breast cancer MCF-7 cells. Cancer Lett 1993;68:95-104.
20. Gao Y, Wang Z, Zhu Y, et al. NOP2/Sun RNA methyltransferase 2 promotes tumor progression via its interacting partner RPL6 in gallbladder carcinoma. Cancer Sci 2019;110:3510-9.
21. Bucci M. Functional phosphorylation. Nat Chem Biol 2018;14:525.
22. Kholodenko BN, Okada M. Reengineering protein-phosphorylation switches. Science 2021;373:25-6.

**Cite this article as:** Fang Z, Li P, Li F. Combining Bulk RNA-seq and scRNA-seq data to identify RNA m5C methyltransferases *NSUN1*: a rising star as a biomarker for cancer diagnosis, prognosis and therapy. Transl Cancer Res 2023;12(9):2336-2350. doi: 10.21037/tcr-23-66

 