NSUN5 is Upregulated and Positively Correlated with Translation in Human Cancers: A Bioinformatics-based Study

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Abstract

The role of RNA m⁵C (5-methylcytosine) and RNA m⁵C methyltransferases (RCMTs, including NSUN1, NSUN2, NSUN3, NSUN4, NSUN5, NSUN6, NSUN7 and TRDMT1) in human cancers remains largely unknown. In this study, GEPIA2 was used to compare the expression of RCMTs in human cancers and that in associated normal tissues, and to analyze the prognosis value of NSUN5 expression. UALCAN was used to compare the methylation level of NSUN5 promoter in human cancers and that in associated normal tissues. LinkedOmics was used perform BPs (biological processes), CCs (cellular components), MFs (molecular functions) and KEGG pathways analyses of NSUN5-correlated genes in each cancer one by one. We found that six RCMTs (NSUN1-NSUN5 and TRDMT1), especially NSUN5, were generally upregulated in human cancers, that the hypomethylation of NSUN5 promoter may be responsible for its upregulation, and that overexpressed NSUN5 predicted poorer prognosis and was positively correlated with translation in human cancers. The function of NSUN5 in human cancers and its mechanism need to be validated by biological experiments.

Introduction

According to MODOMICS (<https://iimcb.genesilico.pl/modomics/>), there are at least 171 types of RNA modifications so far.^[1] m⁶A (N⁶-methyladenosine) is the most pervasive RNA modification, and can affect growth and metastasis in multiple cancers (glioblastoma, lung cancer, hepatocellular carcinoma, et al) by influencing RNA biogenesis, splicing, degradation or translation.^[2] However, the role of other types of RNA modifications in human cancers is poorly studied.

In recent years, m⁵C (5-methylcytosine) has been found to be widespread in human rRNA (ribosomal RNA), tRNA (transfer RNA) and mRNA (messenger RNA).^[3, 4] There are eight kinds of human RNA m⁵C methyltransferases (RCMTs): NSUN1 (NOP2), NSUN2, NSUN3, NSUN4, NSUN5, NSUN6, NSUN7 and TRDMT1 (DNMT2).^[5] Only a small number of studies has reported the role of m⁵C and RCMTs in human cancers. For example, m⁵C and RCMTs (NSUN1, NSUN3 and TRDMT1) mediate chromatin organization and 5-azacytidine response and resistance in leukemia.^[6] m⁵C, induced by upregulated NSUN2, promotes pathogenesis of bladder cancer through stabilizing oncogene mRNAs.^[7] m⁵C regulation and its functions in human cancers remains largely unknown and need further research.

In this study, using public databases, we analyzed the expression, prognostic value and possible function of NSUN5 (the most obviously upregulated RCMT) in multiple human cancers.

Results

The expression of RCMTs, especially NSUN5, is generally upregulated in cancers

GEPIA2^[8] (<http://gepia2.cancer-pku.cn/#index>) was used to explore the expression of RCMTs in cancers. As is shown in Table 1 and Fig. 1, the expression of RCMTs, except NSUN6 and NSUN7, is generally upregulated in cancers, compared to associated normal tissues respectively. In detail, NSUN1, NSUN2, NSUN3, NSUN4, NSUN5, NSUN6, NSUN7 and TRDMT1 were respectively upregulated in 23, 19, 18, 18, 25, 7, 12 and 13 types of cancers out of all the 31 types of cancers. Among them, the number of cancer types with upregulated NSUN5 was the most. (Table 1) NSUN5 was upregulated in 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), KIRP (Kidney renal papillary cell carcinoma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), OV (Ovarian serous cystadenocarcinoma), PAAD (Pancreatic adenocarcinoma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), SKCM (Skin Cutaneous Melanoma), STAD (Stomach adenocarcinoma), TGCT (Testicular Germ Cell Tumors), THYM (Thymoma), UCEC (Uterine Corpus Endometrial Carcinoma) and UCS (Uterine Carcinosarcoma), downregulated in KIRC (Kidney renal clear cell carcinoma), LAML (Acute Myeloid Leukemia) and THCA (Thyroid carcinoma), and not significantly changed in LGG (Brain Lower Grade Glioma), PCPG (Pheochromocytoma and Paraganglioma) and SARC (Sarcoma). (Fig. 1) Therefore, we chose NSUN5 for further study.

Table 1
The expression of RCMTs in 31 types of cancers according to GEPIA2.

RCMTs Cancers	NSUN1	NSUN2	NSUN3	NSUN4	NSUN5	NSUN6	NSUN7	TRDMT1
ACC	Down	NS	Down	Down	Up	Down	Down	Down
BLCA	Up	Up	NS	NS	Up	NS	NS	Down
BRCA	Up	Up	Up	Up	Up	Down	Up	Down
CESC	Up	NS	NS	NS	Up	Down	NS	Down
CHOL	Up	Up	Up	Up	Up	Down	Up	Up
COAD	Up	Up	Up	Up	Up	Up	Up	Up
DLBC	Up	Up	Up	Up	Up	Up	Down	Up
ESCA	Up	Up	Up	Up	Up	NS	NS	Up
GBM	Up	Up	Up	Up	Up	Down	Up	Up
HNSC	Up	Up	Up	Up	Up	Up	Down	NS
KICH	Down	Up	NS	Down	Up	Down	Up	Down
KIRC	Up	Up	NS	NS	Down	NS	Down	NS
KIRP	Up	Up	NS	Up	Up	Up	NS	NS
LAML	Up	Down	Up	Down	Down	Up	Up	Up
LGG	Up	Up	Up	Up	NS	Up	Up	Up
LIHC	Up	NS	Up	Up	Up	Down	NS	Up
LUAD	NS	Up	Up	Up	Up	Down	NS	Down
LUSC	Up	Up	Up	Up	Up	Down	Down	Down
OV	Up	Down	Up	Up	Up	Down	Up	Down
PAAD	Up	Up	Up	Up	Up	NS	Down	Up
PCPG	NS	NS	NS	Down	NS	NS	Down	NS
PRAD	Down	Down	NS	NS	Up	Down	Up	NS
READ	Up	Up	Up	Up	Up	NS	Up	Up
SARC	Up	Up	NS	NS	NS	NS	NS	NS
SKCM	Up	Down	Up	Up	Up	Down	Down	Up
STAD	Up	Up	Up	Up	Up	Down	Down	Up
TGCT	Up	NS	NS	Down	Up	Down	Down	Down
THCA	Down	Down	Down	Down	Down	Down	Down	Down
THYM	Up	Up	Up	Up	Up	Up	Down	Up
UCEC	NS	Down	Down	NS	Up	Down	Up	Down
UCS	NS	Down	Down	NS	Up	Down	Up	Down
Number of Cancer Types (Up: NS: Down)	23:4:4	19:5:7	18:9:4	18:7:6	25:3:3	7:7:17	12:7:12	13:6:12
RCMTs, RNA m ⁵ C methyltransferases; NS, not significant.								

The hypomethylation of NSUN5 promoter may be responsible for its upregulation in cancers

To explore why NSUN5 was generally downregulated in cancers, we compared the methylation level of NSUN5 promoter in cancers and associated normal tissues using UALCAN^[9] (<http://ualcan.path.uab.edu/>). Among the 28 types of cancers with DNA methylation data, the methylation level of NSUN5 promoter was downregulated in BRCA, HNSC, KIRC, LIHC, PAAD, STAD, TGCT, THCA and UCEC. Importantly, in BRCA, HNSC, LIHC, PAAD, STAD, TGCT and UCEC, the

methylation level of NSUN5 promoter was downregulated, while the expression of NSUN5 was upregulated. Notably, the methylation level of NSUN5 promoter had a trend of downregulation in BLCA, CESC, ESCA, KIRP, LUAD, LUSC, PRAD, SARC, SKCM and THYM, although it was not significant. It may be due to the small quantity of normal tissues. (Fig. 2)

To sum up, the methylation level of NSUN5 promoter was generally downregulated in cancers, and may contribute to the upregulation of NSUN5, especially for BRCA, HNSC, LIHC, PAAD, STAD, TGCT and UCEC.

Higher expression of NSUN5 predicts poorer prognosis in multiple cancers

We then used GEPIA2 to explore whether the expression of NSUN5 in cancers was related with the prognosis of cancer patients. Among the 33 types of cancers (ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, UCS and UVM (uveal melanoma)) with prognosis data in GEPIA2, higher expression of NSUN5 predicts lower OS (overall survival) for ACC ($P = 0.0040$), GBM ($P = 0.0018$), KIRC ($P = 0.0079$), LGG ($P = 0.0060$) and PRAD ($P = 0.0330$) patients, (Fig. 3) but predicts higher OS for SARC ($P = 0.0420$) and THYM ($P = 0.0220$) patients. (data not shown) The expression of NSUN5 was not associated with OS for the other 26 types of cancers. ($P > 0.05$) Furthermore, higher expression of NSUN5 predicts lower RFS (recurrence free survival) for ACC ($P = 0.0140$), LGG ($P = 0.0140$), LIHC ($P = 0.0055$), LUSC ($P = 0.0500$) and PRAD ($P = 0.0180$) patients. (Fig. 4) The expression of NSUN5 was not associated with RFS for the other 28 types of cancers. ($P > 0.05$)

Totally, higher expression of NSUN5 predicts poorer prognosis for ACC, LGG, PRAD (both for OS and RFS), GBM, KIRC (for OS), LIHC and LUSC (for RFS) patients.

Higher expression of NSUN5 was positively correlated with translation in cancers

To explore the function of NSUN5 in cancers, using LinkedOmics^[10] (<http://linkedomics.org/login.php>), GO (gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses of NSUN5-correlated genes was performed in each cancer one by one. For example, in ACC, the co-expression analysis of NSUN5 (mRNA sequencing data) was shown in Supplementary Table 1 and Fig. 5A; the top 50 most significantly (according to FDR (false discovery rate)) NSUN5-positively-correlated and NSUN5-negatively-correlated genes were listed in Fig. 5B; the BPs (biological processes), CCs (cellular components), MFs (molecular functions) and KEGG pathways in which NSUN5-positively-correlated genes ($FDR \leq 0.05$ and normalized enrichment score > 0) and NSUN5-negatively-correlated ($FDR \leq 0.05$ and normalized enrichment score < 0) genes were significantly enriched were shown in Fig. 5C. The analyses process of the other 31 types of cancers were similar to that of ACC (data not shown).

The BPs, CCs, MFs and KEGG pathways in which NSUN5-positively correlated genes and NSUN5-negatively-correlated genes were significantly enriched in all the 32 types of human cancers were listed in Supplementary Tables 2–9.

To found the common BPs, CCs, MFs or KEGG pathways in human cancers, the number of cancer types with a same BP, CC, MF or KEGG pathway was calculated. The BPs, CCs, MFs or KEGG pathways were ranked by the times they appeared in cancers. The top 10 BPs, CCs, MFs and KEGG pathways in which NSUN5-positively-correlated genes were significantly enriched were listed in Tables 2–5, respectively. The top 10 BPs, CCs, MFs and KEGG pathways in which NSUN5-negatively-correlated genes were significantly enriched were listed in Supplementary Tables 10–13, respectively.

Table 2
The top 10 BPs in which NSUN5-positively-correlated genes were significantly enriched in cancers.

BP	NADH dehydrogenase complex assembly	translational elongation	mitochondrial respiratory chain complex assembly	rRNA metabolic process	ncRNA processing	nucleoside monophosphate metabolic process	nucleoside triphosphate metabolic process	ribonucleoprotein complex biogenesis	translational initiation
Cancers									
ACC	✓	✓	✓	✓	✓	✓	✓	✓	✓
BLCA	✓	✓	✓	✓	✓	✓	✓	✓	✓
BRCA	×	×	×	×	✓	×	×	×	×
CESC	✓	✓	✓	✓	✓	✓	✓	✓	✓
CHOL	✓	✓	✓	✓	✓	✓	✓	✓	✓
COADREAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
DLBC	✓	✓	✓	✓	✓	✓	✓	✓	✓
ESCA	✓	✓	×	×	×	✓	✓	×	✓
GBM	✓	✓	✓	×	×	✓	✓	×	×
HNSC	×	×	×	×	×	×	×	×	×
KICH	✓	✓	✓	✓	✓	×	×	✓	✓
KIRC	✓	✓	✓	✓	✓	✓	✓	✓	✓
KIRP	✓	✓	✓	✓	✓	×	×	✓	×
LAML	✓	✓	✓	✓	✓	✓	✓	✓	✓
LGG	✓	×	✓	×	×	×	×	×	×
LIHC	✓	✓	✓	✓	✓	✓	✓	✓	✓
LUAD	✓	✓	✓	✓	✓	✓	✓	✓	×
LUSC	✓	✓	✓	✓	✓	✓	✓	✓	✓
MESO	✓	✓	✓	✓	✓	✓	✓	✓	✓
OV	✓	✓	✓	✓	✓	✓	✓	✓	×
PAAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
PCPG	✓	✓	✓	✓	✓	✓	✓	✓	✓
PRAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
SARC	✓	✓	✓	✓	✓	✓	✓	✓	✓
SKCM	✓	✓	✓	✓	✓	✓	✓	✓	✓
STAD	✓	✓	✓	✓	×	×	×	✓	✓
TGCT	✓	✓	×	✓	×	✓	✓	×	✓
THCA	✓	✓	✓	✓	✓	✓	✓	✓	✓
THYM	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCEC	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCS	✓	✓	✓	✓	✓	✓	✓	✓	✓
UVM	✓	✓	✓	✓	✓	✓	✓	✓	✓
Number of cancer types with "✓"	30	29	28	27	26	26	26	26	25
BP analyses of NSUN5-correlated genes in cancers using Linkedomics. The BP in which NSUN5-positively-correlated genes were significantly enriched (FDR ≤ normalized enrichment score > 0) was denoted by "✓", otherwise, it was denoted by "×". The BPs were ranked by the number of cancer types with "✓" and only shown. BP, biological process.									

A previous study showed that loss of the rRNA methyltransferase NSUN5 impairs global protein synthesis (translation) and normal growth.^[11] Interestingly, in this study, we found that higher expression of NSUN5 in most cancers was positively correlated with translational elongation, rRNA metabolic process, ribonucleoprotein complex biogenesis, translational initiation, (Table 2) ribosome, (Tables 3 and 5) rRNA binding and unfolded protein binding, (Table 4) which

were all associated with protein synthesis. For example, in ACC, higher expression of NSUN5 was positively correlated with translational elongation, rRNA metabolic process, ribonucleoprotein complex biogenesis, translational initiation and ribosome. (Table 6) The corresponding gene (mRNA) symbols were listed in Table 6.

Table 3
The top 10 CCs in which NSUN5-positively-correlated genes were significantly enriched in cancers.

CC Cancers	mitochondrial inner membrane	mitochondrial membrane part	mitochondrial protein complex	NADH dehydrogenase complex	organelle envelope lumen	respiratory chain	ribosome	cytochrome complex	mitochondrial matrix
ACC	✓	✓	✓	✓	✓	✓	✓	✓	×
BLCA	✓	✓	✓	✓	✓	✓	✓	✓	✓
BRCA	✓	×	×	×	×	×	×	×	×
CESC	✓	✓	✓	✓	✓	✓	✓	✓	✓
CHOL	✓	✓	✓	✓	✓	✓	✓	✓	✓
COADREAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
DLBC	✓	✓	✓	✓	✓	✓	✓	✓	✓
ESCA	✓	✓	✓	✓	✓	✓	✓	✓	✓
GBM	✓	✓	✓	✓	✓	✓	✓	×	✓
HNSC	✓	✓	✓	✓	✓	✓	✓	✓	✓
KICH	✓	✓	✓	✓	✓	✓	✓	✓	✓
KIRC	✓	✓	✓	✓	✓	✓	✓	✓	✓
KIRP	✓	✓	✓	✓	✓	✓	✓	✓	✓
LAML	✓	✓	✓	✓	✓	✓	✓	✓	✓
LGG	✓	✓	✓	✓	✓	✓	×	✓	×
LIHC	✓	✓	✓	✓	✓	✓	✓	✓	✓
LUAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
LUSC	✓	✓	✓	✓	✓	✓	✓	✓	✓
MESO	✓	✓	✓	✓	✓	✓	✓	✓	✓
OV	✓	✓	✓	✓	✓	✓	✓	×	✓
PAAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
PCPG	✓	✓	✓	✓	✓	✓	✓	✓	✓
PRAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
SARC	✓	✓	✓	✓	✓	✓	✓	✓	✓
SKCM	✓	✓	✓	✓	✓	✓	✓	✓	✓
STAD	✓	✓	✓	✓	✓	✓	✓	✓	✓
TGCT	✓	✓	✓	✓	✓	✓	✓	✓	✓
THCA	✓	✓	✓	✓	✓	✓	✓	✓	✓
THYM	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCEC	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCS	✓	✓	✓	✓	✓	✓	✓	✓	✓
UVM	✓	✓	✓	✓	✓	✓	✓	✓	✓
Number of cancer types with "✓"	32	31	31	31	31	31	30	29	29

CC analyses of NSUN5-correlated genes in cancers using Linkedomics. The CC in which NSUN5-positively-correlated genes were significantly enriched (FDR ≤ normalized enrichment score > 0) was denoted by "✓", otherwise, it was denoted by "×". The CCs were ranked by the number of cancer types with "✓" and only were shown. CC, cellular component.

Table 4
The top 10 MFs in which NSUN5-positively-correlated genes were significantly enriched in cancers.

MF Cancers	oxidoreductase activity, acting on a heme group of donors	structural constituent of ribosome	electron transfer activity	oxidoreductase activity, acting on NAD(P)H	heme- copper terminal oxidase activity	rRNA binding	threonine- type peptidase activity	catalytic activity, acting on DNA	metal cluster binding	snRNA binding	unfolds protein binding
ACC	×	×	×	×	×	×	×	×	×	×	×
BLCA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BRCA	✓	×	×	×	×	×	×	✓	×	×	×
CESC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CHOL	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
COADREAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DLBC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ESCA	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓
GBM	✓	✓	✓	✓	×	×	✓	×	×	×	✓
HNSC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
KICH	×	✓	×	×	×	✓	✓	×	×	×	×
KIRC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
KIRP	✓	✓	✓	✓	✓	✓	✓	×	×	✓	×
LAML	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LGG	✓	×	✓	✓	✓	×	✓	×	×	×	✓
LIHC	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓
LUAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LUSC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
MESO	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
OV	✓	✓	✓	✓	×	×	×	✓	✓	×	✓
PAAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PCPG	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
PRAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SARC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SKCM	✓	✓	✓	✓	✓	✓	×	×	✓	✓	×
STAD	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓
TGCT	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
THCA	✓	✓	✓	✓	✓	✓	✓	×	✓	×	×
THYM	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCEC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UVM	✓	✓	×	✓	✓	✓	×	×	×	✓	×
Number of cancer types with "✓"	30	29	28	28	27	27	27	24	24	24	24

MF analyses of NSUN5-correlated genes in cancers using Linkedomics. The MF in which NSUN5-positively-correlated genes were significantly enriched (FDR ≤ 0.05 and normalized enrichment score > 0) was denoted by "✓", otherwise, it was denoted by "×". The MFs were ranked by the number of cancer types with "✓" and only the top 10 MFs were shown. MF, molecular function.

Table 5
The top 10 pathways in which NSUN5-positively-correlated genes were significantly enriched in cancers.

Pathway Cancers	Oxidative phosphorylation	Parkinson disease	Huntington disease	Non- alcoholic fatty liver disease (NAFLD)	Ribosome	Pyrimidine metabolism	Spliceosome	Alzheimer disease	Base excision repair	Proteasom
ACC	✓	✓	✓	✓	✓	✓	✓	✓	×	×
BLCA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BRCA	×	×	×	×	×	×	×	×	×	×
CESC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CHOL	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
COADREAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DLBC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ESCA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
GBM	✓	✓	✓	×	×	✓	×	×	✓	✓
HNSC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
KICH	✓	✓	✓	✓	✓	✓	✓	✓	×	×
KIRC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
KIRP	✓	✓	✓	✓	✓	×	✓	×	✓	✓
LAML	✓	✓	✓	✓	✓	✓	×	✓	✓	×
LGG	✓	✓	×	✓	×	×	×	✓	×	✓
LIHC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LUAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LUSC	✓	✓	✓	✓	✓	✓	✓	×	✓	✓
MESO	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
OV	✓	✓	✓	×	✓	✓	✓	✓	×	✓
PAAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PCPG	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PRAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SARC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SKCM	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
STAD	✓	✓	✓	✓	✓	✓	✓	×	✓	✓
TGCT	✓	✓	✓	✓	✓	✓	✓	✓	×	✓
THCA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
THYM	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCEC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UCS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UVM	✓	✓	✓	✓	✓	×	✓	✓	✓	×
Number of cancer types with "✓"	31	31	30	29	29	28	28	27	26	26

KEGG pathway analyses of NSUN5-correlated genes in cancers using Linkedomics. The pathway in which NSUN5-positively-correlated genes were significantly enriched ($FDR \leq 0.05$ and normalized enrichment score > 0) was denoted by "✓", otherwise, it was denoted by "×". The pathways were ranked by the number of cancer types with "✓" and only the top 10 pathways were shown. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 6

The translation-associated BPs, CCs, MFs and KEGG pathways in which NSUN5-positively correlated genes were significantly enriched in ACC

Gene set		NES	FDR	Gene (mRNA) symbols
BP	translational elongation	1.708	0.026	AURKAIP1;EEF1A2;EEF1D;EEF2;EEF2K;EEFSEC;EIF5A;EIF5AL1;GADD45GIP1; MRPL11;MRPL14;MRPL20;MRPL21;MRPL22;MRPL27;MRPL3;MRPL32;MRPL34; MRPL36;MRPL4;MRPL41;MRPL42;MRPL47;MRPL51;MRPL52;MRPL54;MRPS11; MRPS12;MRPS15;MRPS17;MRPS2;MRPS21;MRPS24;MRPS26;MRPS33;MRPS34; MRPS36;PSTK;RPLP1;RPLP2;TUFM;ZNF598
BP	rRNA metabolic process	1.962	0.003	BRF1;BYSL;CD3EAP;DDX27;DDX49;DDX56;DEDD2;DHX37;DKC1;EBNA1BP2; ERI3;ERN2;EXOSC1;EXOSC3;EXOSC5;FBL;FRG1;GAR1;GTF3A;GTF3C1;H2AFY; ISG20;KRI1;KRR1;LAS1L;LYAR;MARS;MRPS11;MRT04;NGDN;NHP2;NOB1;NOC4L; NOL12;NOL6;NOL8;NOP10;NOP2;NOP56;NSA2;NSUN5;NVL;PA2G4;PIH1D1;POP4; RIOK2;RPF2;RPL10A;RPL11;RPL26;RPL35;RPL35A;RPL5;RPL7A;RPS14;RPS15;RPS16; RPS17;RPS19;RPS2;RPS21;RPS27;RPS28;RPS6;RPS7;RPS8;RPS9;RRP1;RRP9;RSL1D1; SART1;SBDS;SMARCA4;SUV39H1;TBL3;UTP14A;UTP15;UTP20;WDR36;WDR46;ZNHIT3
BP	ribonucleoprotein complex biogenesis	2.039	0.001	ATXN2;ATXN2L;BCCIP;BRX1;BYSL;CD2BP2;CELF5;CIRBP;DDX23;DDX27;DDX49;DDX56; DENR;DHX37;DKC1;EBNA1BP2;EIF3B;EIF3CL;EIF3G;EIF3I;EIF3K;EIF3M;EIF4H;ERI3;EXOSC1; EXOSC3;EXOSC5;FBL;FRG1;GAR1;GEMIN7;GNL2;GTF3A;GTPBP10;ISG20;KRI1;KRR1;LAS1L; LSM14A;LSM4;LUC7L2;LYAR;MCTS1;MPV17L2;MRPL11;MRPL20;MRPL22;MRPS11;MRPS2; MRT04;MYBBP1A;NGDN;NHP2;NIP7;NOB1;NOC2L;NOC4L;NOL12;NOL6;NOL8;NOM1;NOP10; NOP16;NOP2;NOP56;NPM1;NSA2;NSUN5;NVL;PA2G4;PIH1D1;POP4;PPAN;PPAN-P2RY11;PRMT7; PRPF31;PRPF6;RAN;RBM22;RBMX;RIOK2;RPF2;RPL10;RPL10A;RPL11;RPL12;RPL13A;RPL24;RPL26; RPL26L1;RPL35;RPL35A;RPL38;RPL5;RPL6;RPL7A;RPLP0;RPS10;RPS14;RPS15;RPS16;RPS17;RPS19; RPS2;RPS21;RPS23;RPS27;RPS28;RPS5;RPS6;RPS7;RPS8;RPS9;RRP1;RRP9;RSL1D1;RSL24D1; RUVBL2;SART1;SBDS;SF3A2;SNRPB;SNRPC;SNRPD2;SNRPD3;SNRPF;SNRPG;SRPK2;STRAP; STYXL1;SURF6;SUV39H1;TAF9;TARBP2;TBL3;TRAF7;TXNL4A;UTP14A;UTP15;UTP20;WDR36;WDR46;XAB2
BP	translational initiation	2.207	0.000	ATF4;DENR;EIF1;EIF1AY;EIF2AK1;EIF2B1;EIF3B;EIF3CL;EIF3G;EIF3I;EIF3K;EIF3M;EIF4B; EIF4E1B;EIF4EBP1;EIF4H;EIF6;HSPB1;LARP1;MCTS1;MTFMT;MTIF3;NPM1;PABPC1;PAIP1; PAIP2;PPP1R15A;RPL10;RPL10A;RPL11;RPL12;RPL13;RPL13A;RPL14;RPL17;RPL18;RPL18A; RPL21;RPL22;RPL24;RPL26;RPL28;RPL29;RPL3;RPL30;RPL31;RPL32;RPL34;RPL35;RPL35A; RPL36;RPL36A;RPL37;RPL37A;RPL38;RPL39;RPL4;RPL41;RPL5;RPL6;RPL7A;RPL8;RPL9;RPLP0; RPLP1;RPLP2;RPS10;RPS11;RPS13;RPS14;RPS15;RPS15A;RPS16;RPS17;RPS18;RPS19;RPS2; RPS20;RPS21;RPS23;RPS24;RPS26;RPS27;RPS28;RPS29;RPS3;RPS4X;RPS4Y1;RPS5;RPS6;RPS7; RPS8;RPS9;RPSA;RXRA;UBA52;YTHDF1
CC/MF	ribosome	2.306	0.000	APEX1;AURKAIP1;C12orf65;CHCHD1;DENR;EEF2;GADD45GIP1;HSPA14;LARP4;MCTS1;MPV17L2; MRPL11;MRPL14;MRPL20;MRPL21;MRPL22;MRPL27;MRPL3;MRPL32;MRPL34;MRPL36;MRPL4; MRPL41;MRPL42;MRPL47;MRPL51;MRPL52;MRPL54;MRPS11;MRPS12;MRPS15;MRPS17;MRPS2; MRPS21;MRPS24;MRPS26;MRPS33;MRPS34;MRPS36;MT3;MTG1;NAA10;NDUFA7;NDUFAB1;RBM3; RPL10;RPL10A;RPL11;RPL12;RPL13;RPL13A;RPL17;RPL18;RPL18A;RPL21;RPL24;RPL26;RPL26L1; RPL28;RPL29;RPL3;RPL30;RPL31;RPL32;RPL34;RPL35;RPL35A;RPL36;RPL36AL;RPL37;RPL37A; RPL38;RPL39;RPL4;RPL41;RPL5;RPL6;RPL7A;RPL8;RPL9;RPLP0;RPLP1;RPLP2;RPS10;RPS11; RPS13;RPS14;RPS15;RPS15A;RPS16;RPS17;RPS18;RPS19;RPS2;RPS21;RPS23;RPS26;RPS27; RPS28;RPS29;RPS3;RPS4X;RPS4Y1;RPS4Y2;RPS5;RPS6;RPS6KL1;RPS7;RPS8;RPS9;RRBP1;RSL24D1;SUR

BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; NES, normalized enrichment score discovery rate.

Gene set		NES	FDR	Gene (mRNA) symbols
KEGG	ribosome	2.620	0.000	FAU;MRPL11;MRPL14;MRPL20;MRPL21;MRPL22;MRPL27;MRPL3;MRPL32;MRPL34;MRPL36;MRPL4;MRPS11;MRPS12;MRPS15;MRPS17;MRPS2;MRPS21;RPL10;RPL10A;RPL11;RPL12;RPL13;RPL13A;RPL17;RPL18;RPL18A;RPL21;RPL24;RPL26;RPL26L1;RPL28;RPL29;RPL3;RPL30;RPL31;RPL32;RPL34;RPL35;RPL35A;RPL36;RPL36AL;RPL37;RPL37A;RPL38;RPL39;RPL4;RPL41;RPL5;RPL6;RPL7A;RPL8;RPL9;RPLP0;RPLP1;RPLP2;RPS10;RPS11;RPS13;RPS14;RPS15;RPS15A;RPS16;RPS17;RPS18;RPS19;RPS2;RPS21;RPS23;RPS26;RPS27;RPS28;RPS29;RPS3;RPS4X;RPS4Y1;RPS4Y2;RPS5;RPS6;RPS7;RPS8;RPS9;RSL24D1;UBA52
				BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; NES, normalized enrichment score discovery rate.

In summary, higher expression of NSUN5 was positively correlated with translation-related mRNA, BPs, CCs, MFs and KEGG pathways in human cancers.

Discussion

In the present study, by data mining, we found that out of all the eight kinds of RCMTs, six kinds of RCMTs (NSUN1, NSUN2, NSUN3, NSUN4, NSUN5 and TRDMT1) were generally upregulated in human cancers. It indicates that m⁵C and RCMTs may play important roles widely in human cancers. In fact, it has been reported that in leukemia, NSUN3 and TRDMT1 could bind hnRNPk, NSUN1 could bind BRD4, both leading to formation of 5-Azacididine-sensitive chromatin structure.^[6] While NSUN2 could drive bladder cancer progression by stabilizing the mRNA of HDGF through upregulating its m⁵C level.^[7] However, the function of m⁵C and RCMTs and its mechanism in other human cancers remain largely unclear. Researches on these aspects should be carried on in the future.

It has been reported that NSUN5 is a rRNA methyltransferase, introducing m⁵C3782 into human 28S rRNA.^[11] NSUN5 deficiency altered ribosome function, leading to impaired global protein synthesis and normal growth.^[11] In this work, we revealed that NSUN5 was the most obviously upregulated RCMT, that higher expression of NSUN5 predicted poorer prognosis, and that NSUN5 expression was positively correlated with translation-related mRNAs, BPs, CCs, MFs and KEGG pathways in multiple human cancers. In addition, the hypomethylation of NSUN5 promoter may be responsible for its upregulation in cancers.

To sum up, we assumed that higher expression of NSUN5 could promote growth by inducing global translation through upregulating translation-related mRNAs in human cancers. The role of NSUN5 in human cancers should be confirmed by biological experiments and the mechanism how NSUN5 upregulates translation-related mRNAs needs further exploration.

Conclusions

Totally, bioinformatics analysis showed that NSUN5 was an oncogene, overexpressed NSUN5 predicted poorer prognosis and was positively correlated with translation in human cancers.

Methods

GEPIA2

The “Single Gene Analysis-Boxplots” module in GEPIA2^[8] (<http://gepia2.cancer-pku.cn/#index>) was used to compare the expression of each RCMT one by one in human cancers and that in associated normal tissues. The cancer data were from TCGA (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>) cancer data. The normal tissues data were from TCGA normal data and GTEx (<https://commonfund.nih.gov/GTEX/>) data.

The “Single Gene Analysis-Survival Analysis” module in GEPIA2 was used to analyze the prognostic value of NSUN5 in human cancers. The median expression of NSUN5 was used as the cutoff, which distinguished High-NSUN5-Group from Low-NSUN5-Group.

UALCAN

The “TCGA analysis” module in UALCAN^[9] (<http://ualcan.path.uab.edu/>) was used to compare the promoter methylation level of NSUN5 in human cancers and that in associated normal tissues. DNA methylation level was indicated by Beta value ranging from 0 (unmethylated) to 1 (fully methylated).

LinkedOmics

The “LinkFinder” module in LinkedOmics^[10] (<http://linkedomics.org/login.php>) was used to find NSUN5-correlated genes each cancer one by one. Both “Search Dataset” and “Target Dataset” were chosen as “RNAseq”, and the statistical method was chosen as “Spearman Correlation test”.

NSUN5-correlated genes ("Association Result") was then used to perform enrichment analysis in the "LinkInterpreter-GSEA (Gene Set Enrichment Analysis)" module in LinkedOmics. Biological process, Cellular Component, Molecular Function and KEGG pathway analyses were performed one by one. The "Rank Criteria" was chosen as "Statistic" ("spearman correlation" in this situation), the "Minimum Number of Genes" was chosen as "3" and the "Simulations" was chosen as "500".

Abbreviations

ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BP, biological process; BRCA, Breast invasive carcinoma; CC, cellular component; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; FDR, false discovery rate; GBM, Glioblastoma multiforme; GO, gene ontology; HNSC, Head and Neck squamous cell carcinoma ; KEGG, Kyoto Encyclopedia of Genes and Genomes; 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; m5C, 5-methylcytosine; m6A, N6-methyladenosine; MF, molecular function; mRNA, messenger RNA; OS, overall survival; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; RCMT, RNA m5C methyltransferase; READ, Rectum adenocarcinoma; RFS, recurrence free survival; rRNA, ribosomal RNA; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; tRNA, transfer RNA; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; UVM, Uveal Melanoma.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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Ji-meng Zhao: Software;

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Huan-gan Wu: Conceptualization, Project administration and Funding acquisition.

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Figures

Figure 1

The expression of NSUN5 in 31 types of cancers and associated tissues (GEPIA2). *, P<0.05; **, P<0.01; ***, P<0.001; black and green asterisk indicate upregulation and downregulation in tumor, respectively. NS, not significant. TPM, transcripts per kilobase of exon model per million mapped reads.

Figure 2

The promoter methylation level of NSUN5 in 28 types of cancers and associated normal tissues. (UALCAN) *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; black and green asterisk indicate upregulation and downregulation in tumor, respectively. NS, not significant.

Figure 3

Higher expression of NSUN5 predicts lower overall survival for ACC, GBM, KIRC, LGG and PRAD patients. (GEPIA2)

Figure 4

Higher expression of NSUN5 predicts lower recurrence free survival for ACC, LGG, LIHC, LUSC and PRAD patients. (GEPIA2)

Figure 5

The BPs (biological processes), CCs (cellular components), MFs (molecular functions) and KEGG pathways analyses of NSUN5-correlated genes in ACC. (LinkedOmics)

Supplementary Files

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