SNHG3/miR-148a-3p Axis–mediated High Expression of DNMT1 Correlates with Poor Prognosis and Tumor Immune Infiltration of Hepatocellular Carcinoma

Ninghua Yao
Affiliated Hospital of Nantong University https://orcid.org/0000-0002-7607-2516
Wei Jiang
Wuxi No 2 People's Hospital
Jie Sun
Nantong University Affiliated Hospital: Affiliated Hospital of Nantong University
Chen Yang
Shanghai Cancer Institute
Wenjie Zheng (wenjiezheng@ntu.edu.cn)
Affiliated Hospital of Nantong University https://orcid.org/0000-0002-3073-4596
Yan Wang
Affiliated Hospital of Nantong University

Research article

Keywords: DNA-methyltransferase 1, hsa-miR-148a-3p, hepatocellular carcinoma, tumor immune infiltration, noncoding RNA

Posted Date: September 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-864636/v1

SNHG3/miR-148a-3p axis–mediated high expression of DNMT1 correlates with poor prognosis and tumor immune infiltration of hepatocellular carcinoma

Ninghua Yao[1,2#], Wei Jiang[3#], Jie Sun[1], Chen Yang[4], Weijie Zheng[2], Yan Wang[1]

[1]Department of Cancer Center, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China.

[2]Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China.

[3]Department of Neurology, the Second People's Hospital of Wuxi, Wuxi, Jiangsu, China.

[4]State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Author contributions:

(I) Conception and design: N Yao, W Jiang, and C Yang; (II) Administrative support: Y Wang; (III) Provision of study materials or patients: W Zheng; (IV) Collection and assembly of data: J Sun, and W Jiang; (V) Data analysis and interpretation: W Zheng, and N Yao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to:

Y. Wang. Department of Cancer Center, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong 226001, Jiangsu, China. Tel: +8651381161252.

E-mail: wang-yan1991@126.com.

W. Zheng. Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China. Tel: +8651385052413.

E-mail: wenjiezheng@ntu.edu.cn.

Abstract

Background

Epigenetic reprogramming plays an important role in the occurrence, development, and prognosis of hepatocellular carcinoma (HCC). DNA methylation is a key epigenetic regulatory mechanism, and DNA methyltransferase 1 (DNMT1) is the major enzyme responsible for maintenance methylation. Nevertheless, the role and mechanism of DNMT1 in HCC remains poorly defined.

Methods

In the current study, we conducted pan-cancer analysis for DNMT1's expression and prognosis using The Cancer Genome Atlas (TCGA) data set. We conducted gene Set Enrichment Analysis (GSEA) between high-and-low DNMT1 expression groups to

identify DNMT1-related functional significance. We also investigated the relationship between DNMT1 expression and tumor immune microenvironment, including immune cell infiltration and the expression of immune checkpoints. Through a combination series of computer analyses (including expression analyses, correlation analyses, and survival analyses), the noncoding RNAs (ncRNAs) that contribute to the overexpression of DNMT1 were ultimately identified.

Results

We found that DNMT1 was upregulated in 16 types of human carcinoma including HCC, and DNMT1 might be a biomarker predicting unfavorable prognosis in HCC patients. DNMT1 mRNA expression was statistically associated with age, histological grade, and the level of serum AFP. Moreover, DNMT1 level was significantly and positively linked to tumor immune cell infiltration, immune cell biomarkers, and immune checkpoint expression. Meanwhile, Gene Set Enrichment Analysis (GSEA) revealed that high-DNMT1 expression was associated with epithelial mesenchymal transition (EMT), E_2F target, G_2M checkpoint, and inflammatory response. Finally, through a combination series of computer analyses the SNHG3/hsa-miR-148a-3p/DNMT1 axis was confirmed as the potential regulatory pathway in HCC.

Conclusion

SNHG3/miR-148a-3p axis upregulation of DNMT1 may be related to poor outcome, tumor immune infiltration, and regulated malignant properties in HCC.

Keywords: DNA-methyltransferase 1; hsa-miR-148a-3p; hepatocellular carcinoma; tumor immune infiltration; noncoding RNA

Introduction

Hepatocellular carcinoma (HCC) is the fourth-leading cause of cancer-associated mortality worldwide (1), with over 100,000 new cases diagnosed and deaths each year (2). In China, the 5-year survival rate for HCC is only 14.1%, and the recurrence rate is about 70% (3). Chemotherapy, liver transplantation, and surgery are common treatments, but they are only suitable for early-stage HCC patients (4). Since the molecular pathogenesis of HCC remains to be elucidated, it is essential to identify and characterize novel cancer-promoting genes to enable a better understanding this deadly disease, identify promising prognostic biomarkers, and develop more effective clinical therapies.

Epigenetic reprogramming regulates the malignant properties of HCC. DNA methylation is a key epigenetic regulatory mechanism that determines the pool of cancer stem cells in liver cancer and possibly other solid tumors (5) that are usually catalyzed by DNA methyltransferases (DNMTs) DNMT1, DNMT3a, and DNMT3b (6). Site-specific hypermethylation and silencing of putative tumor-suppressor genes associated with abnormal expression of DNMTs could cause carcinogenesis and tumor progression (7). DNMT1 is considered to be a maintenance DNA methyltransferase

which mainly maintains CpG methylation and is involved in embryonic development and somatic cell survival(8). DNMT1 is universally overexpressed in proliferating cells. Extensive studies have indicated that DNMTI is closely associated with tumorigenesis and metastasis in various cancers, including melanoma (9), prostate cancer (10), pancreatic cancer (11), head and neck squamous carcinoma (12), and breast cancer (13). Overexpression of DNMT1 in tumors indicates a poor prognosis (14). Furthermore, it has been reported that DNMT1 could modulate the immune system by maintaining forkhead box P3 (Foxp3) DNA methylation (15). Nevertheless, comprehensive studies on the expression, prognosis, and mechanisms of DNMT1 in HCC remain to be conducted, and the relationship between DNMT1 and tumor immune infiltration in HCC has been poorly defined.

In the present study, we first conducted expression analyses and survival analyses of DNMT1 in various human cancers. Next, we investigated microRNA (miRNA)-related and long noncoding RNAs (lncRNA)–related DNMT1 regulation in HCC. We ultimately clarified the relationship between DNMT1 expression and tumor immune microenvironment, including immune cell infiltration and the expression of immune checkpoints.

Methods

The Cancer Genome Atlas data acquisition

Level 3 RNA-sequencing (RNA-seq) data (Fragments Per Kilobase per Million [FPKM]) of 33 types of human cancer, including from The Cancer Genome Atlas-Liver Hepatocellular Carcinoma (TCGA-LIHC) data set and corresponding clinical information, were obtained from TCGA Genomic Data Commons (GDC, https://portal.gdc.cancer.gov/). The RNA-seq data (FPKM values) were then transformed to transcripts-per-million reads (TPM) and normalized into log2 (*TPM*+ 1).

Kaplan-Meier plotter analysis

Survival analysis for DNMT1, including overall survival (OS) and relapse-free survival (RFS) in 8 types of human cancer, were analyzed using the Kaplan-Meier plotter (http://kmplot.com/analysis/), which is an online database able to assess the association of RNA expression with survival in 21 cancer types.

Gene Expression Profiling Interactive Analysis database analysis

The correlation between DNMT1 and biomarkers of immune cells in HCC was analyzed using data from TCGA by Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) (16).

Candidate miRNA prediction and starBase database analysis

Upstream binding miRNAs of DNMT1 were predicted by the following miRNA-target prediction programs: PITA (https://tools4mirs.org/software/target_prediction/pita/),

RNA22 (https://cm.jefferson.edu/rna22/), miRmap (https://mirmap.ezlab.org/), microT (https://bio.tools/DIANA-microT), miRanda, PicTar (https://pictar.mdc-berlin.de/), and TargetScan (http://www.targetscan.org/vert_72/). We finally only included those miRNAs appearing in 2 or more of the above programs as candidate miRNAs of DNMT1 for subsequent analysis. The expression correlation between candidate miRNAs and DNMT1 and the expression level of hsa-miR-148a-3p in HCC were analyzed with the starBase v3.0 project (http://starbase.sysu.edu.cn/). In addition, starBase was employed to predict candidate lncRNAs potentially binding to hsa-miR-148a-3p. The expression correlation between hsa-miR-148a-3p and SNHG3 or between SNHG3 and DNMT1 in HCC were also analyzed by starBase.

Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA, http://software.broadinstitute.org/gsea/index.jsp) was conducted between high-and-low DNMT1 expression groups to identify DNMT1-related functional significance based on the Hallmark gene set ("h.all.v7.0.symbols.gmt"). Statistical significance was considered at |NES|>1, adjusted *P* value <0.05, and false discovery rate (FDR) <0.05.

Cell composition fraction estimation

To make reliable immune infiltration estimations, we used "immunedeconv" (https://icbi-lab.github.io/immunedeconv/), an R package that integrates 6 state-of-

the-art algorithms, including xCell, MCP-counter, TIMER, CIBERSORT, EPIC, and quanTIseq. Visualization was performed using the software packages "ggplot2" and "pheatmap".

Additional bioinformatic and statistical analysis

We used R software (version 3.6.3, https://www.r-project.org/) to analyze data and plot graphs. Wilcoxon rank-sum test for unpaired samples and the Wilcoxon signed-rank test was used for paired samples. Visualization was performed using the R package "ggplot2" (http://ggplot2.org). Patient characteristics between groups were compared using the chi-square test and Fisher's exact test. Correlations of all RNAs and those between the levels of RNAs and immune checkpoints in HCC were analyzed using Spearman's correlation and visualized using "ggplot2". Survival analysis for all RNAs in the competing endogenous RNA (ceRNA) network was carried out and visualized using the survival package in R (https://CRAN.R-project.org/package=survival). The differentially expressed genes (DEGs) were determined by limma tests with $|\log2(FC)|>1$ and adjusted *P* value <0.05 (17). The Kruskal-Wallis test was used to analyze the RNA expression among different histological grades. A *P* value <0.05 was considered statistically significant.

Results

Pan-cancer analysis of DNMT1 expression

We first analyzed the expression of DNMT1 in 33 types of TCGA-ALL (normal = 730, tumor = 11,363) database to investigate its possible roles in carcinogenesis. As presented in Fig. 1A, DNMT1 was upregulated in 16 types of human carcinoma, including bladder cancer (BLCA), cervical and endocervical cancer (CESC), breast cancer (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), glioblastoma multiforme (GBM), kidney renal clear cell carcinoma (KIRC), LIHC, lung adenocarcinoma (LUAD), pheochromocytoma and paranglioma (PCPG), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC), and downregulated in kidney chromophobe (KICH), while not significantly altered in kidney renal papillary cell carcinoma (KIRP), pancreatic adenocarcinoma (PAAD), thyoma (THYM), or prostate adenocarcinoma (PRAD), compared with corresponding normal tissue. Next, we verified the expression of DNMT1 in TCGA tumor tissues compared with paired normal tissues. We found that the expression of DNMT1 was statistically increased in UCEC, LIHC, BRCA, ESCA, HNSC, STAD, READ, BLCA, KIRC, LUAD, and LUSC and significantly reduced in THCA (Fig. 1B-1M). In summary, DNMT1 was upregulated in UCEC, LIHC, BRCA, ESCA, HNSC, STAD, READ, BLCA, KIRC, and LUAD, indicating that DNMT1 might play a key regulatory role in the carcinogenesis of these 10 cancers.

The prognostic values of DNMT1 in human cancer

We next performed survival analysis, including OS and RFS, for DNMT1 in UCEC, BLCA, BRCA, HNSC, LIHC, LUSC, THCA, and LUAD. For OS, LIHC patients with higher expression of DNMT1 had a poorer prognosis; however, those with a higher expression with HNSC had a better prognosis (Fig.2). For RFS, increased expression of DNMT1 was correlated with poor clinical outcomes in LIHC and THCA patients (Fig. 3). Taken together, these data suggested that DNMT1 could be used as a biomarker predicting unfavorable prognosis in HCC patients.

Relationship between DNMT1 expression and clinical characteristics in HCC

We downloaded clinical and gene expression data of HCC patients from TCGA database, including gender, age, histologic grade, alpha-fetoprotein (AFP), OS, Child-Pugh grade, T classification, and pathologic stage. Then, associations between clinical characteristics and DNMT1 mRNA expression in HCC were analyzed. Patients were divided into high- and low-expression groups on the basis of median DNMT1 mRNA expression. Our results revealed that DNMT1 mRNA expression was statistically associated with age, histological grade, and the level of serum AFP (all $P < 0.001$,;Table 1).

Prediction and analysis of upstream miRNAs of DNMT1

ncRNAs are responsible for the regulation of gene expression and can be classified into miRNAs, small nucleolar RNAs (snoRNAs), circular RNAs (circRNAs), and lncRNAs (18). To determine whether DNMT1 was regulated by some ncRNAs, we first predicted the upstream miRNAs that might bind to DNMT1 and finally found 14 miRNAs. For better visualization, we established an miRNA-DNMT1 regulatory network using Cytoscape software (https://cytoscape.org/) (Fig.4A). In theory, there should be a negative correlation between miRNA and DNMT1 due to the action mechanism of miRNA. Therefore, we performed expression correlation analysis for miRNA-DNMT1 pairs. As shown in Fig. 4B, DNMT1 was significantly and negatively associated with hsa-miR-148a-3p and positively linked to the other 13 predicted miRNAs in HCC. Then, we assessed the expression of hsa-miR-148a-3p in HCC and normal control samples with starBase and evaluated the prognostic value of hsa-miR-148a-3p in HCC with a Kaplan-Meier plotter. As shown in Fig. 4C and 4D, hsa-miR-148a-3p was significantly downregulated in HCC, and its downregulation was correlated with poor prognosis. Together, these findings suggest that hsa-miR-148a-3p might be the most influential upstream miRNA of DNMT1 in HCC.

Prediction and analysis of upstream lncRNAs of hsa-miR-148a-3p

Next, we predicted the upstream lncRNAs of hsa-miR-148a-3p using the starBase database and obtained a total of 45 possible lncRNAs. Similarly, for better visualization, the lncRNA-hsa-miR-148a-3p regulatory network was established using Cytoscape

228 software (Fig. 5A). We then detected 1548 DEGs between TCGA-LIHC tumor samples
229 and normal tissues using the “limma” R package. A Venn diagram was finally created
230 showing 3 overlaps of the 1454 DEGs and 45 possible lncRNAs (Fig. 5B), indicating
231 that LINC01554, small nucleolar RNA host gene 3 (SNHG3), and H19 were the co-
232 expressed differential lnRNAs found in both cohorts. As is presented in the volcano
233 plot in Fig. 5C, SNHG3 was the upregulated gene and LINC01554 or H19 was the
234 downregulated gene in HCC. Subsequently, the prognostic values of the SNHG3 in
235 HCC were assessed, which revealed that overexpressed SNHG3 indicated poor OS of
236 patients with HCC (Fig. 5D). Based on the above results, we next explored whether
237 SNHG3 could regulate hsa-miR-148a-3p expression as a ceRNA in HCC. According
238 to the ceRNA hypothesis, lncRNAs usually serve as ceRNAs by binding to miRNAs,
239 and the key qualified lncRNAs in the ceRNA subnet should be negatively associated
240 with miRNA and positively linked to mRNA at the same time. The expression
241 correlation between SNHG3 and hsa-miR-148a-3p or DNMT1 in HCC is shown in Fig.
242 5E-F. Furthermore, different expressions of DNMT1, has-miR-148a-3p, and SNHG3
243 were observed in normal and HCC tissues of different histologic grades (Fig. 5 G-I).
244 While the expression of DNMT1 and SNHG3 in HCC was statistically increased, the
245 expression of hsa-miR-148a-3p was significantly reduced compared with
246 corresponding normal tissue. Likewise, while high-grade groups (G2/G3/G4) had
247 significantly higher DNMT1 and SNHG3 expression than low-grade groups (G1), the
248 expression of hsa-miR-148a-3p in low-grade groups (G1) was higher. Cumulatively,

these findings suggested that SNHG3 might serve as a ceRNA to mediate DNMT1 by competitively binding to hsa-miR-148a-3p.

DNMT1 expression was positively related to immune cell infiltration in HCC

Tumor-infiltrating immune cells are independent predictors of cancer survival. As presented in Fig. 6A, there was no significant change in the level of immune cell infiltration with copy number alteration of DNMT1 in HCC. Thus, we conducted a correlation analysis between DNMT1 expression and immune cell infiltration in HCC using TIMER (http://timer.cistrome.org/). The expression of DNMT1 was significantly and positively related to all analyzed immune cells, including B cell (Cor = 0.486; P = 8.03e−22), CD8+ T cell (Cor = 0.331; P = 3.36e−10), CD4+ T cell (Cor = 0.494; P = 1.40e−22), macrophage (Cor = 0.541; P = 2.27e−24), neutrophil (Cor = 0.467; P = 4.67e−20), and dendritic cell (DC, Cor = 0.536; P = 1.22e−26) in HCC (Fig. 6B–G). Then, to further investigate the role of DNMT1 in tumor immunity, HCC patients from TCGA were divided into DMNT1-high and DNMT1-low subgroups. We used XCell (https://xcell.ucsf.edu/) to compare the differences in the abundance of tumor-infiltrating immune cells and extracellular matrix cells between the 2 groups (Fig. S1). Results illustrated that the stromal score was higher in the low-DNMT1 expression group than in the high-DNMT1 expression group ($P < 0.001$). The high-DNMT1 expression group had a significantly higher abundance of CD4+ memory T cells, Th2 cells, gamma delta T cells (Tgd cells), natural killer (NK) T cells, monocytes, and most

DC and B cells, but a significantly lower abundance of M2 macrophages，CD4 central memory T cells，CD8+ naive T cells，hematopoietic stem cells (HSCs)，granulocyte-macrophage progenitor (GMP) cells, and endothelial cells compared to the low-DNMT1 expression group (P <0.05). Finally, we used the GEPIA database to determine the expression correlation of DNMT1 with immune cell biomarkers in HCC. It was revealed that DNMT1 expression had significantly positive correlation with the gene markers of B cells (CD19 and CD79A), CD8+ T cells (CD8A and CD8B), CD4+ T cells (CD4), M1 macrophages (IRF5 and PTGS2), M2 macrophages (CD163, VSIG4, and MS4A4A), neutrophils (ITGAM and CCR7), and DCs (HLA-DPB1, HLA-DRA, HLA-DPA1, CD1C, NRP1, and ITGAX) in HCC. All these results indicated that DNMT1 is positively related to immune cell infiltration.

DNMT1 expression was positively associated with immune checkpoints in HCC

Programmed cell death protein 1 (PD-1; also known as PDCD1), programmed death ligand 1 (PD-L1; also known as CD274）, and cytotoxic T lymphocyte antigen 4 (CTLA4) are important biomarkers of T cell exhaustion (19). Tumors can escape immune surveillance by taking advantage of immune checkpoints. We plotted the relationship of DNMT1 and PD-1, PD-L1, and CTLA-4 to clarify the relationship between DNMT1 and tumor immune escape. Expression of DNMT1 correlated positively with all 3 of these immune checkpoints, showing statistical significance in HCC (Fig.7A,7C,7E). Similar to GEPIA database analysis, we also found that DNMT1

expression was positively related to that of PD-1, PD-L1, and CTLA4 in HCC, with statistical significance indicated with TIMER (Fig. 7B,7D,7F). These findings confirmed that the carcinogenic effects mediated by DNMT1 might be related to the dysfunctional state of T cells and tumor immune escape.

GSEA identified DNMT1-related hallmark pathways

To determine the potential function of DNMT1 in HCC, GSEA analyses were conducted between the high- and low-DNMT1 expression groups. The top 4 hallmark items (adjusted *P* value <0.05) involved in the high-DNMT1 expression group were epithelial–mesenchymal transition, G_2/M checkpoint, E2F_targets, and inflammatory response (Fig. 8A), while the top 4 Hallmark items (adjusted *P* value <0.05) involved in the low-DNMT1 expression group were bile acid metabolism, fatty acid metabolism, oxidative phosphorylation, and xenobiotic metabolism (Fig. 8B). Cyclin-dependent kinase 1 (CDK1) is the key regulator of the G_2/M checkpoint (20). We further analyzed the expression correlation of DNMT1 with CDK1 and E2Fs (E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8) in HCC. As shown in Fig. 8C, DNMT1 expression was significantly and positively correlated with CDK1 expression. Fig. 8D-8K, further shows that DNMT1 expression was positively related to that of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8 in HCC, with statistical significance.

Discussion

The occurrence and development of HCC is a complex, dynamic biological process that involves genetic, epigenetic cell state, and microenvironment alterations. Clarifying the molecular mechanism underlying HCC carcinogenesis may contribute to the development of effective therapeutic targets or valuable prognostic biomarkers. Accumulating evidence has shown that DNMT1 participates in the tumorigenesis and progression of various human cancers, including HCC. However, to date, knowledge about DNMT1 in HCC remains insufficient, and further research is needed.

This study was performed to identify the feasibility of DNMT1 as a promising biomarker in HCC patients. We first performed pan-cancer analysis on the expression of DNMT1 using TCGA database. Then, the survival analysis of DNMT1 in some of the cancer types with statistical significance as analyzed above was conducted, indicating increased expression of DNMT1 to be correlated with poor clinical outcomes in HCC. Moreover, high DNMT1 expression was associated with histological grade. This suggests that upregulated DNMT1 might be involved in malignant transformation, which is in line with previously described results (14).

The ceRNA hypothesis consists of lncRNA mainly regulating mRNA through the ceRNA regulatory mechanism and RNAs affecting each other's levels by competing with a limited pool of miRNAs (21). It has been shown that DNMT1 can inhibit the transcription of tumor-suppressive miRNAs in cancer progression by maintaining their

hypermethylation (22). In our study, we developed a lncRNA–miRNA–mRNA triple regulatory network related to DNMT1 in HCC. Through candidate miRNA prediction conducted by the prediction programs—PITA, RNA22, miRmap, microT, miRanda, PicTar, and TargetScan—and correlation analysis—including expression analysis and survival analysis—we finally identified has-miR-148a-3p as the most likely potential upstream miRNA of DNMT1 in HCC. Has-miR-148a-3p is a member of the miR-148/152 family and has been reported to be a tumor suppressor for various human cancers, including pancreatic cancer (23), esophageal cancer (24), and HCC (25). Recent evidence has confirmed that the reciprocal negative regulation between hsa-miR-148a-3p and DNMT1 contributes to cell proliferation, cell cycle processes, and maintaining cell stemness characteristics in HCC (26).

Next, upstream lncRNAs of has-miR-148a-3p/DNMT1 axis were also predicted, and 45 possible lncRNAs were found. We intersected these lncRNAs with 1548 DEGs between TCGA-LIHC tumor samples and normal tissues to screen for differentially expressed lncRNAs. In the end, on the basis of the volcano map, ceRNA hypothesis, and correlation analyses, we identified SNHG3 as the upstream lncRNA. It has been reported that SNHG3 is an oncogene in many kinds of malignancies (27). Meanwhile, multiple miRNAs in HCC have been shown to promote tumor growth and metastasis through targeting SNHG3 (28, 29). Considering these findings, we identified SNHG3/hsa-miR-148a-3p/DNMT1 axis as the potential regulatory pathway in HCC.

At present, compared with other tumors, immunotherapy for liver cancer is still in its infancy (30). Increased infiltration of immune cells in tumors and high expression of immune checkpoints contribute to the efficacy of immunotherapy (31). Epigenetic modulation could enhance immunotherapy for HCC by upregulating previously repressed neoantigens and increasing cytotoxic T-cell infiltration in the immunosuppressive tumor microenvironment (32). It was found that increased methylation of T-cells is beneficial and that epigenetic control in intratumoral T-cells of Foxp3 can regulate the growth of HCC (33). Our results ultimately revealed DNMT1 expression to be positively associated with immune cell infiltrates, such as DCs, CD4+ T cells, and B cells. Meanwhile, we also found positive correlations between DNMT1 expression and immune checkpoints, which affect T-cell exhaustion and immune escape. Taken together these findings indicate that DNMT1 may be a valuable immunotherapy biomarker, and targeting DNMT1 might enhance the efficacy of immunotherapy in HCC.

To further explore the biological functions of DNMT1, we conducted GSEA analyses between the high- and low-DNMT1 expression groups. The GSEA found that Hallmark gene sets enriched in the high-DNMT1 expression group were mainly related to epithelial–mesenchymal transition (EMT), E2F target, G_2M checkpoint, and inflammatory response. The EMT process is critical for epithelial cell invasion,

resistance to apoptosis, tumor dissemination, and drug resistance (34). It might be that DNMT1-induced epigenetic silencing of SFRP1 causes activation of the Wnt signaling pathway and increases the aggressiveness of HCC by induction of EMT (35). Both the E2F and G_2/M checkpoints are targets associated with the cell cycle. Additionally, E2F activators regulate the transition from the G1 to S phase in the cell cycle and control cell apoptosis and differentiation (36). The key member of the Hallmark G_2M checkpoint gene set is CDK1. Meanwhile, we identified that DNMT1 significantly increased the expression of E2Fs and CDK1. These results amply demonstrated that increased DNMT1 participates in tumor progression via deleterious interaction with cell cycle–related molecules. The GSEA analyses also demonstrated enrichment of genes involved in the inflammatory response, which might account for the increased infiltration of immune cells in tumors. Cumulative, these findings elucidate the way in which DNMT1 participates in HCC, which will help future targeted therapy research.

Conclusions

In summary, we established the SNHG3/hsa-miR-148a-3p/DNMT1 axis as the potential regulatory pathway of hepatocarcinogenesis, which was also identified as a biomarker of poor prognosis. We further found that DNMT1 might exert its oncogenic effect through modulating cell cycle progression by regulating transcription and increasing tumor immune cell infiltration and immune checkpoint expression in HCC. However, these results should be validated by more basic hepatocarcinogenesis-related

experiments in the future.

Declarations

Ethics approval and consent to participate: 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.

Consent for publication： Not applicable.

Availability of data and materia: TCGA Data Poral: https://portal.gdc.cancer.gov/

Competing interests: The authors have no conflicts of interest to declare.

Funding: This study was supported by grants from the National Natural Science Foundation (no. 82070622 and 81702419), the Key Research and Development Plan of Jiangsu Province (no. BE2020668), and the Nantong Science 389 and Technology Project (no. MS12019013 and MSZ20207).

Author contributions: (I) Conception and design: N Yao, W Jiang, and C Yang; (II) Administrative support: Y Wang; (III) Provision of study materials or patients: W Zheng; (IV) Collection and assembly of data: J Sun, and W Jiang; (V) Data analysis and interpretation: W Zheng, and N Yao; (VI) Manuscript writing: All authors; (VII) **Final approval of manuscript:** All authors.

Acknowledgments: We thank the patients and investigators who participated in TCGA for providing the data.

References

1. Akinyemiju T, *et al.* (2017) The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA oncology* **3:** 1683-1691.
2. Bray F, *et al.* (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians* **68:** 394-424.
3. Allemani C, *et al.* (2018) Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet (London, England)* **391:** 1023-1075.
4. Forner A, Reig M, Bruix J. (2018) Hepatocellular carcinoma. *Lancet (London, England)* **391:** 1301-1314.
5. Raggi C, *et al.* (2014) Epigenetic reprogramming modulates malignant properties of human liver cancer. *Hepatology (Baltimore, Md.)* **59:** 2251-2262.
6. Jeltsch A, Jurkowska RZ. (2016) Allosteric control of mammalian DNA methyltransferases - a new regulatory paradigm. *Nucleic acids research* **44:** 8556-8575.
7. Arechederra M, Daian F. (2018) Hypermethylation of gene body CpG islands predicts high dosage of functional oncogenes in liver cancer **9:** 3164.
8. Iwanami N, *et al.* (2020) Transgenerational inheritance of impaired larval T cell development in zebrafish **11:** 4505.
9. Maric H, *et al.* (2019) DNMT1 and DNMT3B genetic polymorphisms affect the clinical course and outcome of melanoma patients. *Melanoma research* **29:** 596-602.
10. Lee E, *et al.* (2016) DNMT1 Regulates Epithelial-Mesenchymal Transition and Cancer Stem Cells, Which Promotes Prostate Cancer Metastasis. *Neoplasia*

445 *(New York, N.Y.)* **18:** 553-566.
446 11. Wong KK. (2020) DNMT1 as a therapeutic target in pancreatic cancer:
447 mechanisms and clinical implications. *Cellular oncology (Dordrecht)* **43:** 779-
448 792.
449 12. Cui J, Zheng L, Zhang Y, Xue M. (2021) Bioinformatics analysis of DNMT1
450 expression and its role in head and neck squamous cell carcinoma prognosis.
451 *Scientific reports* **11:** 2267.
452 13. Vernier M, McGuirk S, Dufour CR, Wan L, Audet-Walsh E. (2020) Inhibition
453 of DNMT1 and ERRα crosstalk suppresses breast cancer via derepression of
454 IRF4 **39:** 6406-6420.
455 14. Saito Y*, et al.* (2003) Increased protein expression of DNA methyltransferase
456 (DNMT) 1 is significantly correlated with the malignant potential and poor
457 prognosis of human hepatocellular carcinomas. *International journal of cancer*
458 **105:** 527-532.
459 15. Sun X, Cui Y, Feng H, Liu H, Liu X. (2019) TGF-β signaling controls Foxp3
460 methylation and T reg cell differentiation by modulating Uhrf1 activity **216:**
461 2819-2837.
462 16. Tang Z*, et al.* (2017) GEPIA: a web server for cancer and normal gene
463 expression profiling and interactive analyses. *Nucleic acids research* **45:** W98-
464 w102.
465 17. Ritchie ME*, et al.* (2015) limma powers differential expression analyses for
466 RNA-sequencing and microarray studies. *Nucleic acids research* **43:** e47.
467 18. Wang J*, et al.* (2019) ncRNA-Encoded Peptides or Proteins and Cancer.
468 *Molecular therapy : the journal of the American Society of Gene Therapy* **27:**
469 1718-1725.
470 19. Wherry EJ, Kurachi M. (2015) Molecular and cellular insights into T cell
471 exhaustion. *Nature reviews. Immunology* **15:** 486-499.
472 20. Liu S*, et al.* (2020) The Severe Fever with Thrombocytopenia Syndrome Virus

NSs Protein Interacts with CDK1 To Induce G(2) Cell Cycle Arrest and Positively Regulate Viral Replication. *Journal of virology* **94**.

21. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. (2011) A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146:** 353-358.

22. Ning X*, et al.* (2015) DNMT1 and EZH2 mediated methylation silences the microRNA-200b/a/429 gene and promotes tumor progression. *Cancer Lett* **359:** 198-205.

23. Fu X*, et al.* (2020) MicroRNA-148a-3p suppresses epithelial-to-mesenchymal transition and stemness properties via Wnt1-mediated Wnt/β-catenin pathway in pancreatic cancer. *J Cell Mol Med* **24:** 13020-13035.

24. Wang Y, Hu Y, Guo J, Wang L. (2019) miR-148a-3p Suppresses the Proliferation and Invasion of Esophageal Cancer by Targeting DNMT1. *Genetic testing and molecular biomarkers* **23:** 98-104.

25. Jung KH*, et al.* (2016) Differentiation therapy for hepatocellular carcinoma: Multifaceted effects of miR-148a on tumor growth and phenotype and liver fibrosis. *Hepatology* **63:** 864-879.

26. Li X*, et al.* (2020) Casticin inhibits stemness of hepatocellular carcinoma cells via disrupting the reciprocal negative regulation between DNMT1 and miR-148a-3p. *Toxicology and applied pharmacology* **396:** 114998.

27. Xu B*, et al.* (2020) LncRNA SNHG3, a potential oncogene in human cancers **20:** 536.

28. Zhao Q*, et al.* (2019) LncRNA SNHG3 Promotes Hepatocellular Tumorigenesis by Targeting miR-326. *The Tohoku journal of experimental medicine* **249:** 43-56.

29. Zhang PF*, et al.* (2019) LncRNA SNHG3 induces EMT and sorafenib resistance by modulating the miR-128/CD151 pathway in hepatocellular carcinoma **234:** 2788-2794.

30. Johnston MP, Khakoo SI. (2019) Immunotherapy for hepatocellular carcinoma:

Current and future. *World journal of gastroenterology* **25:** 2977-2989.

31. Yang Y. (2015) Cancer immunotherapy: harnessing the immune system to battle cancer. *The Journal of clinical investigation* **125:** 3335-3337.

32. Hong YK*, et al.* (2019) Epigenetic modulation enhances immunotherapy for hepatocellular carcinoma. *Cellular immunology* **336:** 66-74.

33. Liu Q*, et al.* (2019) Epigenetic control of Foxp3 in intratumoral T-cells regulates growth of hepatocellular carcinoma. *Aging* **11:** 2343-2351.

34. Kahlert UD, Nikkhah G, Maciaczyk J. (2013) Epithelial-to-mesenchymal(-like) transition as a relevant molecular event in malignant gliomas. *Cancer Lett* **331:** 131-138.

35. Quan H*, et al.* (2014) Hepatitis C virus core protein epigenetically silences SFRP1 and enhances HCC aggressiveness by inducing epithelial-mesenchymal transition. *Oncogene* **33:** 2826-2835.

36. Rowland BD, Bernards R. (2006) Re-evaluating cell-cycle regulation by E2Fs. *Cell* **127:** 871-874.

Figure 1. Pan-cancer expression analysis for DNMT1. (A) The expression of DNMT1 in 33 types of human cancer based on TCGA cancer and normal data. (B–M) DNMT1 expression in TCGA UCEC (B), LIHC (C), BRCA (D), ESCA (E), HNSC (F), THCA (G), STAD (H), READ (I), BLCA (J), KIRC (K), LUAD (L), and LUSC (M) tissues compared with paired normal tissues. *: P value ≤ 0.05; **: P value ≤ 0.01; ***: P value ≤ 0.001; ns: P value > 0.05.

DNMT1, DNA-methyltransferase 1; TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular cancer; BRCA, breast cancer; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; THCA, thyroid carcinoma; STAD, stomach adenocarcinoma; READ, rectum adenocarcinoma; BLCA, bladder cancer; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; KIRC, LUSC, lung squamous cell carcinoma

Figure 2. OS of dichotomized DNMT1 expression in various human cancers. (A–H) The OS plot of DNMT1 in UCEC (A), BLCA (B), BRCA (C), HNSC (D), LIHC (E), LUSC (F), THCA (G), and LUAD (H).

OS, overall survival; DNMT1, DNA-methyltransferase 1; TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular cancer; BRCA, breast cancer; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; THCA, thyroid carcinoma; STAD, stomach adenocarcinoma; READ, rectum adenocarcinoma; BLCA, bladder cancer; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; KIRC, LUSC, lung squamous cell carcinoma

Figure 3. RFS analysis of dichotomized DNMT1 expression in various human cancers. (A–H) The RFS plot of DNMT1 in UCEC (A), BLCA (B), BRCA (C), HNSC (D), LIHC (E), LUSC (F), THCA (G), and LUAD (H).

RFS, relapse-free survival; DNMT1, DNA-methyltransferase 1; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular cancer; BRCA, breast cancer; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; LUSC, lung squamous cell carcinoma; THCA, thyroid carcinoma; BLCA, bladder cancer; LUAD, lung adenocarcinoma

Figure 4. Identification of hsa-miR-148a-3p as a potential upstream miRNA of DNMT1 in HCC. (A)The miRNA-DNMT1 regulatory network established by Cytoscape software. (B) The expression correlation between predicted miRNAs and

549 DNMT1 in HCC analyzed by the starBase database. (C) The expression of hsa-miR-
550 148a-3p in HCC and normal samples in TCGA-LIHC. (D) The prognostic value of hsa-
551 miR-148a-3p in HCC.

552 miRNA, micro RNA; DNMT1, DNA-methyltransferase 1; HCC, hepatocellular carcinoma; LIHC, liver
553 hepatocellular carcinoma; TCGA, The Cancer Genome Atlas

554 **Figure 5. Identification of SNHG3 as a potential upstream lncRNAs of hsa-miR-**
555 **148a-3p in HCC.** (A) The lncRNA-mir-148a-3p regulatory network established by
556 Cytoscape software. (B) Venn diagram showing overlaps of 45 possible upstream
557 lncRNAs of mir-148a-3p (LIST2) with DEGs between TCGA-LIHC tumor samples
558 and normal tissues. (C) Volcano plot of differentially expressed lncRNAs (|log2fold
559 change|≥1 and adjusted *P* value <0.05). Red represents upregulated genes, and blue
560 indicates downregulated genes. The 3 overlapped genes are highlighted and labeled. (D)
561 The prognostic value of SNHG3 in HCC. (E) The correlation of hsa-miR-148a-3p
562 expressions with SNHG3 expressions in HCC from the starBase v. 3.0 project. (F) The
563 correlation of SNHG3 expressions with DNMT1 expressions in HCC from the starBase
564 v. 3.0 project. (G-I) The expression of DNMT1, miR-148a-3p, and SNHG3 in normal
565 and HCC tissues of different histologic grades. **P* value <0.05; ***P* value <0.01; ****P*
566 value <0.001.

567 DNA-methyltransferase 1; ncRNA, long non-coding RNA; HCC, hepatocellular carcinoma; DEGs,
568 differentially expressed genes; TCGA-LIHC, The Cancer Genome Atlas liver hepatocellular carcinoma

Figure 6. Correlation between DNMT1 levels and immune cell infiltration in HCC. (A)The level of immune cell infiltration in HCC under different copy numbers of DNMT1. (B–G) The relationship of DNMT1 expression level in HCC with (B) B cell, (C) CD8+ T cell, (D) CD4+ T cell, (E) macrophage, (F) neutrophil, or (G) DC infiltration level.

DNA-methyltransferase 1; HCC, hepatocellular carcinoma; DC, dendritic cell.

Figure 7. Relationship between the expression of DNMT1 and PD-1, PD-L1, or CTLA-4 in HCC. (A) The expression correlation of DNMT1 with CTLA-4 in HCC. (B) Spearman's correlation between the expression of DNMT1 and CTLA-4 in HCC adjusted for tumor purity using TIMER. (C) The expression correlation of DNMT1 with PDCD1 in HCC. (D) Spearman's correlation between the expression of DNMT1 and PDCD1 in HCC adjusted for tumor purity using TIMER. (E) The expression correlation of DNMT1 with CD274 (PD-L1) in HCC. (F) Spearman's correlation between the expression of DNMT1 and CD274 (PD-L1) in HCC adjusted for tumor purity using TIMER.

DNA-methyltransferase 1; PD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; HCC, hepatocellular carcinoma; CTLA, cytotoxic T lymphocyte antigen 4.

Figure 8. GSEA revealed Hallmark pathways related to DNMT1 in HCC.

A. GSEA results showing differential enrichment of genes in Hallmark with high DNMT1 expression. B. GSEA results showing differential enrichment of genes in HALLMARK with low DNMT1 expression. C. The expression correlation of DNMT1

590 with CDK1 in HCC. D-K. The expression correlation of DNMT1 with E2Fs in HCC:
591 E2F1 (D), E2F2 (E), E2F3 (F), E2F4 (G), E2F5 (H), E2F6 (I), E2F7 (G), and E2F8 (K).
592 DNA-methyltransferase 1; GSEA, Gene Set Enrichment Analysis; HCC, hepatocellular carcinoma.

593 **Figure S1. Immune cell score heat map.** Different colors represent the expression
594 trend in HCC tissues, and the vertical axis represents the gene expression distribution,
595 where different colors represent different groups. Asterisks represent levels of
596 significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).
597 HCC, hepatocellular carcinoma

598 **Table 1. Relationship between DNMT1 expression and clinical characteristics in**
599 **HCC**

600 **Table 2. Correlation analysis between DNMT1 and biomarkers of immune cells in**
601 **HCC determined by the GEPIA database.**

Figures

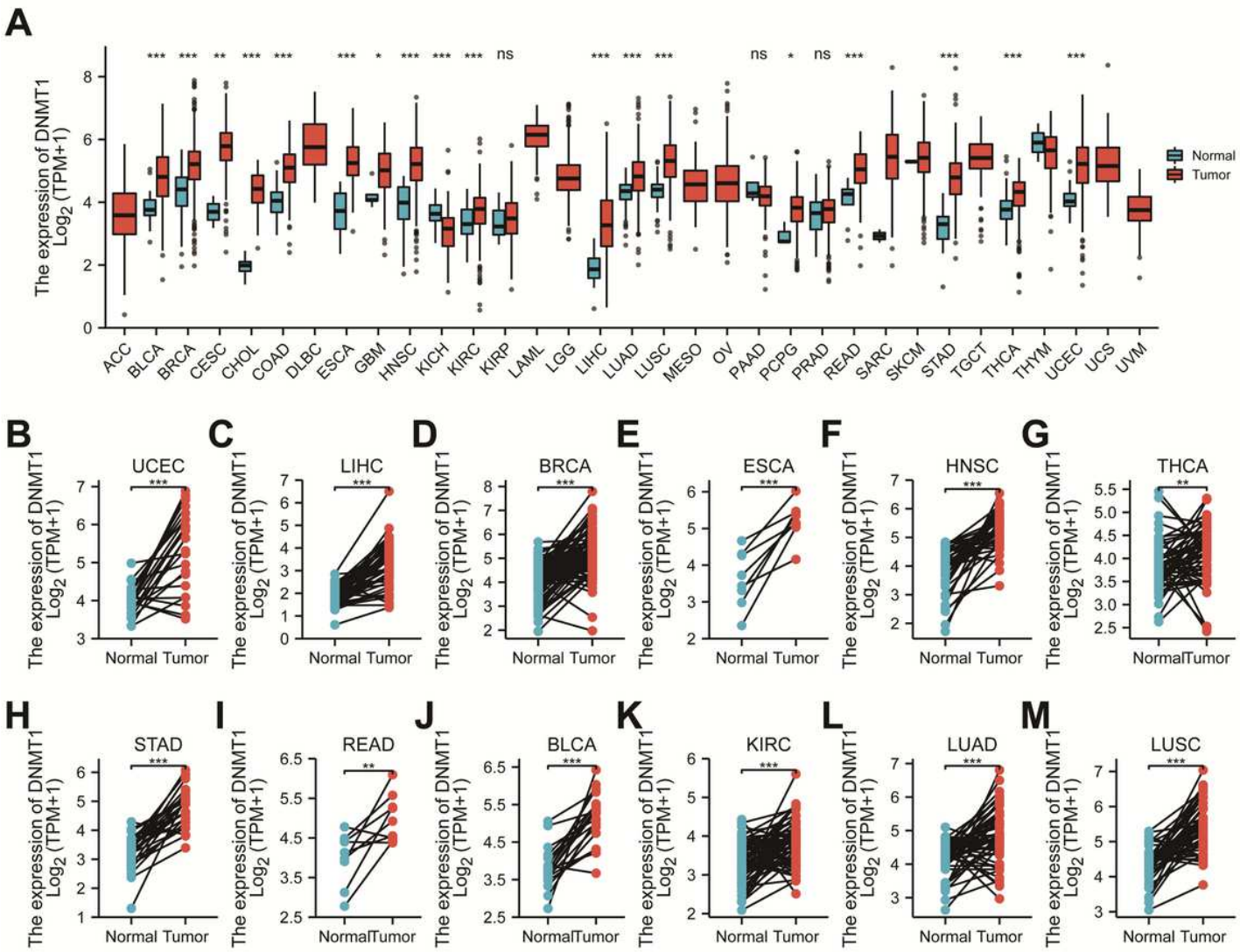


Figure 1

Pan-cancer expression analysis for DNMT1. (A) The expression of DNMT1 in 33 types of human cancer based on TCGA cancer and normal data. (B–M) DNMT1 expression in TCGA UCEC (B), LIHC (C), BRCA (D), ESCA (E), HNSC (F), THCA (G), STAD (H), READ (I), BLCA (J), KIRC (K), LUAD (L), and LUSC (M) tissues compared with paired normal tissues. *: P value ≤ 0.05; **: P value ≤ 0.01; ***: P value ≤ 0.001; ns: P value > 0.05. DNMT1, DNA-methyltransferase 1; TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular cancer; BRCA, breast cancer; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; THCA, thyroid carcinoma; STAD, stomach adenocarcinoma; READ, rectum adenocarcinoma; BLCA, bladder cancer; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; KIRC, LUSC, lung squamous cell carcinoma

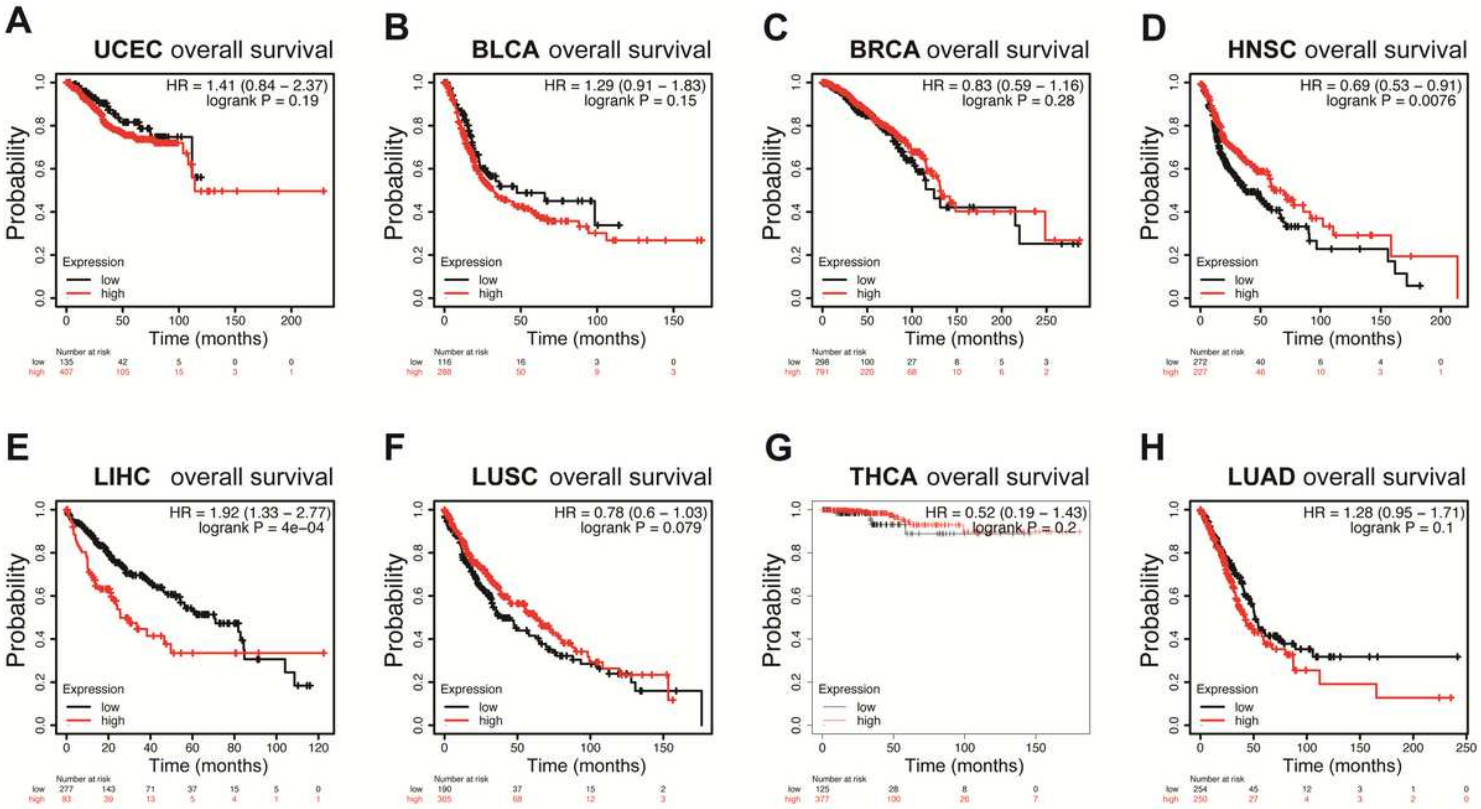


Figure 2

OS of dichotomized DNMT1 expression in various human cancers. (A– H) The OS plot of DNMT1 in UCEC (A), BLCA (B), BRCA (C), HNSC (D), LIHC (E), LUSC (F), THCA (G), and LUAD (H). OS, overall survival; DNMT1, DNA-methyltransferase 1; TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular cancer; BRCA, breast cancer; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; THCA, thyroid carcinoma; STAD, stomach adenocarcinoma; READ, rectum adenocarcinoma; BLCA, bladder cancer; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; KIRC, LUSC, lung squamous cell carcinoma

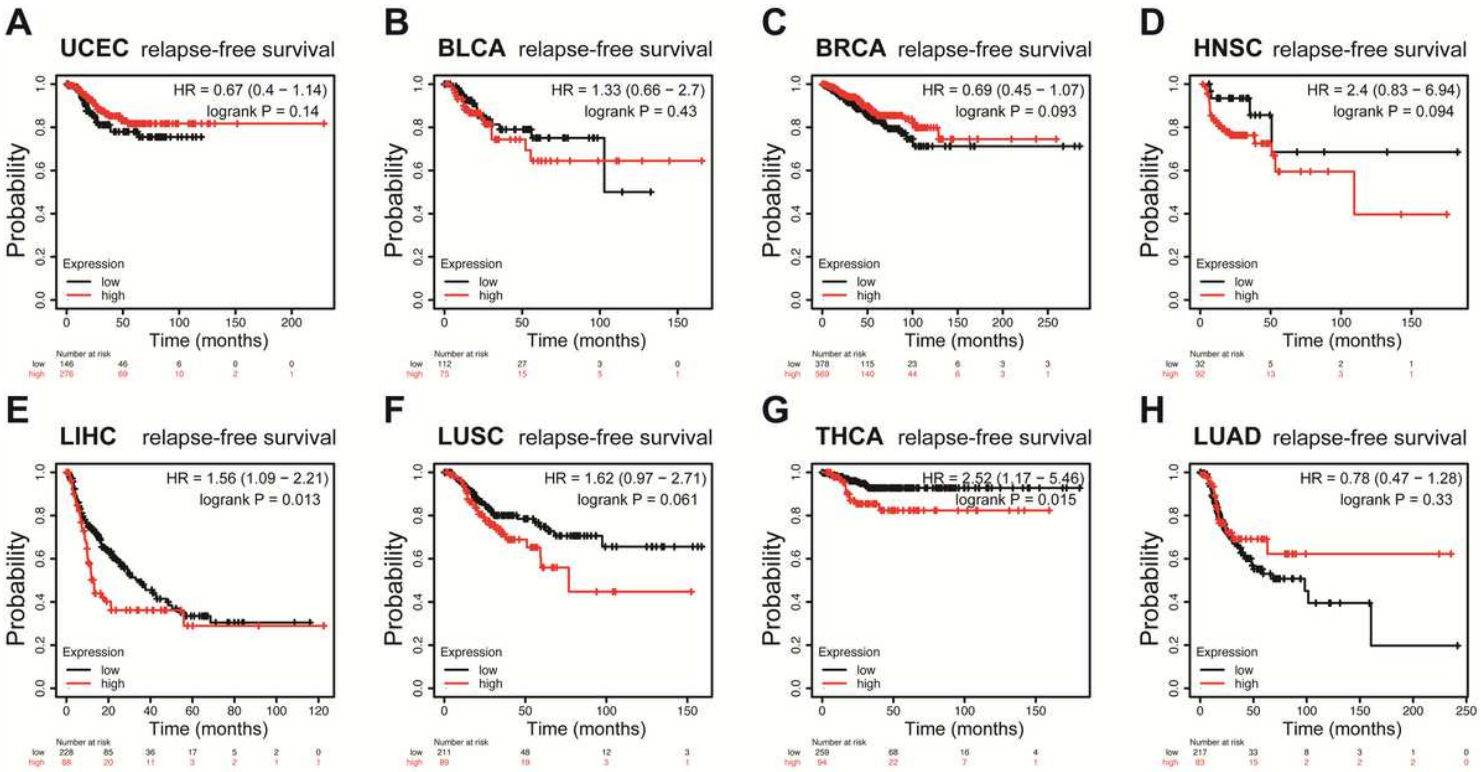
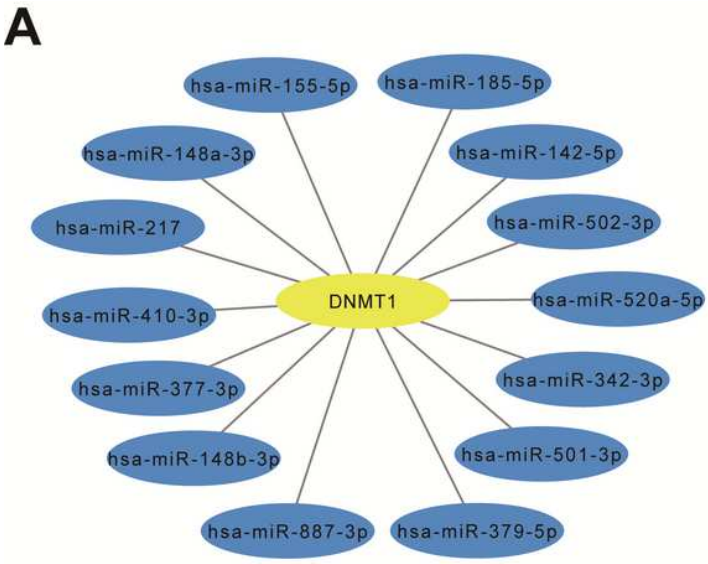


Figure 3

RFS analysis of dichotomized DNMT1 expression in various human cancers. (A–H) The RFS plot of DNMT1 in UCEC (A), BLCA (B), BRCA (C), HNSC (D), LIHC (E), LUSC (F), THCA (G), and LUAD (H). RFS, relapse-free survival; DNMT1, DNA-methyltransferase 1; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular cancer; BRCA, breast cancer; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; LUSC, lung squamous cell carcinoma; THCA, thyroid carcinoma; BLCA, bladder cancer; LUAD, lung adenocarcinoma



B

Gene	MiRNA	R-value	P-value
DNMT1	hsa-miR-148a-3p	-0.224	1.37E-05
DNMT1	hsa-miR-148b-3p	0.331	3.38E-14
DNMT1	hsa-miR-185-5p	0.142	6.18E-03
DNMT1	hsa-miR-342-3p	0.181	4.73E-04
DNMT1	hsa-miR-887-3p	0.201	9.93E-05
DNMT1	hsa-miR-379-5p	0.236	4.52E-06
DNMT1	hsa-miR-217	0.136	8.92E-03
DNMT1	hsa-miR-142-5p	0.265	2.27E-07
DNMT1	hsa-miR-502-3p	0.151	3.51E-03
DNMT1	hsa-miR-377-3p	0.193	1.85E-04
DNMT1	hsa-miR-520a-5p	0.154	2.93E-03
DNMT1	hsa-miR-410-3p	0.209	5.22E-05
DNMT1	hsa-miR-501-3p	0.237	4.00E-06
DNMT1	hsa-miR-155-5p	0.255	6.86E-07

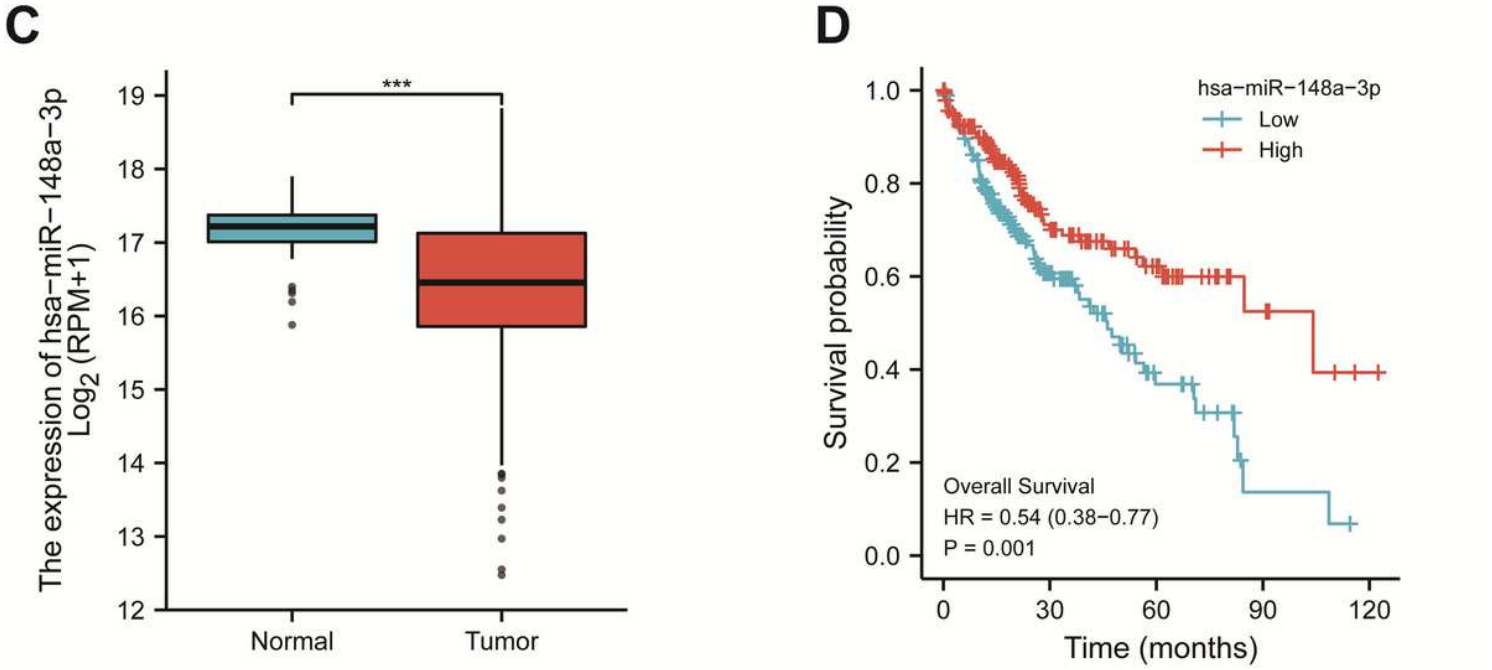


Figure 4

Identification of hsa-miR-148a-3p as a potential upstream miRNA of DNMT1 in HCC. (A)The miRNA-DNMT1 regulatory network established by Cytoscape software. (B) The expression correlation between predicted miRNAs and DNMT1 in HCC analyzed by the starBase database. (C) The expression of hsa-miR-148a-3p in HCC and normal samples in TCGA-LIHC. (D) The prognostic value of hsa-miR-148a-3p in HCC. miRNA, micro RNA; DNMT1, DNA-methyltransferase 1; HCC, hepatocellular carcinoma; LIHC, liver hepatocellular carcinoma; TCGA, The Cancer Genome Atlas

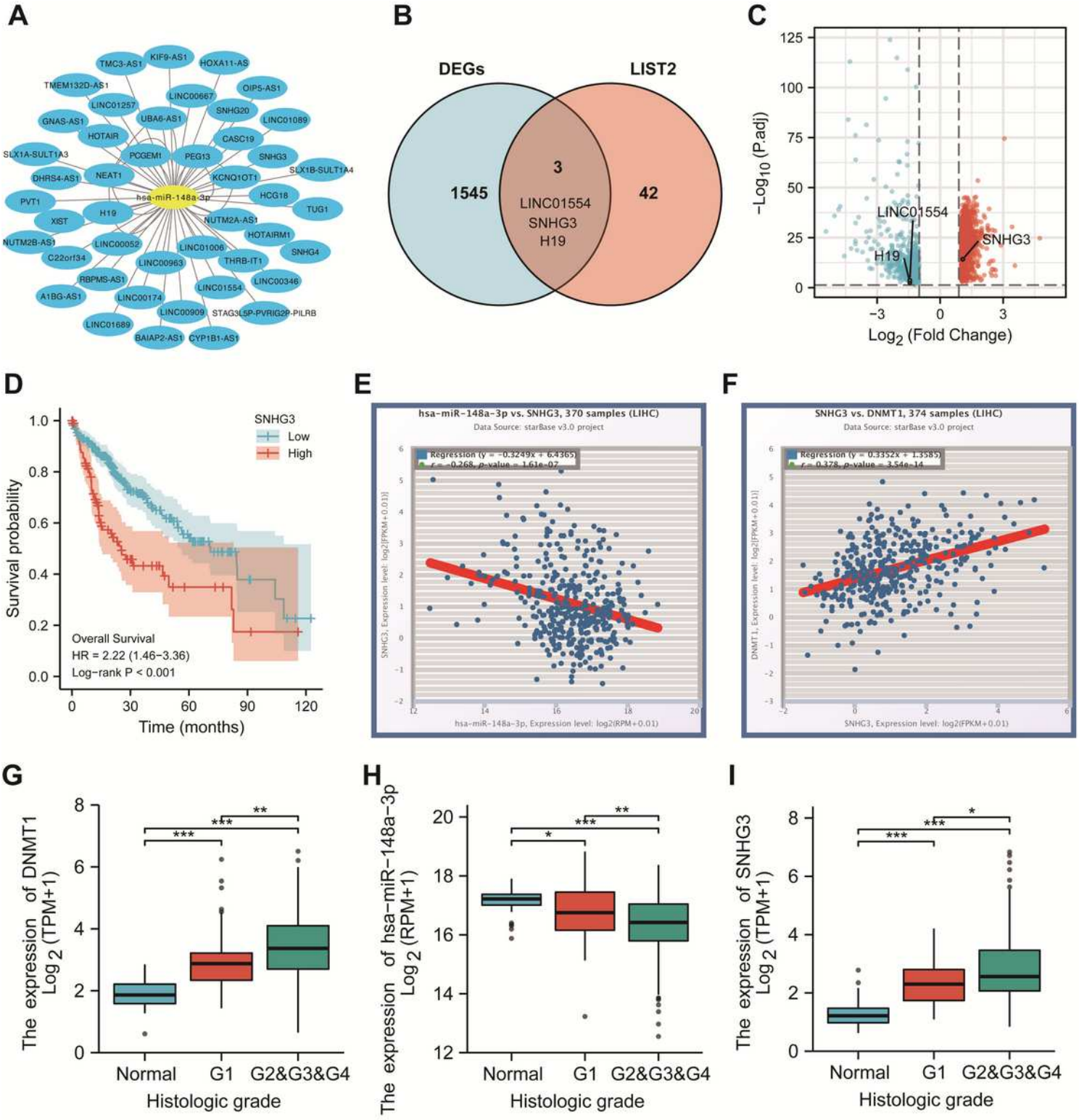


Figure 5

Identification of SNHG3 as a potential upstream lncRNAs of hsa-miR-148a-3p in HCC. (A) The lncRNA-mir-148a-3p regulatory network established by Cytoscape software. (B) Venn diagram showing overlaps of 45 possible upstream lncRNAs of mir-148a-3p (LIST2) with DEGs between TCGA-LIHC tumor samples and normal tissues. (C) Volcano plot of differentially expressed lncRNAs (|log2fold change|≥1 and adjusted P value <0.05). Red represents upregulated genes, and blue indicates downregulated genes. The

3 overlapped genes are highlighted and labeled. (D) The prognostic value of SNHG3 in HCC. (E) The correlation of hsa-miR-148a-3p expressions with SNHG3 expressions in HCC from the starBase v. 3.0 project. (F) The correlation of SNHG3 expressions with DNMT1 expressions in HCC from the starBase v. 3.0 project. (G-I) The expression of DNMT1, miR-148a-3p, and SNHG3 in normal and HCC tissues of different histologic grades. *P value <0.05; **P value <0.01; ***P value <0.001. DNA-methyltransferase 1; ncRNA, long non-coding RNA; HCC, hepatocellular carcinoma; DEGs, differentially expressed genes; TCGA-LIHC, The Cancer Genome Atlas liver hepatocellular carcinoma

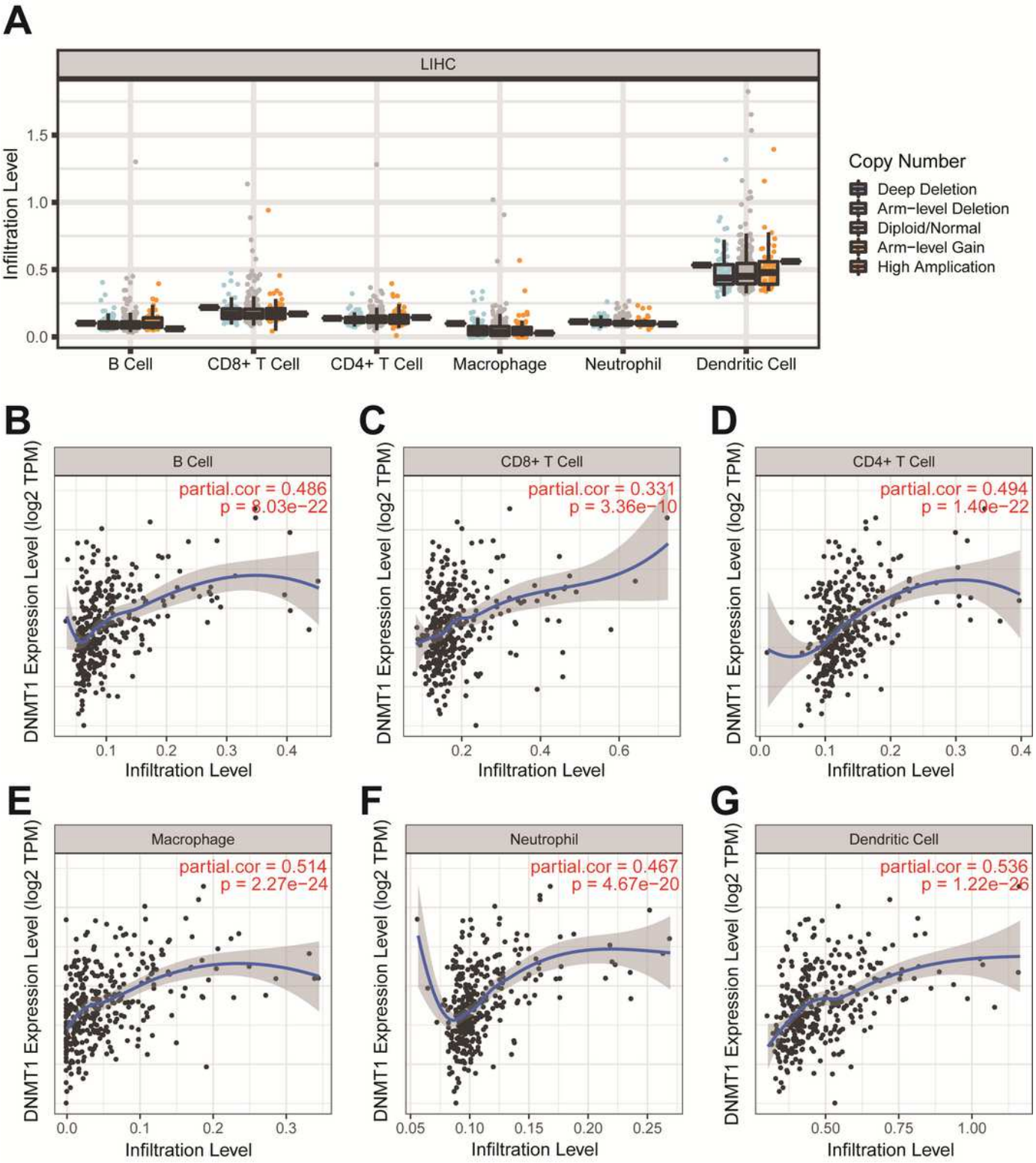


Figure 6

Correlation between DNMT1 levels and immune cell infiltration in HCC. (A)The level of immune cell infiltration in HCC under different copy numbers of DNMT1. (B–G) The relationship of DNMT1 expression level in HCC with (B) B cell, (C) CD8+ T cell, (D) CD4+ T cell, (E) macrophage, (F) neutrophil, or (G) DC infiltration level. DNA-methyltransferase 1; HCC, hepatocellular carcinoma; DC, dendritic cell.

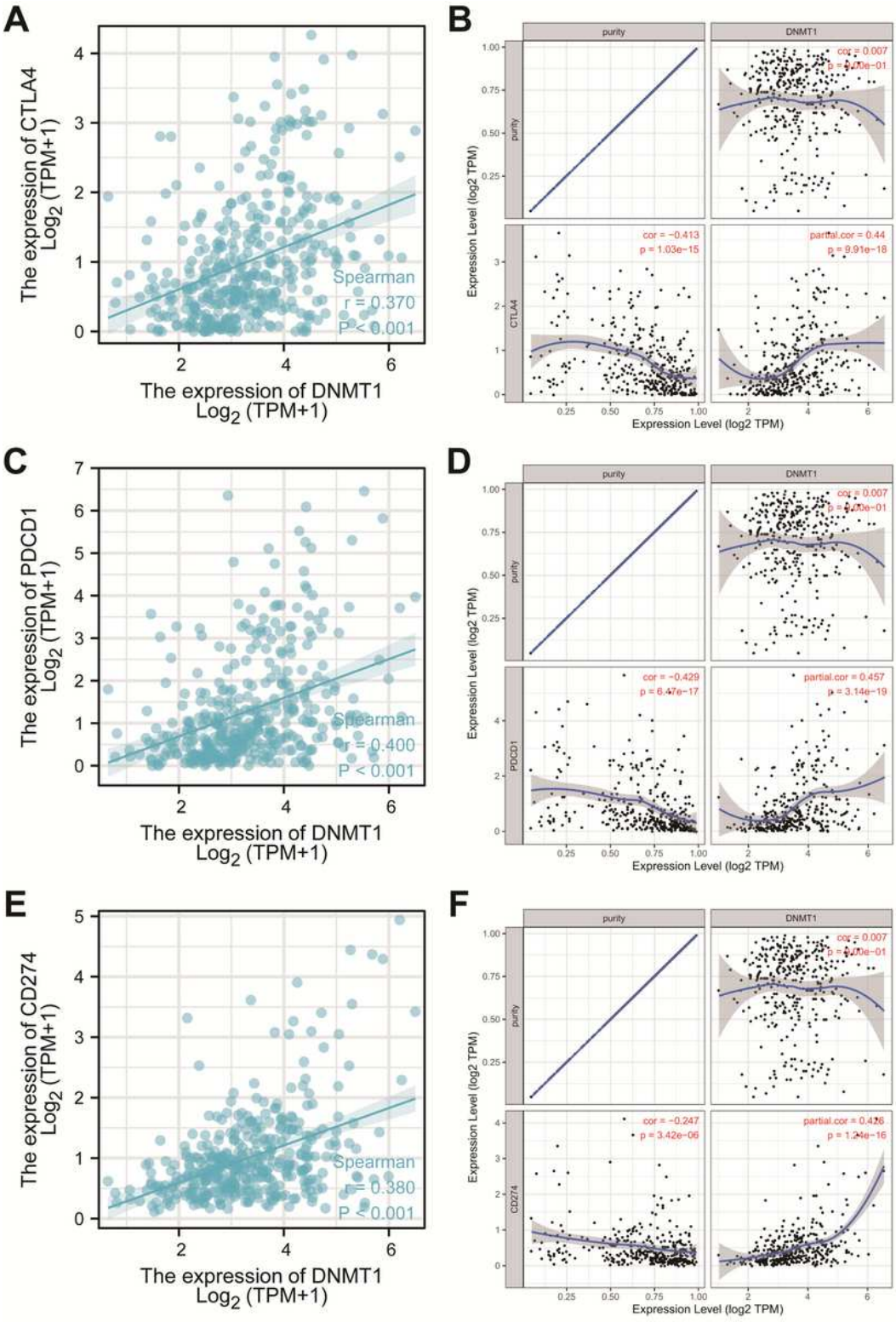


Figure 7

Relationship between the expression of DNMT1 and PD-1, PD-L1, or CTLA-4 in HCC. (A) The expression correlation of DNMT1 with CTLA-4 in HCC. (B) Spearman's correlation between the expression of DNMT1 and CTLA-4 in HCC adjusted for tumor purity using TIMER. (C) The expression correlation of DNMT1 with PDCD1 in HCC. (D) Spearman's correlation between the expression of DNMT1 and PDCD1 in HCC adjusted for tumor purity using TIMER. (E) The expression correlation of DNMT1 with CD274 (PD-L1) in HCC. (F) Spearman's correlation between the expression of DNMT1 and CD274 (PD-L1) in HCC adjusted for tumor purity using TIMER. DNA-methyltransferase 1; PD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; HCC, hepatocellular carcinoma; CTLA, cytotoxic T lymphocyte antigen 4.

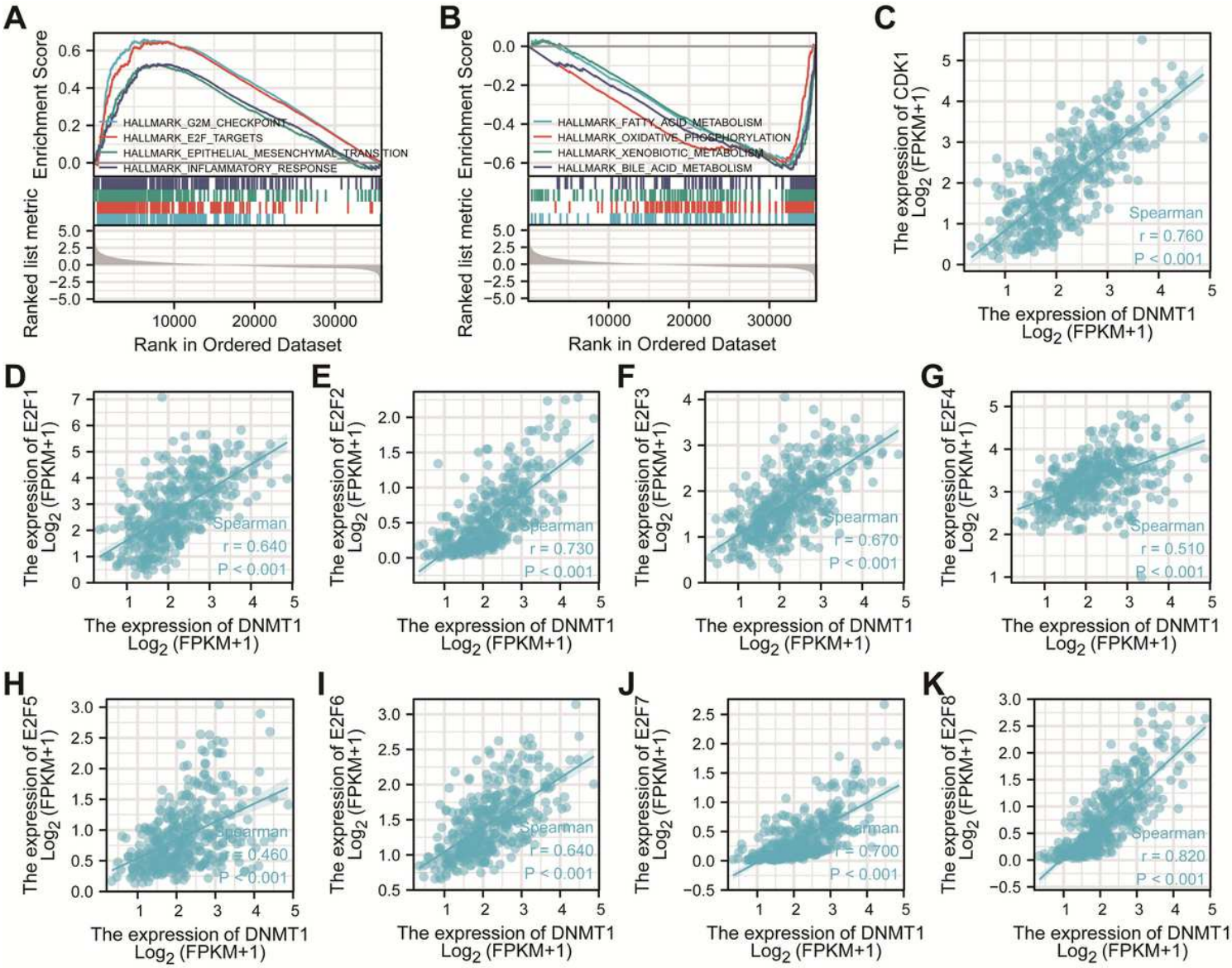


Figure 8

GSEA revealed Hallmark pathways related to DNMT1 in HCC. A□GSEA results showing differential enrichment of genes in Hallmark with high DNMT1 expression. B□GSEA results showing differential enrichment of genes in HALLMARK with low DNMT1 expression. C□The expression correlation of DNMT1 with CDK1 in HCC. D-K. The expression correlation of DNMT1 with E2Fs in HCC: E2F1 (D), E2F2 (E), E2F3

(F), E2F4 (G), E2F5 (H), E2F6 (I), E2F7 (G), and E2F8 (K). DNA-methyltransferase 1; GSEA, Gene Set Enrichment Analysis; HCC, hepatocellular carcinoma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- FigS1.tif