Pan-Cancer Analysis of Molecular Characteristics and Oncogenic Role of PRMT5 in Human Cancers

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**Pan-cancer analysis of molecular characteristics and oncogenic role of PRMT5
in human cancers**

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Abstract

Background

Accumulating evidence supports the correlation of protein arginine methyltransferase 5 (PRMT5) and cancers development. However, the expression and prognostic values of PRMT5 in various cancers have not been clarified.

Methods

Here, based on The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets, we performed a pan-cancer analysis to explore the expression profile, prognostic value landscape, relationship with tumor-infiltrating immune cells, and potential molecular mechanisms of PRMT5 in cancer development. Moreover, CCK8, wound healing and transwell assays, and western blotting analysis were conducted to evaluate how PRMT5 affects the proliferation and migration, and expression of related hallmarks in breast cancer cells.

Results

We found that PRMT5 was upregulated in most cancers and PRMT5 harbored distinct prognostic values across different cancer types. In addition, PRMT5 expression was negatively correlated with CD8⁺T cells in tumors of cervical squamous cell carcinoma and endocervical adenocarcinoma (CSEC) and Skin Cutaneous Melanoma (SKCM), and positively correlated with the cancer-associated fibroblasts in tumors of adrenocortical carcinoma, CESC, cholangio carcinoma, liver hepatocellular carcinoma, pancreatic adenocarcinoma, and SKCM-Primary. Moreover, the enrichment analysis identified that PRMT5 mechanistically regulated cancers development by acting on DNA and RNA metabolism, and stress response related pathways. By further gene silencing experiment, we confirmed that PRMT5 knockdown reduced the proliferative and migrative capacities, as well as the expression of PCNA (proliferating cell nuclear antigen), p21 and HMGB1 (high mobility group box 1 protein) in breast cancer cells.

Conclusion

Collectively, our pan-cancer study highlighted the importance value of PRMT5 in cancer development and prognosis, and pharmacologic targeting at PRMT5 may provide a novel approach for the treatment of cancers.

Keywords: PRMT5, pan-cancer analysis, immune infiltration, prognosis, proliferation

1 Background

Cancer is a group of diseases characterized by the dysregulation of important pathways that control cellular processes involved in DNA repair, cell survival, proliferation and mortality[1]. With the expanding development of epigenetics, researchers recognize that many biological processes associated with cancerogenesis are epigenetically regulated. Epigenetic alterations such as DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated targeting have been demonstrated to drive the onset of cancer[2, 3]. Therefore, deciphering aberrant epigenetic mechanisms of tumorigenesis will yield novel insights into anti-cancer strategies.

Arginine methylation mediated by protein arginine methyltransferase (PRMT) family is a widespread post-translational modification in eukaryotes and plays key roles in many biological processes, such as transcriptional repression[4], RNA splicing [5], ribosome biogenesis[6], cell death and proliferation[7, 8]. Using S-adenosine-methionine (SAM) as the methyl donor, PRMT transfers methyl to the nitrogen atom of protein arginine side chain and produces S-adenosyl-homocysteine and methyl arginine[9]. PRMT5, a main methyltransferase of the nine-member PRMT family, is known to regulate oncogenic processes including tumor intrinsic and cell-extrinsic microenvironmental factors[10]. In the past decade, PRMT5 has attracted increasing attention as an anticancer target, and its dysregulation has been certified in various cancers, including glioblastoma, leukemia/lymphoma[11], prostate cancer, and colorectal carcinoma[10]. However, there is still no comprehensive molecular landscape of PRMT5 across cancer types reported so far. Thus, a pan-cancer analysis of PRMT5 based on large-scale clinical data will help to uncover the epigenetic mechanisms and novel therapeutic targets for the intractable cancers.

The publicly funded TCGA project and the available GEO database contain functional genomics datasets of different tumors [12-14] and thus allow us to conduct pan-cancer analysis. In this study, we firstly evaluated the expression signature and prognostic value of PRMT5 in pan-cancer analysis using The Cancer Genome Atlas

(TCGA) project, and Genotype-tissue expression (GTEx) and Clinical proteomic tumor analysis consortium (CPTAC) dataset. We then explored the potential relationships between PRMT5 expression and immune infiltration levels. Finally, the potential molecular mechanism of PRMT5 in tumorigenesis were explored by bioinformatic analysis including protein-protein interaction, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene ontology (GO) analysis. Together, our first pan-cancer analysis provided an oncogenic landscape of PRMT5 across different cancers.

2 Materials and methods

2.1 Data Acquisition

PRMT5 gene expression, gene alteration, and clinical data of 33 cancer types were obtained from TCGA repository (<http://cancergenome.nih.gov>). The exclusion criteria adopted for the samples were (1) the loss of expression of PRMT5; (2) the loss of follow-up information; and (3) accompanied by other diseases. Other data were obtained from public databases as described in the corresponding parts.

2.2 PRMT5 gene expression analysis

The Tumor Immune Estimation Resource (TIMER 2.0) online tool (<http://timer.comp-genomics.org/>) was used to analyze the expression of PRMT5 between tumor and adjacent normal tissues for different tumors or specific tumor subtypes of TCGA project. For certain tumors of TCGA project without normal or with highly limited normal tissues [e.g., Lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), Brain lower grade glioma (LGG), Thymoma (THYM), and Acute Myeloid Leukemia (LAML) etc.], the expression difference between tumor tissues and corresponding normal tissues of the GTEx database were determined by the “Expression analysis-Box Plots” module of the GEPIA2 (Gene expression profiling interactive analysis, version 2) web server (<http://gepia2.cancer-pku.cn/#analysis>) [15], under the settings of P-value cutoff = 0.01, log₂FC (Fold change) cutoff=1, and “Match TCGA normal and GTEx data”. In addition, GEPIA2 was adopted to obtain violin plots of the PRMT5 expression in different pathological stages (stage I, stage II, stage III, stage IV) for tumors in TCGA project.

UALCAN (<http://ualcan.path.uab.edu/index.html>), a comprehensive web resource for analyzing cancer omics data, provides us opportunity to conduct protein expression analysis of Clinical Proteomic Tumor Analysis Consortium (CPTAC) dataset [16]. In this study, we detected the PRMT5 protein expression between primary tumor and normal tissues of CPTAC dataset. The available datasets of three tumors were selected, namely, colon cancer, Lung adenocarcinoma (LUAD), Uterine corpus endometrial carcinoma (UCEC).

2.3 Survival prognosis analysis

The overall survival (OS) and Disease-free survival (DFS) significance map data of PRMT5 across all TCGA tumors were acquired by calculating the log-rank P-value and the HR (hazard ratio-95% confidence interval) through the “Survival Analysis” module of GEPIA2 [17]. The high-expression and low-expression cohorts were distinguished by cutoff-high (50%) and cutoff-low (50%) values.

2.4 Genetic alteration analysis

The cBio Cancer Genomics Portal (<http://www.cbioportal.org/>) is an open platform for interactive exploration of multidimensional cancer genomics data [18]. We used cBioPortal to analyze PRMT5 alterations across all TCGA tumors. The analysis parameters included alteration frequency, mutation type and copy number variation. The mutated site information of PRMT5 was displayed in the schematic diagram of the protein structure of the three-dimensional (3D) structure via the “Mutations” module. The overall, disease-free, progression-free, and disease-free survival differences for the TCGA cancer cases with or without PRMT5 mutation were analyzed via the “Comparison” module.

2.5 Immune infiltration analysis

The “Immune-Gene module” of TIMER2 web server was employed to analyze the association between PRMT5 expression and immune infiltration across all TCGA cancers. We used the TIMER, EPIC, MCPOUNTER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ and XCELL algorithms to evaluate immune infiltration. The *P*-values and partial correlation (Cor) values were obtained via the purity-adjusted

Spearman's rank correlation test. The data were visualized as a heatmap and a scatter plot.

2.6 PRMT5-related gene enrichment analysis

The online database STRING website (<https://www.string-db.org/>) was used to obtain the available experimentally determined PRMT5-binding proteins. The “Similar Gene Detection” module of GEPIA2 was applied to obtain the top 100 PRMT5-related target genes of TCGA database. Then we performed Pearson correlation analysis of PRMT5 and selected genes via the “correlation analysis” module of GEPIA2. Moreover, heatmap data of selected genes was conducted by the “Gene_Corr” module of TIMER2 (Tumor immune estimation resource, version 2) web, which contains the partial correlation (Cor) and P-value in the purity-adjusted Spearman's rank correlation test.

To further analyze the target genes regulated by PRMT5, we performed an intersection analysis to compare the PRMT5-binding genes and interacted genes via an online Venn diagram tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). Moreover, we combined the two sets of data (PRMT5-binding and correlated genes) to perform KEGG pathway analysis. The enriched pathways were finally visualized with “ggplot2” R package. GO enrichment analysis was conducted by the “clusterProfiler” R package. Two-tailed $P < 0.05$ was considered statistically significant.

2.7 Small interfering RNA transfection

To introduce small interfering RNA (siRNA) into MDA-MB-231 cells, the cells were plated on 6-well plates at 30-50% confluence before transfection. Transfection of cells with siRNA was performed using Lipofectamine 3000 transfection reagent (Invitrogen, CA, USA), following the manufacturer's instructions. Commercially available PRMT5 siRNA (Gene Pharm, Shanghai, China), and negative control siRNA were used for transfection. Gene silencing was monitored by Western blot assay at 48 h post-transfection.

2.8 CCK8 assay

Cells were seeded and cultured at a density of 5×10^3 /well in 100 μ L of medium

into 96-well microplates (Corning, USA). Then, the cells were transfected with PRMT5 siRNA. Cell proliferation at indicated time points (24 h, 48 h, 72 h, 96 h post-transfection) was evaluated by Cell Counting Kit-8 (CCK8, Beyotime, Shanghai, China) according to the manufacturer's protocols. Optical density values at 460 nm were detected using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.9 Transwell Assay

MDA-MB-231 cells migration was analyzed in 24-well Transwell plates (8- μ m poresize, Corning, NY, USA). Briefly, 1×10^4 MDA-MB-231 cells were plated in the upper chamber of the transwell chamber (Corning, USA) in 24-well plate for 24 h at 37°C. Cells on the bottom surface of the chamber were fixed with 3.7% paraformaldehyde for 10 min and stained with 0.1% crystal violet/20% methanol and counted. Images were detected by a microscope (Zeiss Axio Vert A1, Carl Zeiss, Gottingen, Germany) and the migrated cell number was calculated as the number of migrated cells by Image J 1.52q software (National Institutes of Health).

2.10 Wound Healing Assay

MDA-MB-231 cells treated with siRNAs were seeded in 12-well plates. The cell monolayers were wounded by scratching with sterile plastic 200- μ l micropipette tips and photographed using phase-contrast microscopy immediately and 24 h after scratching. The migration areas were measured by counting the number of pixels using Image J.

2.11 Western blot assay

Cells were homogenized in ice-cold lysis buffer RIPA containing protease and phosphatase inhibitor cocktail (Sigma-Aldrich, Shanghai, China). Equal amounts of protein were separated by electrophoresis in SDS-PAGE and then transferred onto nitrocellulose filter membranes (GE whatman, USA). After blocking with 5% nonfat dried milk and then incubated overnight at 4°C with anti-PRMT5 mAb (Cat# ab109451, 1:10,000; Abcam, Shanghai, China), anti-PCNA mAb (Cat# A12427, 1:1000; ABclonal, Wuhan, China), anti-HMGB1mAb (Cat# A19529, 1:1000; ABclonal, Wuhan, China),

anti-p21 mAb (Cat# A19094, 1:1000; ABclonal, Wuhan, China), anti-BAX mAb (Cat# A19684, 1:1000; ABclonal, Wuhan, China), and anti- β -actin mAb (Cat# AC038, 1:50,000; ABclonal, Wuhan, China). After being washed with Tris-buffered saline with 0.1% Tween (TBST), the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit antibody (Cat# 65-6120, dilution 1:5000; Thermo Fisher, USA) for 2 h at room temperature. The reaction was followed by enhanced chemiluminescence reaction (Cat. WBKLS0500, Millipore, USA) and the blots were quantified using Bio-Rad imaging system (Image Lab 6.0) with β -actin as the internal control.

2.12 Statistical analysis

For the PRMT5 silencing experiment, quantitative data are expressed as a mean $\pm$ standard deviation (s.d.). All data were analyzed using GraphPad Prism 8 software (GraphPad, La Jolla, CA, USA). Statistical analysis of differences was performed by the Student's t-test between two groups and one-way analysis of variance followed by Tukey's test when comparing among multiple groups. Significance was assessed at a level of $P < 0.05$.

3. Results

3.1 Pan-cancer analysis of PRMT5 expression levels

To explore the oncogenic role of human PRMT5 (NP_006100.2 for protein), we firstly explored the protein structure conservation of PRMT5. As shown in Figure S1, the protein structure of PRMT5 is conserved among different species (e.g., *H. sapiens*, *P. troglodytes*, *M. mulatta*, etc.) and commonly consists of AdoMet_MTases (cl17173) domain.

To determine whether PRMT5 is aberrant expressed in distinct cancers, we analyzed the expression status of PRMT5 across different cancers of TCGA using the TIMER2. As shown in Figure 1A, PRMT5 was upregulated across most cancer types, such as Breast invasive carcinoma (BRCA), Cholangio carcinoma (CHOL), Colon adenocarcinoma (COAD), Esophageal carcinoma (ESCA), Head and Neck squamous cell carcinoma (HNSC), Kidney chromophobe (KICH), Kidney renal clear cell

carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Liver hepatocellular carcinoma (LIHC), LUAD, Lung squamous cell carcinoma (LUSC), Prostate adenocarcinoma (PRAD), Rectum adenocarcinoma (READ), Stomach adenocarcinoma (STAD), Thyroid carcinoma (THCA) ($P < 0.001$), Glioblastoma multiforme (GBM) ($P < 0.01$) and Bladder urothelial carcinoma (BLCA), Skin Cutaneous Melanoma (SKCM) ($P < 0.05$).

For certain tumors of TCGA project without normal tissues, we integrated tumor samples from TCGA and normal samples from GTEx databases to identify PRMT5 mRNA expression characteristics. As shown in Figure 1B, PRMT5 was markedly upregulated in tumor tissues of DLBC and THYM ($P < 0.01$) while downregulated in LAML. However, no significant difference was observed for other tumors, such as ACC (Adrenocortical carcinoma), OV (Ovarian serous cystadenocarcinoma) or TGCT (Testicular germ cell tumors) (Figure S2A). Furthermore, our protein expression analysis of CPTAC dataset also demonstrated that PRMT5 was upregulated in the primary tissues of colon cancer, LUAD and UCEC (Figure 1C). Additionally, to further confirm the relationship between PRMT5 and cancers development, we evaluated the expression of PRMT5 in multiple cancer cell lines, and found its expression was relatively higher in MDA-MB-231 and HepG2 cells when compared with MGC-803, BGC-823, SGC-7901 and MCF7 cells (Figure 1D).

Moreover, we analyzed the mRNA expression of PRMT5 in different clinical stages of cancers using the “Pathological Stage Plot” module of GEPIA2. As shown in Figure 1D, PRMT5 expression varied significantly in different clinical stages of KICH, KIRC and LIHC ($P < 0.05$). No association was found between PRMT5 expression and cancer stage in other cancers (Figure S2B).

3.2 Prognostic Significance of PRMT5 Expression in Human Cancers

To investigate the prognostic value of PRMT5, we firstly divided the cancer cases of TCGA and GEO database into high and low-expression according to the expression levels of PRMT5, and then investigated the correlation of PRMT5 expression with the prognosis of patients with different tumors by the GEPIA2 tool. As shown in Figure 2A, upregulated expression of PRMT5 was linked to poor prognosis of OS for tumors

including ACC ($P < 0.001$), BLCA ($P = 0.047$), HNSC ($P = 0.047$), LAML ($P = 0.034$), LIHC ($P = 0.0065$), LUAD ($P = 0.048$), PAAD (Pancreatic adenocarcinoma) ($P = 0.013$) and SARC ($P = 0.027$) within the TCGA project. The DFS analysis data (Figure 2B) showed high PRMT5 expression was associated with poor prognosis for TCGA cases including ACC ($P < 0.001$), BLCA ($P = 0.03$), KICH ($P = 0.028$), LIHC ($P = 0.0045$) and PAAD ($P = 0.007$). Conversely, high expression of PRMT5 indicated better OS prognosis for GBM ($P = 0.021$), KIRC ($P = 0.016$), LGG ($P = 0.0039$) and DFS prognosis for GBM ($P = 0.018$), KIRC ($P = 0.0057$), LGG ($P = 0.0082$).

To further examine the prognostic potential of PRMT5 in different cancers, we conducted a correlation analysis between PRMT5 expression and various survival curves using the Kaplan-Meier plotter. As shown in Figure S3A, high expression of PRMT5 was correlated with poor Relapse-free survival (RFS) ($P < 0.001$) prognosis for breast cancer. However, high expression of PRMT5 was linked to good Progress-free Survival (PFS) ($P = 0.022$) for ovarian cancer (Figure S3B) and good OS ($P < 0.001$), First progression (FP) ($P = 0.0014$) and Post Progression Survival (PPS) ($P < 0.001$) prognosis for gastric cancer (Figure S3D). In contrast, highly expressed PRMT5 was related to poor OS ($P = 0.0083$) and FP ($P < 0.001$) prognosis for lung cancer (Figure S3C) and poor OS ($P = 0.0098$), PFS ($P = 0.018$), RFS ($P = 0.0069$), and Disease-specific survival (DSS) ($P = 0.031$) prognosis of liver cancer (Figure S3E). Our meta-analysis data (Figure S4) further confirmed the relationship between PRMT5 expression and prognosis for ovarian cancer, lung cancer, gastric cancer and liver cancer but not breast cancer. These data collectively suggested that PRMT5 expression is differentially associated with the prognosis of cases with various cancers.

3.3 Genetic alteration of PRMT5 and its association with survival prognosis in human cancers

After defining the prognostic value of PRMT5, we next observed the genetic alteration status of PRMT5 in different cancer samples of the TCGA cohorts. As shown in Figure 3A, the highest alteration frequency of PRMT5 ($> 4\%$) appears for patients with melanoma with “mutation” as the primary type. It is worth noting that all uterine CS and sarcoma cases with genetic alteration ($\sim 2\%$) had gene amplification of PRMT5

(Figure 3A). We further analyzed the types, sites and case number of the PRMT5 genetic alteration. As shown in Figure 3B, missense mutation of PRMT5 was the main type of genetic alteration, and R534H/C (translation from R (arginine) to H (histidine) or C (cystine)) alteration of PRMT5 was detected in cases of colon adenocarcinoma (COAD), mucinous stomach adenocarcinoma (MSAD) and uterine corpus endometrial carcinoma (UCEC). R534 site was shown in the 3D structure of PRMT5 protein (Figure 3C). We then determined whether genetic alteration of PRMT5 is related to the clinical survival prognosis of cases with different types of tumor. It was noted that, compared with those cases without PRMT5 alteration, UCEC cases with altered PRMT5 was positively correlated with better prognosis in progression-free ($P = 0.0254$) survival, but not overall ($P = 0.168$), disease-specific ($P = 0.191$) and disease-free ($P = 0.0764$) survival. Together, the above findings indicated that PRMT5 mutation is linked to tumorigenesis and prognosis.

3.4 Correlation of PRMT5 expression with immune infiltration

It has long been recognized that $CD8^+$ T lymphocytes inhibit the initiation, progression or metastasis of cancers[19, 20], and cancer-associated fibroblasts modulate the function of various tumor-infiltrating immune cells[21, 22]. Herein, using the TIMER, EPIC, MCPOUNTER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ and XCELL algorithms, we investigated the potential association between PRMT5 expression and the infiltration levels of $CD8^+$ T and cancer-associated fibroblasts in different cancer types of TCGA. As shown in Figure 4, PRMT5 expression was negatively related to the immune infiltration of $CD8^+$ T cell in tumors of CESC and SKCM base on most algorithms. Additionally, we found that PRMT5 expression was positively correlated with the cancer-associated fibroblasts for the TCGA tumors of ACC, CESC, CHOL, LIHC, PAAD, and SKCM-Primary, but negatively related to LGG (Figure 5A). Moreover, the scatterplot data of each tumor produced by one algorithm (Figure 5B) further demonstrated the association between PRMT5 and fibroblasts infiltration. For instance, the expression level of PRMT5 in ACC is positively related to infiltration level of cancer-associated fibroblasts ($Cor = 0.349$, $P = 2.48e-03$) based on TIDE algorithm.

3.5 Enrichment analysis of PRMT5-related partners

To illustrate the potential mechanisms underlying PRMT5 function in tumorigenesis, the protein-protein interaction network was constructed by assembling the PRMT5 associated proteins using the STRING tool. Based on various experimentally collected data, we obtained a total of 44 proteins that binding to PRMT5 (Figure 6A). In addition, we used GEPIA2 tool to explore the genes that correlated with PRMT5 expression, and found that top 100 genes were correlated with PRMT5 expression. As specially shown in Figure 6B, the expression of PRMT5 was positively correlated with various genes expression including suppressor of Ty 16 homolog (SUPT16H) ($R = 0.69$), 5 proteasome (PSMB5) ($R = 0.64$), Neuroguidin (NGDN) ($R = 0.66$), nucleolin (NCL) ($R = 0.48$), Eukaryotic translation initiation factor 2 subunit 1 (EIF2S1) ($R = 0.52$), and apyrimidinic endonuclease 1 (APEX1) ($R = 0.62$). The corresponding heatmap data also certified a positive association between PRMT5 and the above six genes in most detailed cancer types (Figure 6C). By further Venn diagram analysis, two genes (EIF2S1 and NCL) were found in the intersection of the PRMT5-binding and correlated genes (Figure 6D).

To better understand the biological functions of the above 142 PRMT5-overlapping genes, we performed the GO annotation and KEGG pathway analyses. As shown in Figure 6E, the most significantly enriched KEGG pathways indicated that “systemic lupus erythematosus”, “viral carcinogenesis” and “spliceosome” might be involved in the effect of PRMT5 on tumor pathogenesis. The GO enrichment analysis data further indicated that most of these genes are involved in the DNA and RNA metabolism, and stress response related pathways such as catalytic activity acting on DNA/RNA, ATPase activity, transcription coregulator activity, helicase activity, Hsp90 protein binding, etc. (Figure 6F).

3.6 PRMT5 depletion decreases the proliferative and migratory capacities of breast cancer cells

The above GO and KEGG analysis indicated that the carcinogenic role of PRMT5 is linked to the pathways or cellular biology of DNA and RNA metabolism, which suggested that PRMT5 may promote cancers by stimulating cancer cells proliferation.

To prove this, PRMT5 was knocked down in a representative breast cancer cell line, and we found that the proliferation rates of MDA-MB-231 cells were significantly inhibited following PRMT5 knockdown (Figure 7A). Besides, PRMT5 silencing significantly reduced the migratory and invasive ability of MDA-MB-231 cells as evidenced by wound healing (Figure 7B) and transwell assays (Figure 7C), respectively. Moreover, western blot results showed that PRMT5 knockdown decreased the protein levels of PCNA, p21, and HMGB1 in MDA-MB-231 cells (Figure 7D), which may explain the PRMT5-mediated proliferation and migration in breast cancer cells. Taken together, our data demonstrated that PRMT5 facilitates the proliferation and migration of breast cancer cells.

Discussion

In this study, we systematically analyzed dysregulation of PRMT5 across 33 cancer types based on TCGA, CPTAC and GEO databases. We discovered that PRMT5 was upregulated in most cancers, and distinct associations exist between PRMT5 expression and prognosis of tumor patients. In addition, our pan-cancer analysis demonstrated that PRMT5 expression was negatively correlated with CD8⁺T cells in tumors of CESC and SKCM, and positively correlated with the cancer-associated fibroblasts in tumors of ACC, CESC, CHOL, LIHC, PAAD, and SKCM-Primary. Moreover, our enrichment analysis revealed that PRMT5 involved in carcinogenesis by regulating DNA and RNA metabolism, and stress response related pathways. Altogether, our study identified the importance role of PRMT5 in cancer development and prognosis, and pharmacologic targeting at PRMT5 may provide a novel approach for the treatment of cancers.

Overexpression of PRMT5 has been documented in a wide variety of cancers, such as lymphoma[23], ovarian cancer[24], breast cancer[25], colorectal cancer[26], and lung cancer[27]. Consistently, our pan-cancer analysis across 33 cancer types also demonstrated that PRMT5 was upregulated in most cancers and varied in different clinical stages of cancers, which further highlighted the key role of PRMT5 in cancers development. In this context, most studies have pointed out that PRMT5

overexpression is associated with a decrease in patient survival and an increase in tumor growth[24, 28-30], and nowadays highly potent and selective PRMT5 inhibitors, such as EPZ015666, have been developed and displayed anti-tumor properties in animal models[31]. However, our large-scale database analysis indicated that the expression of PRMT5 can also be associated with prolonged survival for cancers such as GBM, KIRC, LGG, at the opposite of the ACC, BLCA, KICH, LIHC, PAAD. This discrepant relationship can be partially explained by the fact that PRMT5 regulated the expression of genes implicated in both tumor promotion and suppression [32]. In addition, the subcellular localization of PRMT5 regulates its activity and oncogenic properties[33]. Lattouf *et al* (2019) have demonstrated that PRMT5 nuclear expression is associated with prolonged survival in breast cancer [34], and the nuclear fraction of PRMT5 was responsible for inhibiting cell growth, while the cytoplasmic fraction promoted cell growth in prostate cancer[35]. Therefore, the subcellular expression of PRMT5 needs to be considered in future oncogenic investigation and PRMT5-targeted therapies development.

Cytotoxic CD8⁺ T cells are the most powerful effectors in the anticancer immune response and form the backbone of current successful cancer immunotherapies [36, 37]. Interestingly, our multiple immune deconvolution analysis demonstrated a negative correlation between PRMT5 expression and the infiltration of CD8⁺ T cells in CESC and SKCM, indicating immunotherapeutic potential of PRMT5 in certain cancers. Indeed, Pharmacological inhibition of PRMT5 by DS-437 has been proved to be effective in increasing CD8⁺ T infiltration and reducing tumor growth in breast cancer[38]. Another important finding of this study is that PRMT5 expression is correlated with cancer-associated fibroblasts in certain tumors. Consistently, Gu *et al* (2012) found that PRMT5 controls cancer cells growth by targeting at fibroblast growth factor receptor signaling [39]. Cancer-associated fibroblasts have been proved to be associated with cancer cells at all stages of cancer progression, and their production of growth factors, chemokines and extracellular matrix facilitates the angiogenic recruitment of immune cells, endothelial cells and pericytes[40]. Therefore,

deciphering how PRMT5 regulates the function of cancer-associated fibroblasts in future research will help us to deeply understand the pathogenesis of cancers and possible PRMT5 targeting treatments.

The PRMTs are critical regulators of cell homeostasis. By further KEGG and GO analysis, we identified that PRMT5 mechanistically regulates malignant tumors by acting on DNA and RNA metabolism, and stress response related pathways. Recently, increasing evidence suggests that DNA and RNA modification are mis-regulated in human cancers and are ideal targets of cancer therapy [41, 42]. Interestingly, a flourish of recent reports have solidified PRMT5 as a major regulator of epigenetic-mediated gene expression [43], mRNA splicing [44], and the DNA damage response [45] in the setting of tumors. Additionally, Fong *et al* (2019) demonstrated that inhibiting symmetric dimethylation of arginine mediated by PRMT5 reduces RNA splicing catalysis and results in preferential killing of leukemias[46]. Along with DNA/RNA modification, excessive proliferation and migration of cancer cells involve in tumorigenesis and metastasis[47]. Herein, we demonstrated that PRMT5 knockdown repressed the proliferative and migrative capacities of breast cancer cells by modulating critical regulators PCNA, p21 and HMGB1. Consistently, several studies have also linked PRMT5 to breast carcinogenesis by promoting cell growth, migration and invasion[34, 48, 49]. Thus, the above findings together suggested that PRMT5 is a key epigenetic regulator of cancer cells biology and resultant cancer development, which further underscores the importance of PRMT5 in future cancer research.

There were some limitations in our study. First, all the data analyzed in our study were obtained from different online databases, and further studies of large-scale clinical samples are needed to validate our findings due to the substantial phenotypic variability and genetic heterogeneity of tumors. In addition, although we identify the upregulated PRMT5 in most cancers, however the expression profile of PRMT5 in different clinical stages are only available for some cancers, thus the dynamic investigation of PRMT5 in all cancer types will help us to deeply understand the oncogenic role PRMT5 and cancers progression. More importantly, how PRMT5 regulates carcinogenesis and

426 metastasis and gene regulatory networks mediated by PRMT5 needs to be clarified in
427 future studies.

428 In conclusion, our pan-cancer analysis firstly demonstrates that PRMT5 is
429 upregulated in most cancers and its dysregulation is closely related with patient survival
430 prognosis, CD8⁺ T cells and cancer associated fibroblasts infiltration, and mutational
431 burden in certain tumors. In addition, the enrichment analysis identifies that PRMT5
432 mechanistically regulates cancers development by acting on DNA and RNA
433 metabolism, and stress response related pathways. Thus, therapeutic strategies to
434 specifically target PRMT5 might be useful for manipulating human cancers.

435 **Abbreviations**

PRMT5	protein arginine methyltransferase 5	ESCA	Esophageal carcinoma
TCGA	The Cancer Genome Atlas	HNSC	Head and Neck squamous cell carcinoma
GEO	Gene Expression Omnibus	KICH	Kidney chromophobe
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	KIRC	Kidney renal clear cell carcinoma
SKCM	Skin cutaneous melanoma	KIRP	Kidney renal papillary cell carcinoma
SAM	S-adenosine-methionine	LIHC	Liver hepatocellular carcinoma
GTE _x	Genotype-tissue expression	LUSC	Lung squamous cell carcinoma
CPTAC	Clinical proteomic tumor analysis consortium	PRAD	Prostate adenocarcinoma
KEGG	Kyoto Encyclopedia of Genes and Genomes	READ	Rectum adenocarcinoma
GO	Gene ontology	STAD	Stomach adenocarcinoma
DLBC	Diffuse large B-cell lymphoma	THCA	Thyroid carcinoma
LGG	Lower grade glioma	GBM	Glioblastoma multiforme
THYM	Thymoma	BLCA	Bladder urothelial carcinoma
LAML	Acute Myeloid Leukemia	ACC	Adrenocortical carcinoma
GEPIA2	Gene expression profiling interactive analysis, version 2	OV	Ovarian serous cystadenocarcinoma
CPTAC	Clinical Proteomic Tumor Analysis Consortium	TGCT	Testicular germ cell tumors
LUAD	Lung adenocarcinoma	RFS	Relapse-free survival
UCEC	Uterine corpus endometrial carcinoma	PFS	Progress-free Survival
OS	Overall survival	FP	First progression
DFS	Disease-free survival	PPS	Post Progression Survival
TIMER2	Tumor immune estimation resource, version 2	DSS	Disease-specific survival
BRCA	Breast invasive carcinoma	COAD	Colon adenocarcinoma
CHOL	Cholangio carcinoma	MSAD	Mucinous stomach adenocarcinoma
COAD	Colon adenocarcinoma	UCEC	Uterine corpus endometrial carcinoma
PCNA	Proliferating cell nuclear antigen	HMGB1	High mobility group box 1 protein
CCK8	Cell Counting Kit-8		

436 **Availability of data and materials**

437 All data generated during this study are included in this published article. The

datasets generated in the current study are obtained from TCGA repository (<http://cancergenome.nih.gov>).

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Contributions

LH and HLX conceived and designed the study. LH and JKP performed the analysis. HL and PXH wrote the paper.

Consent for publication

All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they no competing interests.

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Figure legends

Figure 1. The expression of PRMT5 in different cancers and pathological stages. (A) The mRNA expression level of PRMT5 between tumor and normal tissues were compared using the TCGA database. (B) The mRNA expression level of PRMT5 in cancers of DLBC, LAML, LGG, and THYM. For the above four cancers of TCGA project without normal tissues, the corresponding normal tissues of the GTEx database were included as controls. (C) The protein expression level of PRMT5 between normal tissues and primary tissues of colon cancer, LUAD and UCEC was analyzed based on CPTAC dataset. (D) PRMT5 expression levels were analyzed at the main pathological stages (stage I, stage II, stage III and stage IV) of KICH, KIRC and LIHC based on the TCGA dataset. Log 2 (TPM+1) was applied for log-scale. (E) The expression level of PRMT5 was evaluated in various cancer cell lines by Western blot. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2. Correlation between PRMT5 expression and survival prognosis of cancers using the TCGA dataset. GEPIA2 was used to determine the correlation of PRMT5 expression with overall survival (A) and disease-free survival (B) prognosis across cancer types. The survival map and Kaplan-Meier curves with positive results are given.

Figure 3. Mutation feature of PRMT5 in various cancers of TCGA. cBioPortal tool was used to analyze the mutation features of PRMT5 for the TCGA cancers. (A) Alteration frequency and mutation types; (B) Mutation site; (C) Mutation site with the highest alteration frequency (R534) was displayed in the 3D structure of PRMT5. (D) The correlation between mutation status of PRMT5 and OS, DSS, DFS and PFS of UCEC.

Figure 4. Correlation analysis between PRMT5 expression and infiltration of CD8⁺ T cells.

Figure 5. Correlation analysis between PRMT5 expression and infiltration of cancer-associated fibroblast.

Figure 6. PRMT5-related gene enrichment analysis. (A) The available experimentally

determined PRMT5-binding proteins. (B) The expression correlation of PRMT5 and selected targeting genes from the top 100 PRMT5-correlated genes in TCGA projects. (C) The corresponding heatmap data in the detailed cancer types. (D) An intersection analysis of PRMT5 correlated and -binding genes. KEGG pathway (E) and GO analysis (F) based on the PRMT5 correlated and binding genes.

Figure 7. Knockdown of PRMT5 inhibited the proliferation and migration of breast cancer cells. MDA-MB-231 cells were transfected with PRMT5 siRNA (siPRMT5) or scrambled siRNA (siScr) as described in Materials and Methods. (A) The proliferation of MDA-MB-231 cells was examined by CCK-8 assay. The migration of MDA-MB-231 cells was examined by transwell assay (B) and wound healing assay (C). (D) Western blot and quantitative analyses of PCNA, p21 and HMGB1 in control and PRMT5-silenced MDA-MB-231 cells. Data were shown as mean $\pm$ s.d.. * P < 0.05, ** P <0.01, *** P <0.001.

Figure S1. Conserved domains of PRMT5 protein among different species.

Figure S2. Expression level of the PRMT5 gene in different tumors and pathological stages. (A) The expression status of the PRMT5 gene in ACC, OV, SARC, SKCM, TGCT, UCS in TCGA project were compared with the corresponding normal tissues of the GTEx databases. (B) Expression levels of the PRMT5 gene in different pathological stages of ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, HNSC, KIRP, LUAD, LUSC, OV, PAAD, READ, SKCM, STAD, TGCT, THCA, UCEC.

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Figure S4. Meta-analysis on the correlation between PRMT5 expression and cancer prognosis.

Figures

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