Journal on Oncology

www.journalononcology.org

Research Article

Open Access, Volume 4

Comprehensive Analysis Reveals Genomics and Clinical Characteristics of the DNMT Family and TET Enzyme Family in Pan-Cancer

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Abstract

The purpose of this study is to comprehensively analyse the specific roles of DNA Methyltransferase (DNMT)/Ten-Eleven-Translocation (TET) family genes in pan-cancer. The expression, mutation, Copy Number Variations (CNVs), cancer-related pathways, immune cell infiltration correlation and prognostic potential of DNMT/TET genes were systematically investigated in 33 cancer types using next-generation sequencing data from The Cancer Genome Atlas database. DNMT3B was overexpression in the majority of tumor tissues than in normal tissues and was associated with worse prognoses across cancers. Most DNMT/TET genes were frequently mutated in UCS, and TET1 and TET2 showed higher mutation frequencies in various cancer types. DNMT3B exhibited inclusive copy number amplification in almost all cancers. DNMT/TET genes were mainly involved in cancer-related pathways such as TGF beat signaling. DNMT/TET genes were significantly correlated with NK cells, CD4+T cells, and Tfh cells. Furthermore, TRIM8 and CIRBP were DNMT3B-related methylated genes common in most cancers, and the latter were mainly enriched in the MAPK signaling pathway. Additionally, overexpression of most DNMT genes, except for DMAP1, was associated with worse prognoses across cancers. In a word, this study reveals the genomic alteration and clinical characteristics of DNMT/TET genes across 33 cancers, which might help to clarify the relationship between DNA methylation and tumorigenesis, and contribute to the identification of potential predictors for risk and prognosis in pan-cancer.

Keywords: DNA methylation; DNMT; TET; Pan-cancer; Prognosis.

Manuscript Information: *Received: Oct 24, 2024; Accepted: Nov 22, 2024; Published: Nov 29, 2024*

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Citation: *Liu Z, Li Q, Chen S, Ji F, Piao Y, et al. Comprehensive Analysis Reveals Genomics and Clinical Characteristics of the DNMT Family and TET Enzyme Family in Pan-Cancer. J Oncology. 2024; 4(2): 1158.*

Introduction

Traditionally, it is believed that cancer is caused by genetic alterations including deletions, mutations, genomic instability, Copy Number Variations (CNVs), insertions, recombination, and single-nucleotide polymorphisms [1]. However, increasing evidence suggests that cancer occurrence, development and progression are results of the interaction of genetic and epigenetic changes [2,3]. Recent evidence has shown that half of tumour suppressor gene inactivation is the result of epigenetic rather than genetic mechanisms [4].

DNA methylation is the most common epigenetic regulatory mechanism and plays a critical role in parental imprinting, DNA replication, regulation of gene expression, X chromosome inactivation, and stable gene silencing [1,5,6]. The levels and patterns of DNA methylation are regulated by both DNA methyltransferase (DNMT, including DNMT1, DNMT3A and DNMT3B) and the Ten-Eleven-Translocation (TET) family of dioxygenases (TET1, TET2 and TET3) [7-10]. DNMTs are responsible for transferring a methyl group to the fifth carbon of a cytosine residue to form 5-methylcytosine (5mC) and mediate active DNA methylation [11]. In contrast, TET family oxidizes 5mC to form 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), thereby regulating active DNA demethylation [11,12]. DNA methylation and demethylation are processes of dynamic regulation and mutual transformation mediated by changes in DNMT/TET activity, which are critical in tumorigenesis and development [13-16]. reported that DNMT down-regulated the expression level of Barx2, thereby promoting the proliferation and invasion of gastric cancer cells [16]. Su Jung et al. demonstrated that miR-22 could inhibit demethylation of miR-200 through direct targeting of the TET family of methylcytosine dioxygenases, thereby exerting breast cancer metastatic potential [17]. In recent years, the development of high-throughput sequencing has led to a drastic increase in genetic and epigenetic research in diverse cancers. Numerous mutations in DNMT and TET have been identified in carcinogenic processes, highlighting the importance of DNA methylation mechanisms in the occurrence, development and prognosis of human cancer. However, the specific implication concerning DNMT/TET genes in tumorigenesis has not been fully elucidated.

Given the role of DNA methylation in cancer, it is of great interest to elucidate the whole landscape of expression, mutation, and copy number variation of DNMT/TET genes, as well as their oncogenic pathway activity, immune cell infiltration and prognostic potential. This study was designed to analyse DNMT/TET gene data from 33 types of cancers in The Cancer Genome Atlas (TCGA). We postulate that this work will generate fresh insight into the way DNMT/TET genes influence cancer.

Materials and methods

Collection of DNMT/TET genes

We collected six key DNMT family members (DNMT1, TRDMT1, DNMT3A, DNMT3B, DNMT3L, DMAP1) and three key TET family members (TET1, TET2, TET3) from published review papers [18-20]. We manually converted the gene symbols into Human Genome Organisation Gene Nomenclature Committee (HGNC) symbols and Ensemble gene IDs from GeneCards. (https://www.genecards.org/).

Pan-cancer genome-wide omics data and tumour types

We obtained omics datasets from TCGA database (http://cancergenome.nih.gov/) aimed at TPM (transcripts per kilobase million) expression, mutation, CNVs, methylation data and clinical information (survival status, survival time) from 33 different types of cancers, including CHOL: cholangiocarcinoma; KIRC: kidney renal clear cell carcinoma; DLBC: lymphoid neoplasm diffuse large b-cell lymphoma; KIRP: kidney renal papillary cell carcinoma; UVM: uveal melanoma; KICH: kidney chromophobe; MESO: mesothelioma; LGG: brain lower grade glioma; THYM: thymoma; GBM: glioblastoma multiforme; TGCT: testicular germ cell tumors; BRCA: breast cancer; ESCA: esophageal carcinoma; LUSC: lung squamous cell carcinoma; PAAD: pancreatic adenocarcinoma; LUAD: lung adenocarcinoma; READ: rectum adenocarcinoma; LAML: acute myeloid leukemia; COAD: colon adenocarcinoma; SARC: sarcoma; UCS: uterine carcinosarcoma; PCPG: pheochromocytoma and paraganglioma; UCEC: uterine corpus endometrial carcinoma; ACC: adrenocortical carcinoma; OV: ovarian serous cystadenocarcinoma; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; HNSC: head and neck squamous carcinoma; LIHC: liver hepatocellular carcinoma; THCA: thyroid carcinoma; BLCA: bladder urothelial carcinoma; PRAD: prostate adenocarcinoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma. All of the TCGA data were downloaded via UCSC XENA (https://xenabrowser.net/).

mRNA differential expression analysis in pan-cancer

mRNA Seq data were collected from TCGA database. Only 17 cancer types with more than three pairs of tumour and normal samples were included in the analysis, including BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA and UCEC. The mRNA values were expressed as normalized RSEM values. The genes with $|\text{Log2 fold change}| > 1$ and adjusted P values < 0.05 were reserved for further analysis. TBtools software was used to draw a heatmap [21].

Protein-wide omics data across 20 cancer types from protein expression data

Protein expression data were derived from Human Protein Atlas Datasets (HPA)(https://www.proteinatlas.org/). In the HPA database, the protein expression of each gene was divided into four groups, high, medium, low and not detected, among which one group with a sample number <3 was not included in the subsequent analysis. DNMT/TET protein expression was systematically analysed in 20 cancer types, including SKCA, BRCA, ENCA, RACA, STCA, PACA, carcinoid, OV, URCA, LIHC, CECA, THCA, melanoma, PRCA, COCA, lymphoma, TECA, glioma, LUCA, HNSC. The results were visualized by GraphPad Prism version 8 (GraphPad Software, La Jolla, CA, USA).

Genome-wide mutation analysis and CNV analysis

Raw data on DNMT/TET gene mutations and CNVs across 33 cancer types were obtained from the TCGA database. The mutation frequency was calculated. In the CNV module analysis, the CNV data were divided into two groups, amplification and deletion, and processed using GISTICS 2.0. The percentage of CNVs and the correlation of raw CNV data and mRNA RSEM data were

calculated. The P-value was adjusted by the FDR.

Oncogenic pathway activity analysis

The correlation between gene expression and cancer-related pathways was determined using Gene Set Variation Analysis (GSVA). This is a Gene Set Enrichment (GSE) method to estimate pathway activity variation based on a sample expression matrix in an unsupervised, non-parametric manner. The Spearman Correlation Coefficient (SCC) was calculated to assess the correlation between gene expression and pathway activity. |SCC| > 0.25 and adjusted P value < 0.05 were considered to indicate a significant correlation. The results were visualized by Cytoscape v3.7.1.

Immune cell infiltration analysis

The major immune cell-related genes are shown in Supplementary Table 1. We calculated the SCC between the expression of DNMT/TET genes and immune-related genes to explore their association. |SCC| > 0.25 and adjusted P value < 0.05 were considered to indicate a significant correlation. The results were visualized by Cytoscape v3.7.1.

DNA methylation and KEGG analysis

The LinkedOmics database (http://www.linkedomics.org.) was used to identify DNMT3B-related methylation genes in cancers, including BLCA, BRCA, COAD/READ, ESCA, STAD, HNSC, KIRP, LIHC, LUAD, LUSC and UCEC. The correlation between the expression of DNMT3B and the methylation level of genes was considered to be statistically significant with |SCC| > 0.3 and adjusted P value < 0.05. Next, to investigate the anomalous pathways by dysregulated DNMT3B-related methylated genes in cancers, enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was conducted using the clusterProfiler R package [22]. P < 0.05 was considered to be statistically significant.

Survival analysis

DNMT/TET gene mRNA expression data and corresponding clinical survival data in pan-cancer were merged for survival analysis. The median gene expression was selected as a cut-off value to divide tumour samples into high and low groups. Kaplan-Meier analysis with the log-rank test was used to compare survival rates between the two groups. Additionally, gene expression and prognostic value associations were analysed using the GEPIA2 (Gene Expression Profiling Interactive Analysis 2) database. A P value < 0.05 was considered as statistically significant.

Statistical analysis

All statistical analyses were performed using R software v4.1.0 (http://www.r-project.org) and GraphPad Prism version 8 (GraphPad Software, La Jolla, CA, USA). Spearman correlation test was used for correlation analysis. If not otherwise stated, the rank-sum test was used to test two sets of data, and a P value < 0.05 was considered statistically significant.

Results

DNMT/TET gene expression profiles in pan-cancer

We summarized the chromosomal positions of all DNMT/TET family members across the published literature (Table 1). We then analysed the gene expression differences in TCGA. The results showed heterogeneous expression of DNTM and TET genes in different cancer types. DNMT3B was highly expressed in 13 of the 17 tumours analysed; DNMT3L was expressed at low levels in CHOL, KICH, KIRP, and KIRC and had increased expression in UCEC READ, ESCA, and LUSC. (Figure 1a). The log2FC and adjusted P value of the results are listed in Table 2. The differential expression of DNMT3B in different cancers is visualized (Figure 1b). DNMT3B expression is up-regulated in many cancer types, including BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD and UCEC.

Table 1: Basic characteristics of DNMT/TET genes.

HGNC ID (gene)	GENE ID	Approved symbol	Synonym(s)	Exon	Chromosomal location
2976	1786	DNMT1	AIM DNMT MCMT CXXC9 HSN1E ADCADN m.HsaI	41	19p13.2
2977	1787	TRDMT1	DMNT2 DNMT2 PUMET RNMT1 MHSAIIP	15	10p13
2978	1788	DNMT3A	TBRS HESJAS DNMT3A2 M.HsaIIIA	34	2p23.3
2979	1789	DNMT3B	ICF ICF1 M.HsaIIIB	24	20q11.21
2980	29947	DNMT3L	-	12	21q22.3
18291	55929	DMAP1	EAF2 SWC4 MEAF2 DNMAP1 DNMTAP1	11	1p34.1
29484	80312	TET1	LCX CXXC6 bA119F7.1	20	10q21.3
25941	54790	TET2	MDS KIAA1546	15	4q24
28313	200424	TET3	BEFAHRS hCG_40738	17	2p13.1

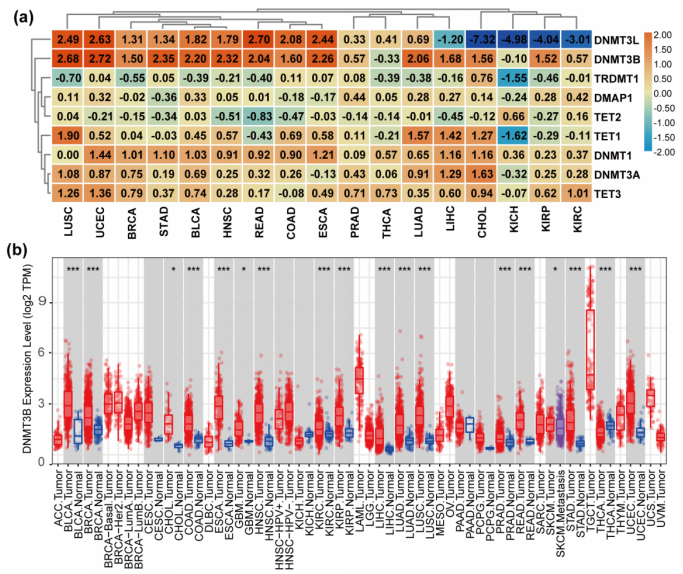

Figure 1: DNMT/TET gene mRNA expression profiles across different cancer types. **(a)** DNMT/TET gene expression in different cancers and normal samples. The colour in the heatmap represents the log2-fold change value between cancer and normal tissues. Blue represents low expression in cancer, while red represents high expression in cancer. **(b)** DNMT3B expression in cancer and normal tissues in 16 cancer types. Red represents cancer tissue, and blue represents normal tissue. *: p < 0.05, **: p < 0.01, ***: p<0.001.

Furthermore, we analysed DNMT/TET protein expression levels in different cancer types. In COAD, STAD, LIHC and PAAD, DNMT3A, TRDMT1, and TET2 were highly expressed, while TET3 expression was significantly reduced (Figure 2a). Detailed DNMT/TET protein expression information in 20 cancers is shown in Supplementary Table 3. DNMT3B protein expression in different human cancers is shown in Figure 2b.

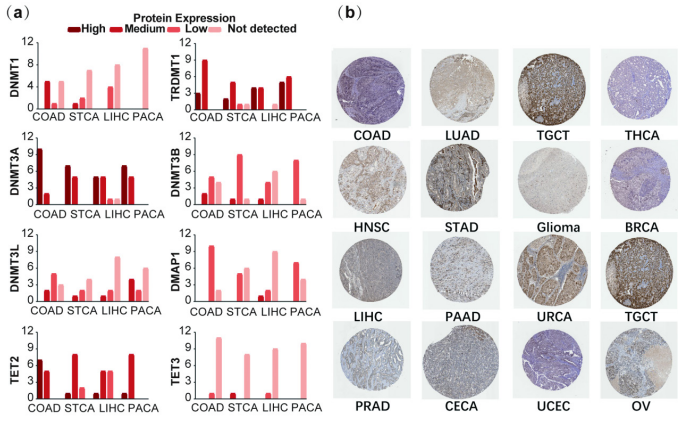

Figure 2: DNMT/TET protein expression profiles across different cancer types. **(a)** DNMT/TET protein expression in esophageal cancer, stomach cancer, liver cancer and pancreatic cancer. For each cancer, gene expression is divided into four groups: high expression, medium expression, low expression, and not detected. The depth of the colour represents the level of expression, where the darker the colour is, the higher the expression. **(b)** DNMT3B protein expression in 16 cancer types based on immunohistochemistry staining.

Additionally, we elucidated the correlations between DNMT and TET gene expression in pan-cancer. Significant correlations were identified between DNMT3A and TET1 (r=0.57, p<0.05), TET2 and TET3 (r=0.55, p<0.05), and TET1 and TET3 (r=0.50, p<0.05) (Figure 3).

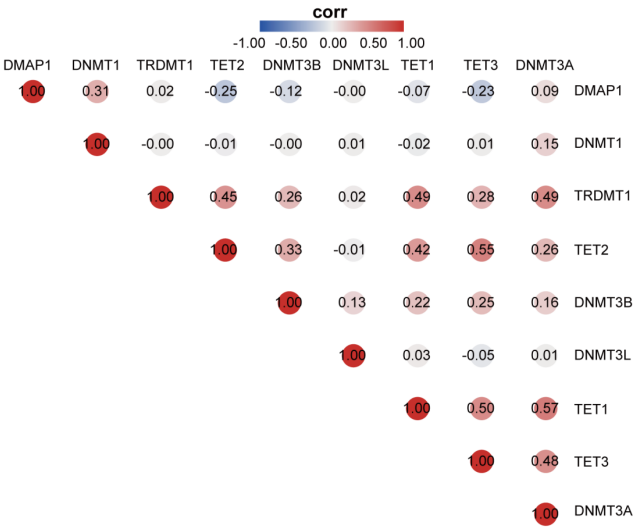

Figure 3: Correlation between DNMT/TET gene expression levels. The numbers on the circles represent the correlation coefficients, and the darker the colour of the circles is, the stronger the correlation.

DNMT/TET genetic alterations in pan-cancer

We examined the DNMT/TET gene mutation frequency in 33 cancer types. Frequent mutations were observed in UCEC, including TET1, TET2, TET3, DNMT1, DNMT3A, and DNMT3B, and rare mutations were observed in CHOL, PCPG, and THCA. TET1 and TET2 had higher mutation frequencies than other genes in pan-cancer, and the overall average mutation frequency of DNMT/TET genes in different cancer types ranged from 0 to 34.9% (Figure 4a). We also investigated DNMT/TET gene CNVs across cancers (Figure 4b). DNMT3B exhibited inclusive copy number amplification in STAD, COAD, READ, BLCA, ESCA, and LUSC, but almost no copy number deletions in multiple cancer types. Most DNMT/TET genes displayed copy number deletions in BLCA, ESCA, LUSC and LUAD but copy number variations were rarely observed in LIHC and LAML.

Next, we analysed the relationship between DNMT/TET gene mutations and expression levels in human cancers and visualized the statistically significant results (Figure 5). Correlation analysis revealed that the expression of genes was positively associated with mutations, especially DNMT1 in COAD, ESCA, STAD, UCEC, and PRAD; DNMT3A in LIHC, LUAD and SARC; and DNMT3L and TET2 in COAD. However, DNMT3A in STAD and TET1 in READ showed a negative correlation. The details are shown in Table 4. The associations between CNVs and mRNA expression in multiple cancer types are shown in Figure 6. We found that almost all expression changes in DNMT/TET genes were associated with CNVs.

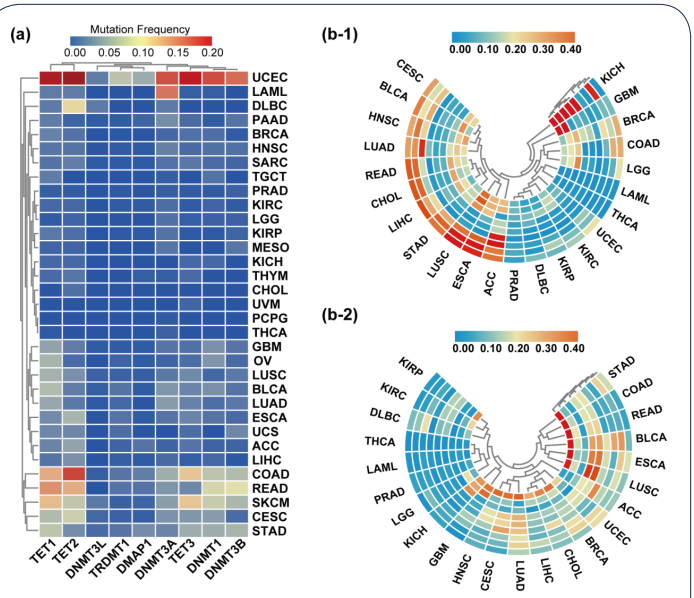

Figure 4: Pan-cancer genetic alterations in DNMT/TET genes. **(a)** DNMT/TET gene pan-cancer mutation frequency. **(b-1)** DNMT/TET gene copy number amplification. Each loop from the outside to the inside represents a gene (TET1, TRDMT1, DNMT3L, DMAP1, TET3, DNMT3A, TET2, DNMT1, and DNMT3B). **(b-2)** DNMT/TET gene copy number deletion. Each loop from the outside to the inside represents a gene (TET2, DNMT3L, DNMT1, DNMT3B, TET3, DNMT3A, DMAP1, TET1, and TRDMT1). The colour in the heat map represents the frequency; the redder the colour is, the greater the frequency.

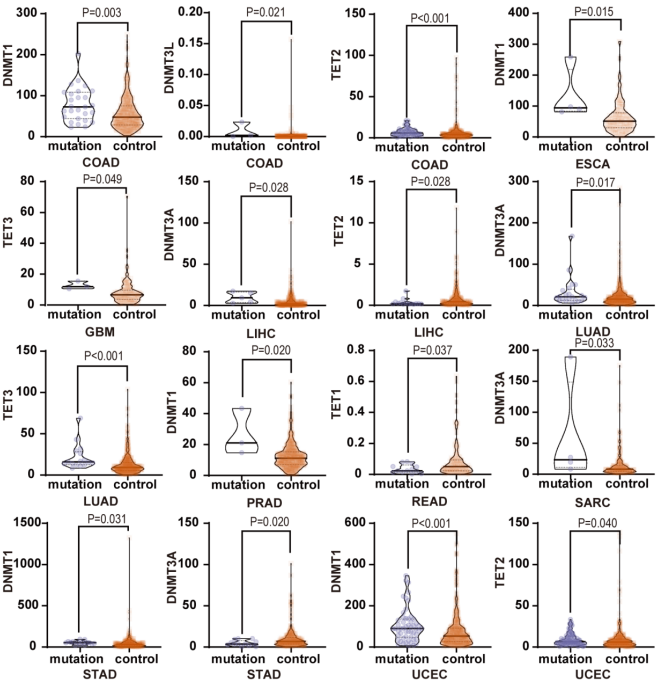

Figure 5: Mutation correlation with DNMT/TET gene expression. In each cancer, the samples are divided into two groups according to the mutation and non-mutation of each gene.

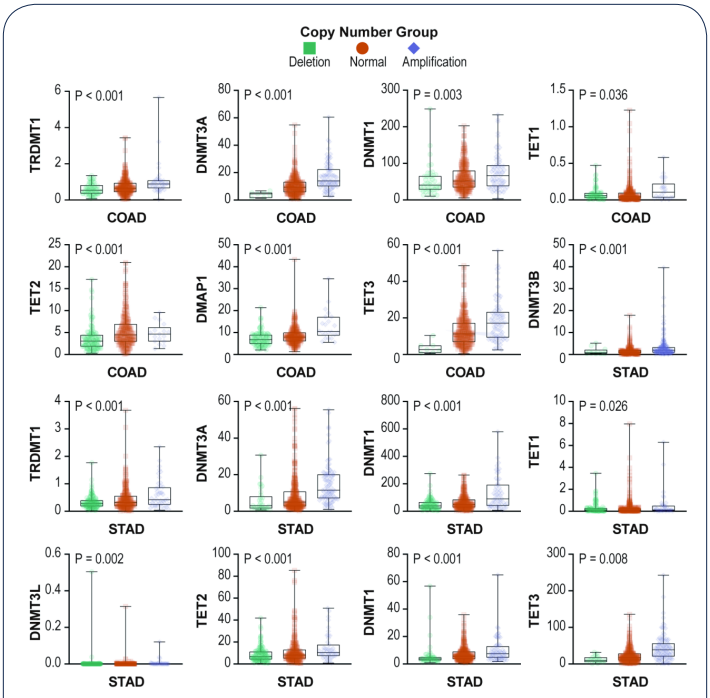

Figure 6: CNV correlation with DNMT/TET gene expression. In each cancer, samples are divided into an amplification group, a normal group and a deletion group according to the CNV of each gene.

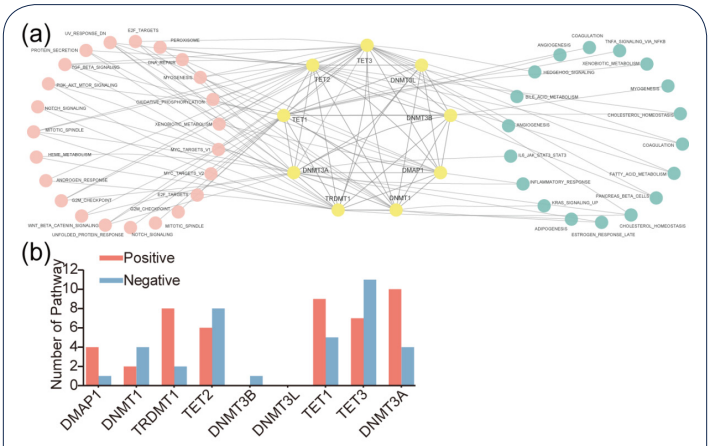

Figure 7: Association between DNMT/TET genes and cancer-related pathways. **(a)** Network diagram demonstrating the correlations between DNMT/TET genes and cancer-related pathways. Green nodes represent negative correlation pathways, while red nodes represent positive pathways. **(b)** The number of correlated pathways in individual DNMT/TET genes.

Correlation between DNMT/TET genes and cancer-related pathways

We investigated the correlation between DNMT/TET gene expression and cancer-related pathways to clarify the molecular significance of DNMT/TET genes in carcinogenesis. DNMT/TET genes were significantly correlated with multiple carcinogenesis pathways, playing both activation and suppression roles (Figure 7a). We concluded that DNMT/TET genes were mainly involved in the following cancer-related pathways: UV response DN, mitotic spindle, cholesterol homeostasis, TGF beta signaling, xenobiotic metabolism, G2/M checkpoint, and E2F targets (Table 5). We also found that DNMT3A, TET1, TET2, and TET3 were more likely to be

related to tumour occurrence and progression pathways (Figure 7b).

Correlation between DNMT/TET genes and immune cell infiltration

The correlation between DNMT/TET gene expression and immune cell infiltration across cancers was assessed. DNMT/TET genes were significantly correlated with NK cells, CD4+T cells, and Tfh cells. Notably, the correlation between DNMT/TET genes and immune cell infiltration tended to be positive. In particular, TET2, TRDMT1, and TET3 showed a stronger correlation (Figure 8).

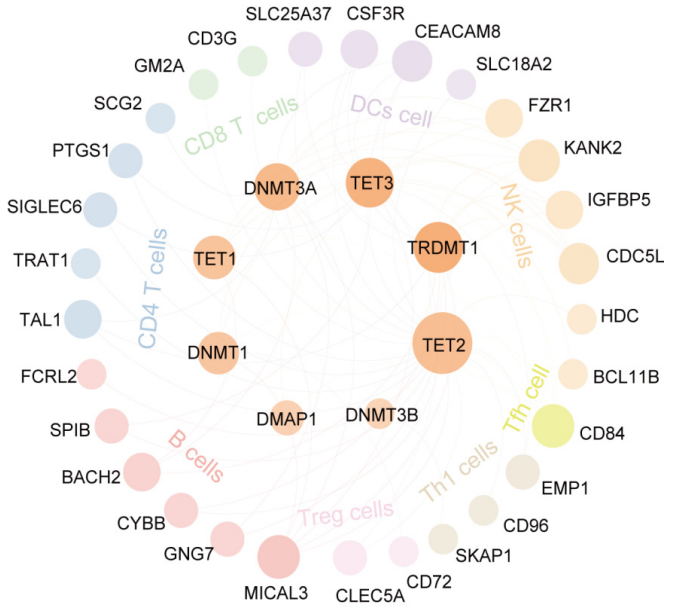

Figure 8: Correlation between DNMT/TET genes and immune cell infiltration. The genes in the outer circle represent genes within individual immune cells. Inner circles are formed by DNMT/TET genes. The size of each circle represents the number of connections, where the larger the circle is, the greater the number of related genes.

Identification and KEGG analysis of DNMT3B-related methylated genes

We identified DNMT3B-related methylated genes in BLCA, BRCA, CHOL, COAD/READ, ESCA, STAD, HNSC, KIRP, LIHC, LUAD, LUSC and UCEC (Table 6). Then, we manually retained genes with protein-coding functions in the results. The online Venn diagram tool was used to identify the shared DNMT3B-related methylated genes. TRIM8 and CIRBP were found to be common in 58% cancers. The expression of TRIM8 and CIRBP in these cancers and their para-carcinoma tissue are shown in Figure 9. Compared with normal tissues, TRIM8 was highly expressed in ESCA, while CIRBP was significantly expressed at lower levels in COADREAD, ESCA, STAD and UCEC. To further understand the pathways involved in these methylation genes, KEGG analysis was performed. We found that Salmonella infection, bacterial invasion of epithelial cells and the MAPK signalling pathway were involved in 42% of cancers (Table 7). The MAPK signalling pathway is involved in 5 out of the 12 cancers we analysed, including BRCA, KIRP, LUAD, LUSC and STAD.

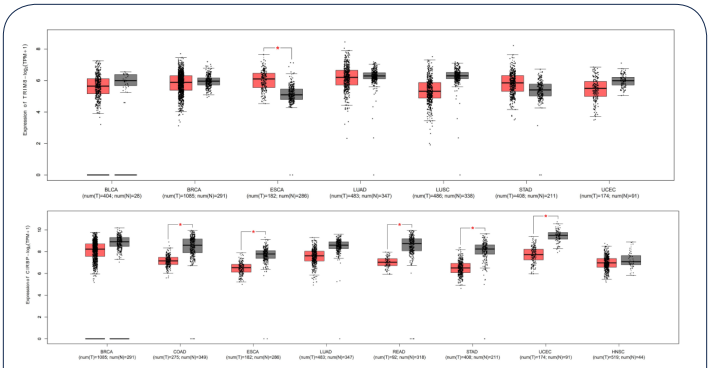

Figure 9: The expression of TRIM8 and CIRBP in cancers. Red represents tumour tissue, and grey represents normal tissue. The expression data were converted into log2 (TPM+1) values for differential expression analysis. *: $p < 0.05$.

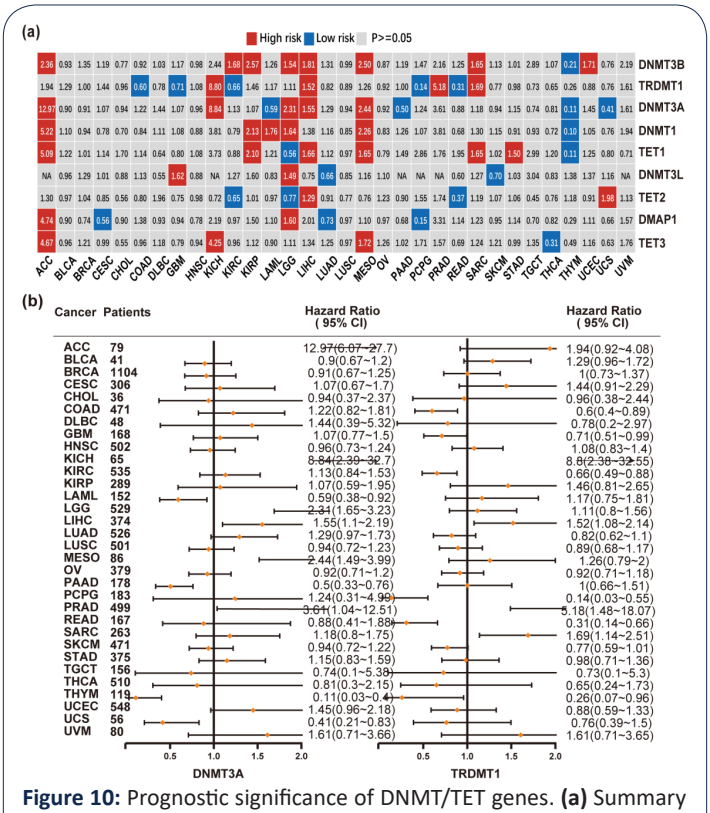

Figure 10: Prognostic significance of DNMT/TET genes. **(a)** Summary of the correlation between?

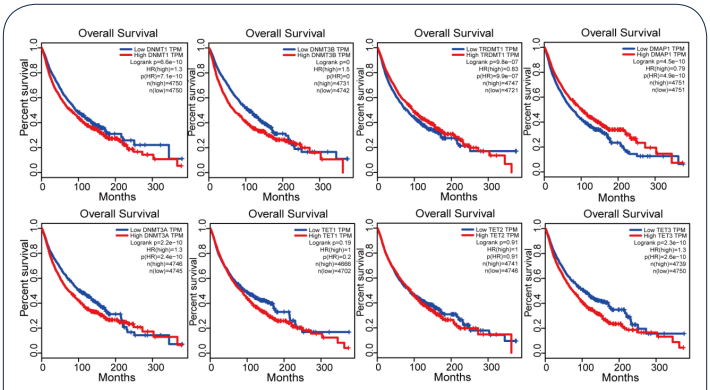

Figure 11: Survival analysis of DNMT/TET genes in pan-cancer. The median expression level was selected as the cut-off value.

Prognostic analysis of DNMT/TET genes in pan-cancer

The association between DNMT/TET gene expression and prognosis in patients with different cancers is shown in Figure 10a. DNMT3B, which showed increased expression in ACC, KIRC, KIRP, LGG, LIHC, MESO, SARC, and UCEC; TRDMT1 in KICH, LIHC and PRAD; DNMT3A in ACC, KICH, LIHC,PRAD and SARC; DNMT1 in ACC, KIRP, LAML, LGG and MESO; TET1 in ACC, KIRP, LIHC, MESO, SARC and STAD; DNMT3L in GBM and LGG; TET2 in LIHC and UCS; DMAP1 in ACC and LGG; and TET3 in ACC, KICH and MESO were associated with poor survival. The following genes with decreased expression were associated with poor survival: DNMT1, DNMT3A, DNMT3B and TET1 in THYM; TRDMT1 and TET2 in READ and KICH; TRDMT1 and DMAP1 in PCPG; DNMT3L and DMAT1 in LUAD; TET1 and TET2 in LGG; DNMT3A in UCS, PAAD and LAML; TET3 in THCA; DNMT3L in SKCM; TRDMT1 in GBM and COAD; and DMAP1 in CESC.

TRDMT1 and DNMT3A had clearly different effects on prognosis in various cancer types (Figure 10b). TRDMT1 was a predictor of poor prognosis in ACC, KICH, LIHC, PRAD, and SARC but not in COAD, GBM, KIRC, PCPG, and READ. Meanwhile, DNMT3A expression was associated with poor prognosis in ACC, KICH, LGG, LIHC, and MESO but not in LAML, PAAD, THYM, and UCS. Moreover, high or low DNMT gene expression was significantly related to patient survival status (Figure 11). Increased DNMT gene expression, except that of DMAP1, was associated with worse prognoses across cancers. Compared to other genes, DNMT3B had the worst prognosis in cancer patients. No significant correlation was observed between the expression level of TET1 or TET2 and overall cancer survival. These results showed that dysregulated DNA methylation might be related to tumorigenesis.

Discussion

To illustrate the pivotal role of the DNA methylation regulatory gene family in cancer, we performed this thorough pan-cancer analysis of the core genes belonging to the DNMT and TET families using data obtained from TCGA, genomic and transcriptomic landscapes. The results suggested that the expression of DNMT/TET was heterogeneous in certain cancers and para-cancer tissues and affected patient prognosis. The identified correlation between DNMT/TET and immune cell infiltration, as well as cancer-related pathways, highlighted the significance of DNMT/TET genes in tumorigenesis.

Our systematic analysis of TPM data of nine key DNMT/TET genes from TCGA database provided an overview of DNMT/TET gene expression profiles in 33 human cancer types. DNMT/TET gene expression in different cancer types was heterogeneous, and DNMT3B was expressed in most cancer types. A review of the available literature supports our results, showing that the expression of DNMT genes is increased in a host of malignant tumours, including PRCA, leukaemia, BRCA, PACA, and COAD [23]. Additionally, TET gene expression is substantially decreased in BRCA, LIHC, LUAD, PACA, and PRCA [24]. DNMT3B expression was increased in BRCA [25], PRCA [26], and COAD [27]. We found that DNMT3L expression levels were increased in COAD, READ, and LUCA and decreased in CHOL, PRAD, and BLCA. These results are consistent with those showing that DNMT3L is highly expressed in LUAD [28] and gastrointestinal stromal tumour [29]. Notably, our findings show that overexpression of DNMT3B and DNMT3L may have important impacts on numerous cancers. The underlying mechanisms of DNMT3B and DNMT3L in cancer deserve further investigation and may provide new ideas for cancer treatment.

DNMT or TET gene mutations may be responsible for the occurrence and progression of tumours [30,31]. By analysing DNMT/TET gene mutations in multiple cancers, we found that the total average mutation frequency ranged from 0 to 34.9% and that TET1 and TET2 had relatively high mutation frequencies in pan-cancer. TET genes are frequently mutated in various cancers. TET gene mutations were previously detected in haematopoietic malignancies, melanoma, and some solid tumours [19,23]. TET2 mutations were frequently detected in myeloid malignancies (~15%) [33]. Moreover, genetic studies have shown that TET2 is essential for the self-renewal and differentiation of haematopoietic stem cells in mouse models and have confirmed that TET2 inactivation leads to the development of myeloid malignancies [34-36]. TET2 mutations have been detected in AML, CMML, MPD, and MDS [37], and the mutation frequency of TET2 is significantly higher than that of TET1 and TET3. In solid tumours, TET gene mutations were observed in COAD [38] and Clear-Cell Renal Cell Carcinoma (CCRCC) [39]. Interestingly, most DNMT/TET genes showed high mutation frequencies in UCEC, which is a cancer type with a high global mutation burden [40]. DNMT/TET gene mutations were rare in CHOL, PCPG, and THCA. Furthermore, TET genes usually function as cancer suppressors. Indeed, TET genes were reported to be the targets of oncogenic miRNAs [17], and reduced TET gene expression is often detected in cancer cells [24]. Furthermore, TET gene mutations may interfere with TET gene expression, which plays a pivotal role in human tumours [41,42]. Indeed, our results showed that TET2 and TET3 mutations were significantly related to their expression in some cancers.

Sporadic CNVs significantly affect genomic stability in cancer, and CNVs are observed in nearly 80% of cancers [43,44]. We noticed that DNMT3B exhibited extensive CNV in different cancers, and almost all DNMT/TET genes showed copy number deletions in BLCA, ESCA, LUSC and LUAD. Additionally, significant copy number changes were usually related to alterations in the expression of the corresponding genes [45]. We found that CNV and DNMT/TET gene expression levels were associated with multiple cancer types. Together, these findings strongly suggest that genetic changes might contribute to DNMT/TET gene expression changes in carcinogenesis. The potential regulatory mechanism related to this deserves further exploration.

We found that most DNMT/TET genes were positively correlated with each other, especially DNMT3A with TET1, TET2 with TET3, and TET1 with TET3. DNMT/TET genes might have a synergistic effect in tumorigenesis, although additional investigation is necessary to determine the mechanism of interaction between these genes in oncogenesis.

Our results indicate that DNMT/TET gene expression is significantly associated with multiple cancer-related pathways, especially in UV response DN, mitotic spindle, cholesterol homeostasis, TGF beat signalling, xenobiotic metabolism, G2/M checkpoint, and E2F targets. Individual DNMT/TET genes presented different associations with distinct cancer-related pathways, suggesting that each DNMT/TET gene may have different functions. These results are consistent with those of a previous study showing

that DNMT/TET genes have distinct expression patterns and functions [9,46]. The methylation of the mouse oocyte genome was less than 50%, which is significantly lower than that of the sperm genome and is related to DNMT1 cytoplasmic retention [47,48]. TET1 was highly expressed in mouse ESCs. Unlike TET1 expression, TET3 expression was mainly confined to oocytes and zygotes, where it appeared to contribute to the active demethylation or conversion of 5 mC to 5 hmC in male prokaryotes after fertilization [49]. Further investigations are required to analyse the common and diverse functions of these genes in tumorigenesis.

Analysis of the relationship between pan-oncogene expression and immune cell infiltration showed that NK, CD4+ T, and Tfh cells were most significantly associated with DNTM/TET genes and with TET2, TRDMT1, and TET3 in particular. The TET protein family has an important influence on maintaining immune system homeostasis by driving Treg cells, and changes in this family may result in the occurrence of cancers [50,51].

Another study suggested that TET2 regulates Th1 cells, playing a pivotal role in the prevention of excessive inflammation in Experimental Autoimmune Encephalomyelitis (EAE) [52]. Together, these findings suggest that further studies of DNMT/TET genes may provide novel anti-cancer therapeutics.

TRIM8 and CIRBP are DNMT3B-related methylated genes shared among most cancers with different DNMT3B expression. TRIM8 plays a dual role as a tumor suppressor gene and an oncogene. Consistent with the findings of previous studies, in our results, the TRIM8 expression level showed a downregulation trend in BRCA [53].

Tian et al. found that TRIM8 is downregulated in BRCA and that knocking out TRIM8 can significantly enhance the proliferation and migration of BRCA cells. CIRBP is a stress response protein that promotes cancer development [54]. Chen et al. found that CIRBP is a DNA methylation marker associated with occult lymph node metastasis in NSCLC, which has important value in predicting the prognosis of early NSCLC [55,56].

CIRBP is significantly decreased in UCEC patients and is a major risk factor for survival. The DNA methylation event of CIRBP is a prognostic indicator of gynaecological cancers [57,58]. In BRCA, CIRBP promotes proliferation and clonogenesis, which is associated with a poor prognosis. Next, we found that DNMT3B-related methylated genes were mainly enriched in Salmonella infection, bacterial invasion of epithelial cells and the MAPK signalling pathway. The MAPK signalling pathway, in particular, is involved in 5 out of the 12 cancers we analysed. The MAPK signalling pathway regulates various aspects of cell function and is often changed in cancers. Previous studies found that hypermethylation inhibits the expression of different genes in different cancers and regulates the MAPK signalling pathway to promote the development of caners [59]. In cervical cancer, DNMT3B mediates PTPRR hypermethylation, inhibits the MAPK signalling pathway, and promotes metastasis [60]. IRAK3 hypermethylation activates the MAPK signalling to promote glioma development [61]. In liver cancer, abnormally methylated DEGs were also mainly enriched in the MAPK signalling pathway [62,63]. Current evidence suggests that the MAPK signalling pathway is a viable target for cancer therapy, while for MAPK inhibitor-resistant cells, epigenetic therapy may bring great hope for the development of new and effective therapies.

Finally, we evaluated the value of DNMT/TET genes in pan-cancer prognosis. Most DNMT/TET genes were correlated with a poor prognosis in ACC, MESO, and LIHC and were correlated with a good prognosis in THYM. Survival analysis also showed that high DNMT gene expression, except that of DMAP1, was significantly associated with poor prognosis in pan-cancer and that TET1 and TET2 showed no statistically significant relationship with overall survival in pan-cancer. Early studies showed that DNMT genes were poor prognostic factors in diverse cancers, including AML [64], STAD [65] and LUAD [66]. More interestingly, our results revealed that TRDMT1 and DNMT3A showed distinctly disparate prognoses across cancers. DNMT3A expression is related to a poor survival rate in haematologic cancer [67] and CMML [68], and in AML, DNMT3A acts as a tumour suppressor [69]. This could be explained by different DNMT3A expression patterns among cancers. Taken together, these findings suggest that DNMT genes can serve as prognostic predictors in pan-cancer. Future studies are needed to reveal the critical role of DNMT/TET genes in the prognosis of various tumours.

Table 3:

	Breast cancer				Carcinoid			
Protein expression gene symbol	**High**	**Medium**	**Low**	**Not.detected**	**High**	**Medium**	**Low**	**Not detected**
DNMT1	0	3	5	3	0	0	3	1
TRDMT1	1	9	1	0	2	2	0	0
DNMT3A	11	1	0	0	1	3	0	0
DNMT3B	0	2	6	2	0	0	2	2
DNMT3L	0	0	4	8	0	0	3	1
DMAP1	0	4	8	0	0	0	3	1
TET2	7	5	0	0	0	4	0	0
TET3	1	0	1	10	0	0	0	4
TET1	NA	NA	NA	NA	NA	NA	NA	NA

	Cervical cancer				Colorectal cancer			
Protein expression gene symbol	High	Medium	Low	Not.detected	High	Medium	Low	Not.detected
DNMT1	0	3	4	5	0	5	1	5
TRDMT1	0	9	3	0	3	9	0	0
DNMT3A	9	2	0	1	10	2	0	0
DNMT3B	0	3	7	2	0	2	5	4
DNMT3L	0	3	6	1	0	2	5	3
DMAP1	0	0	6	6	0	0	10	2
TET2	10	2	0	0	7	5	0	0
TET3	0	1	4	6	0	0	1	11
TET1	NA	NA	NA	NA	NA	NA	NA	NA

Table 4:

Cancer type	Gene symbol	Mutation group (Mean±SD)	Control group (Mean±SD)	P value
COAD	DNMT1	78.24±41.74	56.72±39.53	0.003
COAD	DNMT3L	0.01±0.01	0.00±0.01	0.021
COAD	TET2	7.11±4.80	5.94±9.37	0.001
ESCA	DNMT1	132.20±84.82	62.85±51.08	0.015
GBM	TET3	12.68±2.58	8.10±7.49	0.049
LIHC	DNMT3A	9.81±6.47	4.51±7.58	0.014
LIHC	TET2	0.37±0.43	0.79±1.14	0.028
LUAD	DNMT3A	32.77±36.23	20.54±23.48	0.017
LUAD	TET3	21.99±15.54	12.53±11.80	＜0.001
PRAD	DNMT1	26.42±15.04	12.37±7.92	0.02
READ	TET1	0.04±0.03	0.09±0.11	0.037
SARC	DNMT3A	61.02±85.90	14.30±20.17	0.033
STAD	DNMT1	51.51±22.37	46.51±79.44	0.031
STAD	DNMT3A	4.68±3.42	9.79±11.37	0.02
UCEC	DNMT1	105.60±81.18	75.34±79.02	＜0.001
UCEC	TET2	9.37±7.66	8.60±12.78	0.04

Table 5:

Gene	Cancer-related pathway	Corr	P value
DMAP1	XENOBIOTIC_METABOLISM	0.289	5.05E-199
DMAP1	OXIDATIVE_PHOSPHORYLATION	0.280	1.9536E-186
DMAP1	MYOGENESIS	0.259	9.7535E-159
DMAP1	DNA_REPAIR	0.372	0
DMAP1	INFLAMMATORY_RESPONSE	-0.269	3.5184E-172
DNMT1	PEROXISOME	0.279	7.2224E-185
DNMT1	E2F_TARGETS	0.321	3.8397E-249
DNMT1	UV_RESPONSE_DN	-0.305	8.9777E-224
DNMT1	PROTEIN_SECRETION	-0.310	4.6683E-231
DNMT1	KRAS_SIGNALING_UP	-0.314	1.2484E-236
DNMT1	ADIPOGENESIS	-0.311	8.3374E-232
TRDMT1	UV_RESPONSE_DN	0.516	0
TRDMT1	TGF_BETA_SIGNALING	0.399	0
TRDMT1	PROTEIN_SECRETION	0.400	0
TRDMT1	PI3K_AKT_MTOR_SIGNALING	0.321	5.5843E-249
TRDMT1	NOTCH_SIGNALING	0.261	3.5191E-161
TRDMT1	MITOTIC_SPINDLE	0.424	0
TRDMT1	HEME_METABOLISM	0.431	0
TRDMT1	ANDROGEN_RESPONSE	0.335	8.8135E-272
TRDMT1	ESTROGEN_RESPONSE_LATE	-0.305	1.0938E-222
TRDMT1	CHOLESTEROL_HOMEOSTASIS	-0.380	0
TET2	MITOTIC_SPINDLE	0.405	0
TET2	G2M_CHECKPOINT	0.250	0
TET2	UV_RESPONSE_DN	0.371	3.0393E-219
TET2	TGF_BETA_SIGNALING	0.401	5.8313E-119
TET2	PROTEIN_SECRETION	0.302	4.94712E-35
TET2	ANDROGEN_RESPONSE	0.308	0
TET2	XENOBIOTIC_METABOLISM	-0.339	2.6013E-28
TET2	PEROXISOME	-0.327	4.4254E-227
TET2	PANCREAS_BETA_CELLS	-0.294	0.000743703
TET2	OXIDATIVE_PHOSPHORYLATION	-0.425	0
TET2	MYOGENESIS	-0.370	0
TET2	FATTY_ACID_METABOLISM	-0.333	7.26E-268
TET2	COAGULATION	-0.321	1.25E-248
TET2	CHOLESTEROL_HOMEOSTASIS	-0.369	0
DNMT3B	MYOGENESIS	-0.259	4.328E-159
TET1	WNT_BETA_CATENIN_SIGNALING	0.328	7.1372E-261
TET1	UV_RESPONSE_DN	0.441	0
TET1	UNFOLDED_PROTEIN_RESPONSE	0.307	4.1181E-226
TET1	TGF_BETA_SIGNALING	0.338	2.2766E-277
TET1	PI3K_AKT_MTOR_SIGNALING	0.265	2.7704E-166
TET1	NOTCH_SIGNALING	0.254	7.4858E-153
TET1	MITOTIC_SPINDLE	0.510	0
TET1	G2M_CHECKPOINT	0.450	0
TET1	E2F_TARGETS	0.306	4.8289E-224
TET1	XENOBIOTIC_METABOLISM	-0.290	4.0068E-201
TET1	TNFA_SIGNALING_VIA_NFKB	-0.285	5.6479E-193
TET1	COAGULATION	-0.356	0
TET1	CHOLESTEROL_HOMEOSTASIS	-0.338	6.0664E-278
TET1	ANGIOGENESIS	-0.327	6.0915E-259
TET3	UNFOLDED_PROTEIN_RESPONSE	0.441	0
TET3	TGF_BETA_SIGNALING	0.316	1.207E-239
TET3	MYC_TARGETS_V2	0.385	0
TET3	MYC_TARGETS_V1	0.338	5.7629E-277
TET3	MITOTIC_SPINDLE	0.590	0
TET3	G2M_CHECKPOINT	0.608	0
TET3	E2F_TARGETS	0.420	0
TET3	HEDGEHOG_SIGNALING	-0.274	9.8594E-178
TET3	XENOBIOTIC_METABOLISM	-0.506	0
TET3	PEROXISOME	-0.345	1.6353E-290
TET3	PANCREAS_BETA_CELLS	-0.350	1.7035E-299
TET3	OXIDATIVE_PHOSPHORYLATION	-0.373	0
TET3	MYOGENESIS	-0.530	0
TET3	FATTY_ACID_METABOLISM	-0.440	0
TET3	COAGULATION	-0.529	0
TET3	CHOLESTEROL_HOMEOSTASIS	-0.322	8.3792E-251
TET3	BILE_ACID_METABOLISM	-0.415	0
TET3	ANGIOGENESIS	-0.456	0
DNMT3A	WNT_BETA_CATENIN_SIGNALING	0.342	4.2753E-284
DNMT3A	UV_RESPONSE_DN	0.383	0
DNMT3A	UNFOLDED_PROTEIN_RESPONSE	0.323	1.7172E-252
DNMT3A	PI3K_AKT_MTOR_SIGNALING	0.265	1.7437E-166
DNMT3A	NOTCH_SIGNALING	0.259	7.5755E-159
DNMT3A	MITOTIC_SPINDLE	0.516	0
DNMT3A	HEME_METABOLISM	0.268	1.6763E-170
DNMT3A	G2M_CHECKPOINT	0.434	0
DNMT3A	E2F_TARGETS	0.318	3.4526E-244
DNMT3A	DNA_REPAIR	0.305	6.2327E-223
DNMT3A	INFLAMMATORY_RESPONSE	-0.258	5.6146E-157
DNMT3A	IL6_JAK_STAT3_STAT3	-0.324	7.7177E-253
DNMT3A	ESTROGEN_RESPONSE_LATE	-0.270	1.8872E-172
DNMT3A	CHOLESTEROL_HOMEOSTASIS	-0.295	7.4933E-208

Table 6:

Gene	SCC	P-value	FDR (BH)
SLC9A3R1	0.314627914	2.63E-05	0.003381348
ELOVL5	0.334658343	7.22E-06	0.001562081
UBTF	0.35363752	1.95E-06	0.000697805
GOT2	0.323280351	1.52E-05	0.002615726
UHRF1	0.304869794	4.77E-05	0.004656639
PWWP2B	0.308118265	3.92E-05	0.00423937
PCBP2	0.304843852	4.78E-05	0.004656639
CCDC17	0.314474064	2.65E-05	0.003381348
SLC27A6	0.348929803	2.72E-06	0.000838196
MARCKSL1	0.303971256	5.04E-05	0.004873547
ABHD2	0.347647825	2.97E-06	0.000859503
C18orf1	0.3001247	6.33E-05	0.005589898
BUB3	0.320803731	1.78E-05	0.002779214
RAB24	0.323670324	1.48E-05	0.002582359
LRIG1	0.342539436	4.23E-06	0.001051741
CDC42SE2	0.306724557	4.27E-05	0.004426819
RARA	0.358553118	1.37E-06	0.00053952
CREB3L2	0.300978956	6.51E-05	0.005666182
DCDC2B	0.330388507	9.58E-06	0.001987768
IRS1	0.3016041	5.80E-05	0.005282358
CCDC92	0.303642085	5.14E-05	0.004923191
DEPDC6	0.330390681	9.58E-06	0.001987768
MUC6	0.307047204	4.19E-05	0.004386785
VWA3A	0.339285462	5.29E-06	0.001236993
ZFP36L2	0.361205626	1.13E-06	0.00049306
P4HB	0.344182652	3.78E-06	0.001011127
TSC22D4	0.301922657	5.69E-05	0.005206876
WDR6	0.376562787	3.56E-07	0.000223656
HSBP1	0.317284987	2.23E-05	0.003116204
SUGT1L1	0.317543928	2.19E-05	0.003116204
ADORA2B	0.329212483	1.04E-05	0.002125464
EMP2	0.311515492	3.19E-05	0.003702523
SMAD9	0.300077413	6.35E-05	0.005589898
SIPA1L2	0.322492701	1.60E-05	0.002638416
TBC1D10B	0.423686493	6.99E-09	1.59E-05
ATN1	0.33804574	5.75E-06	0.001329891
SH3BP4	0.350020341	2.52E-06	0.000816701
ACSS1	0.310002335	3.50E-05	0.003887062
ZNF747	0.313008605	2.91E-05	0.00352036
ZFYVE28	0.365401079	8.28E-07	0.00039639
ANKRD13D	0.341099586	4.67E-06	0.00111333
TMEM87B	0.424405545	6.55E-09	1.59E-05
SSR3	0.31300797	2.91E-05	0.00352036
PXK	0.302637242	5.46E-05	0.005129935
BTG2	0.3438707	3.86E-06	0.001011127
KIAA1984	0.321027586	1.76E-05	0.002761302
CDK2AP1	0.36227087	1.04E-06	0.000476748
DUSP6	0.323705509	1.48E-05	0.002582359
PTCH1	0.327013285	1.20E-05	0.002334521
TTRAP	0.334892825	7.11E-06	0.00155451
DHX58	0.341396057	4.58E-06	0.001110038
ARID4B	0.35776736	1.45E-06	0.00056013
NFKBIA	0.316643574	2.32E-05	0.003171761
SIDT1	0.300569208	6.17E-05	0.005541295
XBP1	0.472734385	6.64E-11	9.01E-07
CCDC57	0.344803545	3.87E-06	0.001011127
WDR51B	0.303244557	5.54E-05	0.005133756
KIAA0649	0.373229895	4.59E-07	0.000263988
TENC1	0.377811476	3.23E-07	0.000209648
ZNF787	0.312794906	2.95E-05	0.003526748
TRIM8	0.345536928	3.44E-06	0.00095444
MYCN	0.308047696	3.94E-05	0.00423937
HNRNPA3	0.336197961	6.51E-06	0.001455993
TRDMT1	0.357068316	1.52E-06	0.000567324
PTCHD2	0.39135818	1.11E-07	0.000117058
BBS2	0.321969143	1.65E-05	0.002706064
CPEB3	0.300791821	6.09E-05	0.005493291
TP53INP2	0.386606615	1.62E-07	0.000130303
EEPD1	0.311181784	3.25E-05	0.003720413
DCAF5	0.312559807	2.99E-05	0.00353627
KIAA1737	0.370626122	6.80E-07	0.000333523
MED18	0.314968262	2.57E-05	0.003355768
CLK1	0.374101831	4.30E-07	0.000261962
FAM63A	0.347690276	2.96E-06	0.000859503
NPDC1	0.34876126	2.75E-06	0.000838196
BTF3	0.336708758	6.29E-06	0.001422658
ARL4D	0.300047934	6.36E-05	0.005589898
ZMAT3	0.303185424	5.28E-05	0.005035451
LRRC14	0.326664654	1.22E-05	0.002342537
CTNNA1	0.323523994	1.59E-05	0.002638416
LRP5L	0.319623272	1.92E-05	0.002863759
BTN3A3	0.305847976	4.50E-05	0.004550621
HMGXB3	0.318237401	2.10E-05	0.003034495
C10orf95	0.30677027	4.26E-05	0.004426819
CIB1	0.386979693	1.57E-07	0.000130303
C2orf62	0.38976923	1.26E-07	0.000126427
LOC222699	0.32596787	1.28E-05	0.002358883

MADD	0.369940493	5.89E-07	0.000307611
NDRG2	0.322687038	1.58E-05	0.002638416
ICK	0.313354948	2.84E-05	0.003511089
CD74	0.347109962	3.09E-06	0.000874555
RBM4	0.305430726	4.62E-05	0.004643157
CALR	0.412203848	1.93E-08	2.77E-05
MBLAC1	0.302961946	5.35E-05	0.005079013
PKP4	0.370473047	5.66E-07	0.000307611
KIAA1324	0.387296399	1.53E-07	0.000130303
RPSA	0.351680073	2.24E-06	0.000740765
UBE2N	0.34545357	3.46E-06	0.00095444
MYO5C	0.358680137	1.36E-06	0.00053952
TREX1	0.41620649	1.36E-08	2.28E-05
C14orf2	0.307915174	3.97E-05	0.004248671
KCNK15	0.308738793	3.78E-05	0.004175847
C16orf58	0.32849787	1.08E-05	0.00220475
MDM4	0.324605194	1.40E-05	0.002509249
NUMA1	0.305009758	4.73E-05	0.004656639
PDDC1	0.443789473	1.08E-09	5.42E-06
SNAP23	0.31303709	2.90E-05	0.00352036
UNC119B	0.45392783	4.00E-10	2.68E-06
GNMT	0.31706355	2.26E-05	0.003116204
AKTIP	0.31541187	2.50E-05	0.003313101
TMEM109	0.407574416	2.87E-08	3.61E-05
IFNGR1	0.31961568	1.92E-05	0.002863759
HNRNPH1	0.413333506	1.75E-08	2.70E-05
RSL1D1	0.300833294	6.71E-05	0.005772691
ISOC1	0.32054648	1.81E-05	0.002779889
FXC1	0.320393518	1.83E-05	0.002779889
UQCRH	0.315696997	2.46E-05	0.003276186
ERGIC1	0.30520786	4.68E-05	0.004656639
TRPM7	0.302552341	5.48E-05	0.005131977
ABCC11	0.301103303	5.98E-05	0.005417148
NACA	0.305001403	4.74E-05	0.004656639
UAP1L1	0.341004272	4.70E-06	0.00111333
SCAMP4	0.349471666	2.62E-06	0.000829093
UQCRHL	0.387182969	1.55E-07	0.000130303
GPBP1L1	0.307660923	4.03E-05	0.004248671
LOC400931	0.379306523	2.88E-07	0.000199565
CYB561	0.343277202	4.03E-06	0.001025044
RBM5	0.326387353	1.24E-05	0.002355402
RPS7	0.32593101	1.28E-05	0.002358883
C10orf104	0.324312243	1.60E-05	0.002638416
SFRS4	0.360891226	1.15E-06	0.000493794
LRRC41	0.300435483	6.54E-05	0.00566901

CEL	0.344775133	3.63E-06	0.000986842
TTC28	0.379721595	2.79E-07	0.000199565
EID2B	0.317123517	2.25E-05	0.003116204
GUF1	0.312183905	3.06E-05	0.003598133
RPS3	0.422699085	7.64E-09	1.59E-05
HPS1	0.308045429	3.94E-05	0.00423937
HFE	0.315794501	2.44E-05	0.003276186
RP5-1022P6.2	0.302426019	5.53E-05	0.005133756
FLJ25006	0.317627894	2.18E-05	0.003116204
LMAN1	0.361841221	1.28E-06	0.000526319
RSPH1	0.351632128	2.25E-06	0.000740765
B4GALT1	0.321577085	1.70E-05	0.002752275
APOL2	0.321233711	1.73E-05	0.002755711
USP54	0.325840212	1.29E-05	0.002358883
TXNL4B	0.398130286	6.35E-08	7.52E-05
MIR200B	0.306110296	4.43E-05	0.00451502
SNORD1A	0.34756195	2.99E-06	0.000859503
C1orf194	0.387296399	1.53E-07	0.000130303
IL6ST	0.311437483	3.20E-05	0.003702523
EEF1B2	0.359952809	1.24E-06	0.000517782
CIRBP	0.42233583	7.89E-09	1.59E-05
RPS8	0.324877011	1.37E-05	0.002487842
KRTAP5-7	0.306143133	4.42E-05	0.00451502
TAPBPL	0.310334317	3.43E-05	0.003829978
ACOX1	0.352616138	2.09E-06	0.000726544
SERP1	0.349355076	2.64E-06	0.000829093
ARFIP2	0.320393518	1.83E-05	0.002779889
HEATR5B	0.314893231	2.59E-05	0.003355768
DNAJB11	0.42946264	4.13E-09	1.39E-05
OGFOD1	0.373560765	4.48E-07	0.000263988
SNORD20	0.411042417	2.13E-08	2.86E-05
MIR93	0.310445973	3.40E-05	0.003829978
LOC100129726	0.361205626	1.13E-06	0.00049306
MIR25	0.36497935	8.54E-07	0.000399474
SNORD49A	0.321217918	1.84E-05	0.002779889
SNORD19B	0.311746429	3.14E-05	0.003675005
LOC100129387	0.31292075	2.92E-05	0.00352036
SNORA33	0.311099057	3.27E-05	0.003720413
SNORD4B	0.324296661	1.42E-05	0.002536993
B3GALT2	0.303754107	5.10E-05	0.0049137
SNORD34	0.322559724	1.59E-05	0.002638416
RPL13AP5	0.328605786	1.74E-05	0.002755711
SNORD35A	0.323299601	2.03E-05	0.002971055
MYCNOS	0.308047696	3.94E-05	0.00423937
LOC100289511	0.393554066	9.25E-08	0.000103372

MIR26A2	0.307683691	4.03E-05	0.004248671
NUDT22	0.371053204	5.42E-07	0.000302779
MIR1306	0.383322253	2.28E-07	0.000176472
SCARNA12	0.335040004	7.04E-06	0.00155451
NDUFA8	0.318686678	2.04E-05	0.002971055
SNORD33	0.314361856	2.67E-05	0.003381348
RPS15AP10	0.307660923	4.03E-05	0.004248671
C1orf189	0.342151856	4.35E-06	0.00106691
SNORD124	0.36944685	6.12E-07	0.000307611
MIR26B	0.435527444	2.36E-09	9.49E-06
SNORD37	0.417111858	1.26E-08	2.28E-05
SNORA4	0.327954102	1.12E-05	0.002239222
MIR1282	0.333739563	7.68E-06	0.001643124
SNORD18C	0.332207607	8.50E-06	0.001799934
MIR1280	0.32674342	1.22E-05	0.002342537
RPL12	0.300365959	6.24E-05	0.00555867
LOC440926	0.382179759	3.19E-07	0.000209648
H3F3A	0.468647024	8.96E-11	9.01E-07
SNORD32A	0.317034071	2.26E-05	0.003116204
MIR27A	0.306640929	4.29E-05	0.004426819
MIR23A	0.315984784	2.42E-05	0.003275745
SNORA15	0.353427625	1.98E-06	0.000697805
SNORD51	0.343391786	3.99E-06	0.001025044
SNORA1	0.354183482	1.87E-06	0.000685207
LOC645851	0.327657507	1.15E-05	0.002260574
SNORA53	0.313805467	2.77E-05	0.003435438
SNORD19	0.323613914	1.49E-05	0.002582359
SNORA65	0.300365959	6.24E-05	0.00555867
UGT2B17	0.304993069	4.74E-05	0.004656639

Table 7: The anomalous pathways enriched by DNMT3B-related methylated genes in cancers.

Pathway	Cancers
Salmonella infection	BLCA BRCA COADREAD LUSC STAD
Bacterial invasion of epithelial cells	BLCA ESCA LUAD LUSC STAD
MAPK signaling pathway	BRCA KIRP LUAD LUSC STAD
Ribosome	CHOL ESCA HNSC UCEC
Th17 cell differentiation	CHOL LUAD STAD UCEC
Leishmaniasis	CHOL LIHC LUAD UCEC
Gastric acid secretion	BLCA COADREAD ESCA KIRP
Endocytosis	BLCA COADREAD LUSC STAD
Neurotrophin signaling pathway	BLCA ESCA LUAD LUSC
Apoptosis Yersinia infection	BLCA LUAD LUSC STAD

Declarations

Authorship contributions: YP designed the research and provided the technical supports. ZZL, QHL and SPC conducted data retrieval and analysis, and wrote the manuscript text. FHJ and COY prepared the figures and tables. All authors reviewed the manuscript.

Fundings: This study was supported by grants from the China Postdoctoral Science Foundation (2021MD703842) and Liaoning Provincial Natural Science Foundation (2024-MS-16).

Data availability: All the data used to support the results of this study can be found in the public databases:

Gene Cards: https://www.genecards.org/;

TCGA database: https://cancergenome.nih.gov/;

UCSC XENA: https://xenabrowser.net/;

Human Protein Atlas Datasets (HPA): https://www.proteinatlas.org/;

Linked Omics database: https://www.linkedomics.org.;

R software v4.1.0: https://www.r-project.org.

Conflicts of interest: The authors declare that there is no conflicts of interest regarding the publication of this article.

References

1. You JS, PA Jones. Cancer genetics and epigenetics: two sides of the same coin? Cancer cell. 2012; 22(1).
2. Baylin SB, PA Jones. A decade of exploring the cancer epigenome - biological and translational implications. Nature reviews. Cancer. 2011; 11(10): 726-734.
3. Verma M. Cancer control and prevention: nutrition and epigenetics. Current opinion in clinical nutrition and metabolic care. 2013; 16(4): 376-384.
4. Issa JP. Cancer prevention: Epigenetics steps up to the plate. Cancer prevention research (Philadelphia, Pa.). 2008; 1(4): 219-222.
5. Kelly AD, JPJ Issa, The promise of epigenetic therapy: Reprogramming the cancer epigenome. Current opinion in genetics & development. 2017; 42: 68-77.
6. Nowacka-Zawisza M, E Wiśnik. DNA methylation and histone modifications as epigenetic regulation in prostate cancer (Review). Oncology reports. 2017; 38(5): 2587-2596.
7. Suzuki MM, A Bird. DNA methylation landscapes: provocative insights from epigenomics. Nature reviews. Genetics. 2008; 9(6): 465-476.
8. Pastor WA, L Aravind, A Rao. TETonic shift: Biological roles of TET proteins in DNA demethylation and transcription. Nature reviews. Molecular cell biology. 2013; 14(6): 341-356.
9. Shen L, et al. Mechanism and function of oxidative reversal of DNA and RNA methylation. Annual review of biochemistry. 2014; 83: 585-614.
10. Uysal F, G Akkoyunlu, S Ozturk. Dynamic expression of DNA methyltransferases (DNMTs) in oocytes and early embryos. Biochimie. 2015; 116: 103-113.

11. Ito S, et al. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature. 2010; 466(7310): 1129-1133.

12. Rasmussen KD, K Helin. Role of TET enzymes in DNA methylation, development, and cancer. Genes & development. 2016; 30(7): 733-750.

13. Gu T, et al. DNMT3A and TET1 cooperate to regulate promoter epigenetic landscapes in mouse embryonic stem cells. Genome biology. 2018; 19(1): 88.

14. Li Y, et al. Epigenetically deregulated miR-200c is involved in a negative feedback loop with DNMT3a in gastric cancer cells. Oncology reports. 2016; 36(4): 2108-2116.

15. Thienpont B, et al. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. Nature. 2016; 537(7618): 63-68.

16. Ma J, et al. BARX2 expression is downregulated by CpG island hypermethylation and is associated with suppressed cell proliferation and invasion of gastric cancer cells. Oncology reports. 2020; 43(6): 1805-1818.

17. Song SJ, et al. MicroRNA-antagonism regulates breast cancer stemness and metastasis via TET-family-dependent chromatin remodeling. Cell. 2013; 154(2): 311-324.

18. Zhang J, et al. DNA Methyltransferases in Cancer: Biology, Paradox, Aberrations, and Targeted Therapy. Cancers. 2020; 12(8).

19. Gujar H, DJ Weisenberger, G Liang. The Roles of Human DNA Methyltransferases and Their Isoforms in Shaping the Epigenome. Genes. 2019; 10(2).

20. Uysal F, S Ozturk. DNA Methyltransferases in Mammalian Oocytes. Results and problems in cell differentiation. 2017; 63: 211-222.

21. Chen C, et al. TB tools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. Molecular plant. 2020; 13(8): 1194-1202.

22. Yu G, et al. cluster Profiler: An R package for comparing biological themes among gene clusters. Omics: A journal of integrative biology. 2012; 16(5): 284-287.

23. Wang LH, et al. Downregulation of miR-29b targets DNMT3b to suppress cellular apoptosis and enhance proliferation in pancreatic cancer. Molecular medicine reports. 2018; 17(2): 2113-2120.

24. Yang H, et al. Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation. Oncogene. 2013; 32(5): 663-669.

25. Tavakolian S, H Goudarzi, E Faghihloo. E-cadherin, Snail, ZEB-1, DNMT1, DNMT3A and DNMT3B expression in normal and breast cancer tissues. Acta biochimica Polonica. 2019; 66(4): 409-414.

26. Singal R, et al. Polymorphisms in the DNA methyltransferase 3b gene and prostate cancer risk. Oncology reports. 2005; 14(2): 569-573.

27. Afgar A, et al. MiR-339 and especially miR-766 reactivate the expression of tumor suppressor genes in colorectal cancer cell lines through DNA methyltransferase 3B gene inhibition. Cancer biology & therapy. 2016; 17(11): 1126-1138.

28. Kiehl S, et al. Epigenetic silencing of downstream genes mediated by tandem orientation in lung cancer. Scientific reports. 2017; 7(1): 3896.

29. He M, et al. Expression of DNMTs and MBD2 in GIST. Biomedical reports. 2013; 1(2): 223-227.

30. Cypris O, T Božić, W Wagner. Chicken or Egg: Is Clonal Hematopoiesis Primarily Caused by Genetic or Epigenetic Aberrations? Frontiers in genetics. 2019; 10: 785.

31. Han M, et al. Epigenetic Enzyme Mutations: Role in Tumorigenesis and Molecular Inhibitors. Frontiers in oncology. 2019; 9: 194.

32. Wang D, et al. Increased 5-hydroxymethylcytosine and Ten-eleven Translocation Protein Expression in Ultraviolet B-irradiated HaCaT Cells. Chinese medical journal. 2017; 130(5): 594-599.

33. Delhommeau F, et al. Mutation in TET2 in myeloid cancers. The New England journal of medicine. 2009; 360(22): 2289-2301.

34. Ko M, et al. Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(35): 14566-14571.

35. Quivoron C, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. Cancer cell. 2011; 20(1): 25-38.

36. Moran-Crusio K, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer cell. 2011; 20(1): 11-24.

37. Dawson MA, T Kouzarides. Cancer epigenetics: from mechanism to therapy. Cell. 2012; 150(1): 12-27.

38. Seshagiri S, et al. Recurrent R-spondin fusions in colon cancer. Nature. 2012; 488(7413): 660-664.

39. Sato, Y, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. Nature genetics. 2013; 45(8): 860-867.

40. Zhang Q, et al. Integrative Analysis of Hypoxia-Associated Signature in Pan-Cancer. iScience. 2020; 23(9): 101460.

41. Lian H, WB Li, WL Jin. The emerging insights into catalytic or non-catalytic roles of TET proteins in tumors and neural development. Oncotarget. 2016; 7(39): 64512-64525.

42. D'Aniello C, et al. Vitamin C in Stem Cell Biology: Impact on Extracellular Matrix Homeostasis and Epigenetics. Stem cells international. 2017; 2017: 8936156.

43. Pang AW, et al. Towards a comprehensive structural variation map of an individual human genome. Genome biology. 2010; 11(5): R52.

44. Zack TI, et al. Pan-cancer patterns of somatic copy number alteration. Nature genetics. 2013; 45(10): 1134-1140.

45. Lynch M. Genomics. Gene duplication and evolution. Science (New York, N.Y.). 2002; 297(5583): 945-947.

46. Yen RW, et al. Isolation and characterization of the cDNA encoding human DNA methyltransferase. Nucleic acids research. 1992; 20(9): 2287-2291.

47. Li Y, et al. Stella safeguards the oocyte methylome by preventing de novo methylation mediated by DNMT1. Nature. 2018; 564(7734): 136-140.

48. Kobayashi H, et al. Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. PLoS genetics. 2012; 8(1): e1002440.

49. Inoue A, Y Zhang. Replication-dependent loss of 5-hydroxymethyl-

cytosine in mouse preimplantation embryos. Science (New York, N.Y.). 2011; 334(6053): 194.

50. Yang R, et al. Hydrogen Sulfide Promotes Tet1- and Tet2-Mediated Foxp3 Demethylation to Drive Regulatory T Cell Differentiation and Maintain Immune Homeostasis. Immunity. 2015; 43(2): 251-263.

51. Yue X, et al. Loss of TET2 and TET3 in regulatory T cells unleashes effector function. Nature communications. 2019; 10(1): 2011.

52. Ichiyama K, et al. The methylcytosine dioxygenase Tet2 promotes DNA demethylation and activation of cytokine gene expression in T cells. Immunity. 2015; 42(4): 613-626.

53. Tian Z, et al. TRIM8 inhibits breast cancer proliferation by regulating estrogen signaling. American journal of cancer research. 2020; 10(10): 3440-3457.

54. Chen Z, et al. DNA methylation markers that correlate with occult lymph node metastases of non-small cell lung cancer and a preliminary prediction model. Translational lung cancer research. 2020; 9(2): 280-287.

55. Hamid AA, et al. Expression of cold-inducible RNA-binding protein in the normal endometrium, endometrial hyperplasia, and endometrial carcinoma. International journal of gynecological pathology: Official journal of the International Society of Gynecological Pathologists. 2003; 22(3): 240-247.

56. Shang C, et al. The Prognostic Value of DNA Methylation, Post-Translational Modifications and Correlated with Immune Infiltrates in Gynecologic Cancers. Pharmacogenomics and personalized medicine. 2021; 14: 39-53.

57. Huang R, et al. The Construction of Bone Metastasis-Specific Prognostic Model and Co-expressed Network of Alternative Splicing in Breast Cancer. Frontiers in cell and developmental biology. 2020; 8: 790.

58. Indacochea A, et al. Cold-inducible RNA binding protein promotes breast cancer cell malignancy by regulating Cystatin C levels. RNA (New York, N.Y.). 2021; 27(2): 190-201.

59. Su PH, et al. Epigenetic silencing of PTPRR activates MAPK signaling, promotes metastasis and serves as a biomarker of invasive cervical cancer. Oncogene. 2013; 32(1): 15-26.

60. Wu X, et al. Hypermethylation of the -Activated MAPK Signaling Pathway to Promote the Development of Glioma. Cancer management and research. 2020; 12: 7043-7059.

61. Fan G, et al. DNA methylation biomarkers for hepatocellular carcinoma. Cancer cell international. 2018; 18: 140.

62. Lee S, J Rauch, W Kolch. Targeting MAPK Signaling in Cancer: Mechanisms of Drug Resistance and Sensitivity. International journal of molecular sciences. 2020; 21(3).

63. Khaliq M, M Fallahi-Sichani. Epigenetic Mechanisms of Escape from BRAF Oncogene Dependency. Cancers. 2019; 11(10).

64. Shen Y, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011; 118(20): 5593-5603.

65. Cao XY, et al. DNA methyltransferase3a expression is an independent poor prognostic indicator in gastric cancer. World journal of gastroenterology. 2014; 20(25): 8201-8208.

66. Gao Q, et al. Deletion of the de novo DNA methyltransferase Dnmt3a promotes lung tumor progression. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(44): 18061-18066.

67. Szablewski V, et al. An epigenetic regulator-related score (EpiScore) predicts survival in patients with diffuse large B cell lymphoma and identifies patients who may benefit from epigenetic therapy. Oncotarget. 2018; 9(27): 19079-19099.

68. Patnaik MM, et al. DNMT3A mutations are associated with inferior overall and leukemia-free survival in chronic myelomonocytic leukemia. American journal of hematology. 2017; 92(1): 56-61.

69. Belani R, et al. ASXL1 and DNMT3A mutation in a cytogenetically normal B3 thymoma. Oncogenesis, 2014; 3: e111.