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RAPID COMMUNICATION

Pan-cancer analysis of DNA methyltransferase family with potential implications in prognosis and immunology in human cancer

DNA methylation, as a key epigenetic modification affecting gene expression, is catalyzed by DNA methyltransferase (DNMT). *DNMT1*, *DNMT3A*, and *DNMT3B* are the main members of the DNMT family, which have been found to regulate many biological processes.[1] Aberrant DNA methylation in the promoter of tumor suppressor genes is considered a biomarker in cancer. However, no evidence of regulatory mechanisms and pathways relating to DNMTs and immune abnormalities has been provided. Herein, we reported a bioinformatic analysis to explore the effect of DNMTs in pan caner. DNMTs are associated with different expressions and poor prognosis in various cancers, especially in LIHC. In addition, we found that DNMTs expression-correlated genes are involved in pathways related to the cell cycle and epithelial–mesenchymal transition (EMT). DNMTs is significantly related to the expression of major immune checkpoints. Furthermore, DNMTs probably engaged in the process of drug resistance. A risk score based on the expression of DNMTs in LIHC was constructed and found that high risk patients have a poor prognosis. Taken together, our study comprehensively analyzed the DNMT family in pan-cancer and demonstrated that DNMTs could be used as prognosis biomarkers and potential therapeutic targets for the development of drugs.

DNMT family includes *DNMT1*, *DNMT2*, *DNMT3A*, *DNMT3B*, and *DNMT3L*. In the present study, we explored the main DNMTs (*DNMT1*, *DNMT3A*, and *DNMT3B*) mRNA expression in tumor tissues and normal tissues in the TCGA and GTEx database, we found differences in DNMTs expression in 30 tumors. Figure 1A showed that there were significant differences between the expression of DNMTs in various cancers and that in normal tissues. The high expression of *DNMT1* was found in 26 cancers, and the expression of *DNMT3A* and *DNMT3B* in tumor samples was significantly higher than the normal samples in 21 and 24 cancers, respectively. These results suggested that the high expression of *DNMT* subtypes may be related to the occurrence and progression of different tumors. To further evaluate the probability of survival differences between groups with different DNMTs expressions, we analyzed survival differences between three subtypes of genes. As shown in Figure 1B, S1 and S2, we found that the high *DNMT1* expression leads to poor prognosis in 12 cancers. The high expression of *DNMT3A* and *DNMT3B* was associated with poor prognosis in 11 and 12 cancers, respectively. In LGG, KIRP, LIHC, MESO, UVM, and PRAD, the high expression of all three subtypes was a high-risk factor for patients. Based on previous findings, we further examined the Receiver Operating Curve (ROC) of *DNMT1*, *DNMT3A*, and *DNMT3B* in cancer. The results suggested that *DNMT1* is a high-risk factor for prognosis in patients with LGG and LIHC. *DNMT3A* and *DNMT3B* may be high-risk factors in LIHC, PRAD, BRCA, and UCEC (Fig. S3A–D). The immunohistochemical results (Fig. S3E–G) and RT-qPCR (Fig. 1C) validated the high expression in these cancers.

Alteration of DNMTs is an important causative factor of aberrant genomic methylation. Based on our previous findings, we used the cBioportal website to analyze alteration frequency and sites in *DNMTs* (Fig. S4A–C). We found that "amplification" and "mutation" were primary types in *DNMTs*. Our results suggested that amplification of genes probably causes overexpression of DNMTs in cancers and lead to poor prognosis (Fig. 1D). In addition, the mutation in the particular domain of DNMTs may lead to changes in downstream amino acids that affect enzyme function. The additional research is required to further

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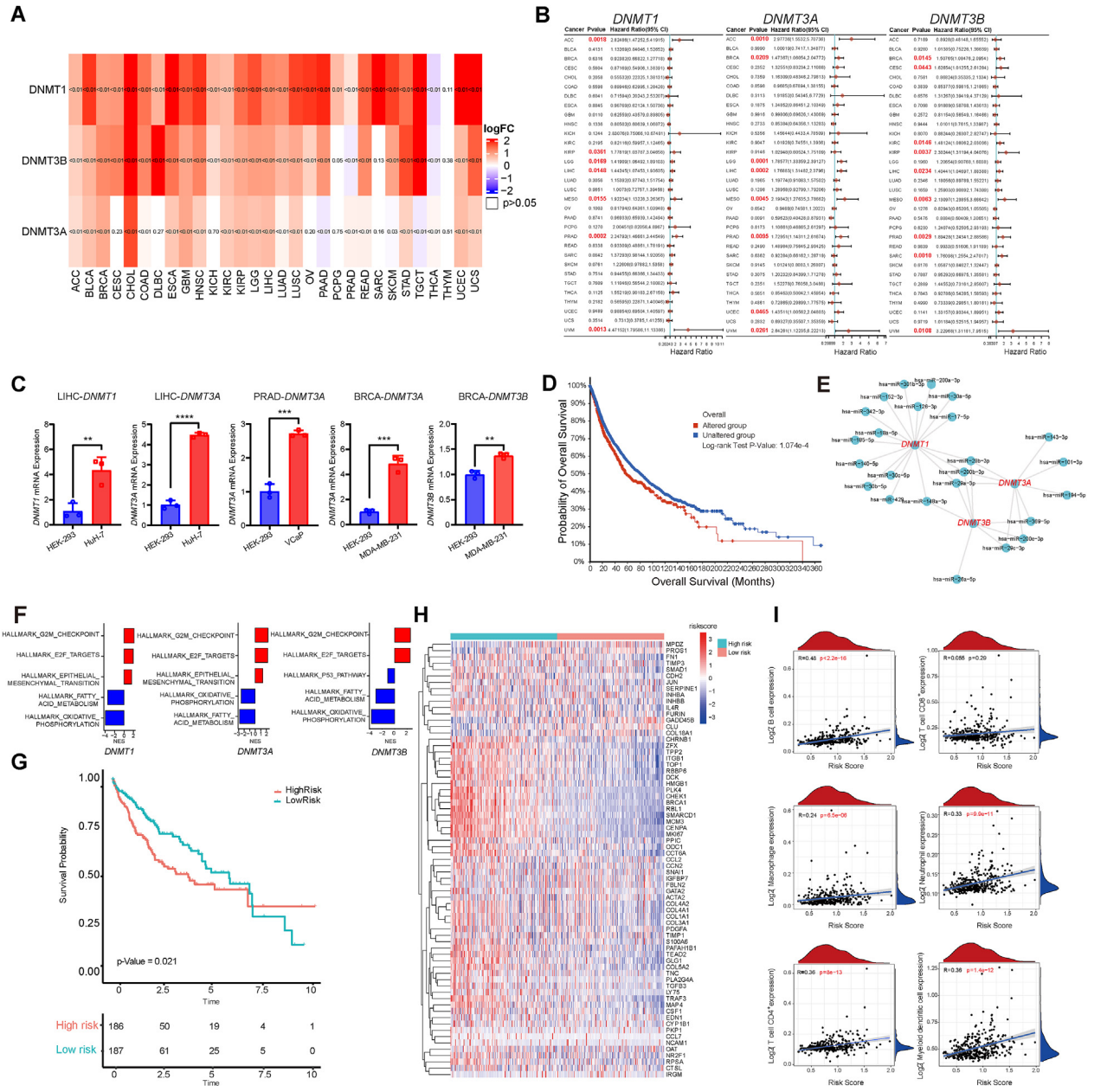

Figure 1 Expression, survival, alternation, regulation, pathway analysis of DNMT family genes in human cancer and risk score model of DNMT family genes in LIHC. **(A)** Heatmap of differential expression of DNMTs between tumor and normal samples in pan-cancer. The color of each box represents the size of logFC value, white box represents *P* value > 0.05, which has no significant difference. **(B)** Progression free survival analysis of DNMTs by Cox univariate regression in pan-cancer samples with significance indicated in red **(C)** Expression of *DNMT1* in HEK-293 and HuH-7 cells; *DNMT3A* in HEK-293 and HuH-7 cells, VCaP cells, MDA-MB-231 cells; *DNMT3B* in HEK-293 and MDA-MB-231 cells by RT-qPCR. The results from three independent experiments were statistically analyzed using *t*-test method. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. **(D)** Overall Survival of DNMTs alternation group and non-alternation group **(E)** Network plot showed microRNAs and transcription factors regulating DNMTs. **(F)** GSEA enrichment of the DNMTs correlated genes in LIHC. Red: NES >0, $P < 0.05$; blue: NES <0, $P < 0.05$. **(G)** Overall survival between high riskscore group and low riskscore group. **(H)** Heatmap of relationship between high and low riskscore groups with the expression of EMT key genes. **(I)** Correlation of riskscore and expression of immune cells in LIHC.

evaluate the mechanism of the effect of alteration in *DNMT* in differential cancers. Moreover, we found that all *DNMT1*, *DNMT3A* and *DNMT3B* were targeted by hsa-miR-29b-3p, hsa-miR-200b-3p,[2] and hsa-miR-29a-3p (Fig. 1E),[3] but the specific regulation of these miRNAs remains to be explored.

To further explore the mechanism of DNMTs in carcinogenesis, protein—protein interactions of DNMT1, DNMT3A, and DNMT3B were analyzed. Figure S5 and S6 show the gene names of the top 20 proteins associated with DNMTs proteins. We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment with

the genes of proteins associated with the three subtypes of DNMTs from STRING. Results of GO enrichment showed that DNMTs related genes were mainly significantly enriched in pathways such as methyltransferase activity, chromosomes, nuclei, chromatin modification, transcriptional regulation, and methylation. etc. KEGG enrichment results showed that related genes were significantly enriched in microRNA in cancer, cell cycle, and other pathways. Based on previous studies, we found that *DNMT1*, *DNMT3A*, and *DNMT3B* are highly expressed in LIHC and have a poor prognosis. To further investigate potential downstream target genes for DNMTs in LIHC, we examined for genes that were significantly negatively associated with DNMT subtypes (Fig. S7A, B). We found *LINC01554*, *SULT1A1*, *PCK1*, *MT2A*, *OGDHL*, and *SLC27A5* might be down-regulated genes. In addition, GSEA analysis of genes differentially expressed with DNMTs in LIHC using HALLMARK and C2 gene sets showed that DNMTs related genes were mainly enriched in E2F_TARGETS, G2_M_CHECKPOINT, EPITHELIAL_MESENCHYMAL_TRANSITION pathways (Fig. 1F; Fig. S7C, S8). Previous studies have shown that DNMTs were associated with epithelial−mesenchymal transition (EMT) related genes. For instance, DNMT3B leads to hypermethylation of CpG sites at the 5' promoter of E-cadherin and further inhibits E-cad protein expression, which mediates the occurrence of EMT.[4] The results and existing researches suggested that the mechanism of effects of DNMTs might be associated with cell cycle processes and EMT.

Immune cells are important parts of the tumor microenvironment and are closely related to the development and metastasis of tumors. Herein, we analyzed the correlation between DNMTs expression and six types of infiltrating immune cells, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells by the TIMER online website. We found that the expression of DNMTs is associated with the infiltration of immune cells and immune checkpoints (Fig. S9), which suggested that DNMTs are probably involved in the immune escape of tumors through a response to the immune checkpoint. Furthermore, we established the risk score model of DNMTs in LIHC by Lasso regression (Fig. S10A, B). Risk score model for DNMTs in LIHC: Risk score = (0.1513) * DNMT1 + (0.1708) * DNMT3A + (0.0082) * DNMT3B. The model suggested that the high-risk group leads to a poor prognosis in LIHC (Fig. 1G). On the basis of this result, we found that 68 EMT-related genes[5] (Fig. 1H) and the expressions of myeloid dendritic cell, B cell, T cell $CD4^+$, neutrophil, and macrophage were significantly positively correlated with high-risk group (Fig. 1I) which confirmed our previous results. The results suggested DNMTs are probably involved in EMT signaling and immune cell infiltration in LIHC. The correlation between DNMTs and sensitivity of first-line chemotherapeutic drugs and reported active small molecules was analyzed based on the GSCA database (Fig. S10C, D). The result suggested that resistance of some chemotherapeutic drugs is probably implicated in DNMTs. Our finding may be helpful in the study of the mechanism of resistance to chemotherapy drugs.

In summary, our study analyzed DNMTs in pan cancer, indicating that DNMTs are involved in the tumorigenesis process, immune escape, and drug resistance that affect the development of cancers and the survival of patients. Therefore, DNMTs can be expected as a potential diagnostic biomarker and powerful therapeutic target for the treatment of cancer.

Author contributions

Conception and design: Jiayu Ding, Xiao Wang, Peng Yang. Acquisition of data (investigation, bioinformatics data mining and process, provided facilities, etc.): Jiayu Ding, Hao Shen, Dawei Wang, Wenbin Kuang, Liping Wang, Xiao Wang, Peng Yang. Analysis and interpretation of data (e.g., statistical analysis, bioinformatics analysis, computational analysis): Jiayu Ding, Hao Shen, Xiao Wang, Peng Yang. Writing-review and editing: Jiayu Ding, Hao Shen, Xiao Wang, Peng Yang. Administrative, technical, or material support: Xiao Wang, Peng Yang.

Conflict of interests

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.07.016.

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