Identification of PRIM2 as a new prognostic and immune-related marker in cancer based on a comprehensive pan-cancer analysis

Jinqun Jiang
Yue Bei People's Hospital

Hai Lu (✉ hysa1985@163.com)
The first Hospital of ShaoGuan

Article

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Abstract

DNA Primase Subunit 2 (PRIM2) encodes a large subunit (p58C) of DNA primase, which plays an important role in DNA replication. However, the role of PRIM2 in cancer and immune regulation has not been elucidated. Therefore, in the present study, the expression profile of PRIM2 in cancer was investigated using publicly available databases like the Cancer Genome Atlas (TCGA), the Genotype-Tissue Expression (GTEx), the Broad Institute Cancer Cell Line Encyclopedia (CCLE) from the University of California Santa Cruz (UCSC) Xena website. PRIM2 expression was analyzed in paired normal to the adjacent tumor and unpaired cancer and normal tissues. The prognosis of PRIM2 in various cancers was studied using Cox regression and Kaplan-Meier analysis. The relationship between PRIM2 and tumor staging, tumor mutation burden (TMB), and microsatellite instability (MSI) was analyzed. Algorithms like cell type identification by estimating relative subsets of RNA transcripts (CIBERSORT), xCell, ssGSEA, and MCP-counter were used to study the correlation between PRIMA2 and tumor immune microenvironment, immune infiltrating cells, and immune-related genes (antigen processing and presentation genes, chemokines, chemokine receptors, HLA-related genes, immune checkpoints). Further, the correlation between PRIM2 and methyltransferase (DNMT1, DNMT3A, DNMT3B) from different databases. The biological processes and signaling pathways associated with PRIM2 in various tumors were studied. Finally, the correlation between the PRIM2 and the sensitivity of multiple drugs was analyzed using the National Cancer Institute (NCI)-60 database. The results show that PRIM2 was up-regulated in most tumors, high PRIM2 expression was associated with the different stages of cancer, and poor prognosis was observed. The results indicate that PRIM2 could potentially be used as a prognostic and immunotherapy target in tumors.

Introduction

Cancer is the second leading cause of mortality worldwide. In 2020, the number of cancer cases increased by approximately 19.3 million globally, and about 10 million cancer-related death were reported, as per the latest data released by the International Agency for Research on Cancer. This puts an unimaginable burden on the global economy. Breast cancer has surpassed lung cancer as the leading cancer in women worldwide, which calls for attention to women's health 1. With the emergence of targeted immunotherapy, marked improvement in survival has been observed in lung cancers 2, glioblastoma multiforme 3, and melanomas 4. Cancer is a heterogeneous disease due to which many patients with pancreatic cancer 5, hepatocellular carcinoma, and cholangiocarcinoma 6 fail to respond to immunotherapy tumors. The cancer heterogeneity also affects the diagnosis and prognosis of the patients 7. Hence, there is an urgent need for early diagnosis and prognostic biomarkers.

PRIM2 is located on chromosome 6 and encodes for DNA primase subunit 2. It forms a DNA primase complex with the PRIM1 and participates in DNA replication 8. Studies have shown single nucleotide polymorphism in PRIMA2 can affect the prognosis of patients with non-small cell carcinoma 9. Dihydroartemisinin inhibits cell proliferation and colony formation of colonies by targeting PRIMA2 and is used in treating lung cancer 10. High-throughput sequencing of circRNA in liver cancer cells treated with
Nitidine Chloride (NC) shows PRIM2 acts as a "miRNA sponge" with hsa_circ_0088364 and hsa_circ_0090049. In cervical cancer, PRIM2 is regulated by Sine oculis homeobox homolog 1 (SIX1), which promotes DNA synthesis and increases cell proliferation in cervical cancer. It may be the therapeutic target of muaddil sapra in treating acute liver injury. These studies provide insight into the role of PRIM2 in cancer; however, additional research is required to understand the association between PRIM2 and cancer.

In this study, the expression of PRIM2 in different cancers was studied using different databases. The relationship between PRIM2 and tumor stage, the prognostic significance in cancer, and its correlation with tumor mutation burden (TMB), microsatellite instability (MSI), and DNA methyltransferase (DNMT1, DNMT3A, DNMT3B) were analyzed. Further, the association of PRIMA2 and immune cells, infiltrating immune cell score, and immune-related genes were evaluated. The biological processes and the pathways enrichment by PRIM2 in the top ten cancers with the highest morbidity and mortality were assessed. Finally, based on the CellMiner database, the correlation between PRIM2 and anticancer drugs of 60 cancer cells was analyzed.

Methods And Materials

Data source and difference analysis

The RNA expression data, tumor mutations, MSI, and clinical data from the Cancer Genome Atlas (TCGA) pan-cancer, cancer cell line encyclopedia (CCLE), and the Genotype-Tissue Expression (GTEx) were retrieved from the University of California, Santa Cruz University (UCSC) Xena browser (http://xena.ucsc.edu/) 14. The duplicates and samples with low expression values were removed from the analysis. Dataset with immunotherapy results was obtained from IMvigor210 (Urothelial carcinoma) 16.

Paired normal to adjacent tumor samples were retrieved from TCGA database to analyze the expression of PRIM2 in tumor and adjacent tumor tissues. Few paracancerous samples for some tumors were available with the TCGA database. Hence data from TCGA was combined with the normal tissues in the GTEx database to analyze the difference in expression of PRIM2 in normal tissues and cancer tissue. p < 0.05 is considered statistically significant.

Prognostic value of PRIM2 and its correlation with clinical characteristics

Cox regression analysis was used to evaluate the correlation between PRIM2 expression (as a continuous variable) and overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). Kaplan-Meier analysis was used to evaluate the correlation between PRIM2 (as a classification variable) and OS, DSS, DFI, and PFI. R package "survival" and "survminer" was to represent Kaplan-Meier results, and R package "forestplot" was to display Cox regression analysis results.
R package “ggpubr” was used to analyze the correlation between \textit{PRIM2} and tumor staging. \( p < 0.05 \) was considered statistically significant.

The correlation between \textit{PRIM2} and TMB (ordered by a single nucleotide) and MSI was analyzed.

\textbf{Correlation between \textit{PRIM2} and DNA methyltransferase family}

The expression data of genes from the DNA methyltransferase family (\textit{DNMT1}, \textit{DNMT3A}, \textit{DNMT3B}) in pan-cancer tissues and normal tissue were extracted from TCGA, CCLE, and GTEx, respectively, from the UCSC Xena website, to calculate the correlation between \textit{PRIM2} and \textit{DNMT1}, \textit{DNMT3A} \textit{DNMT3B}. \( p < 0.05 \) was considered statistically significant.

\textbf{Correlation analysis between \textit{PRIM2} and immune microenvironment, immune cells, immune-related genes}

The adjacent sample data from all tumor types were deleted in the pan-cancer tissues. The R package "estimate"17 was used to calculate the immune cell score, stromal cell score, total score, and tumor purity of each sample. Further, their correlation with the \textit{PRIM2} in different tumor types was calculated with \( p < 0.05 \) and visualized using the R package "ggpur."

Cell type identification by estimating relative subsets of RNA transcripts (CIBERSORT) was used to calculate the proportion of 22 immune cell subtypes in each sample 18. xCell estimated the abundance score of 64 immune cell types. Single-sample Gene Set Enrichment Analysis (ssGSEA) was used for single samples that were ineligible for GSEA. ssGSEA was used to extract the enriched fraction of 24 immune cells, and the MCP-counter method was used to calculate the absolute abundance of eight immune cells and two stromal cells 19. CIBERSORT, xCell, GSVA, and MCPcounter algorithm were used to calculate the immune cell score of each tumor tissue in TCGA pan-cancer. The correlation between the expression levels of \textit{PRIM2} and the immune cells score was calculated and visualized using the heat map. The correlation p-value and R-value of the four algorithms were used to show the correlation.

The expression data for chemokines, chemokine receptors, antigen processing and presentation, and HLA-related genes were retrieved from https://www.immport.org. The expression data for classic immune checkpoint genes such as PD-1 (programmed death receptor 1), PD-L1 (programmed death-ligand 1), CTLA4 (cytotoxic T lymphocyte-associated protein 4), LAG3 (lymphocyte activation gene 3), TIGIT (T cell immune receptor with immunoglobulin and ITIM domain), and TIM-3 (T lymphocyte immunoglobulin mucin 3) was obtained from TCGA pan-cancer for correlation analysis with \textit{PRIM2} and visualized using a heat map.

\textbf{The biological function of \textit{PRIM2} in different tumors}

The biological function of \textit{PRIM2} in different tumors was assessed. The samples for each cancer were divided into high and low expression groups according to the mean value of \textit{PRIM2}. The differences between the high and low expression groups were analyzed, and log FC > 1 was selected and corrected.
Genes with $p < 0.05$ were considered significantly different, and GO analysis was conducted on these genes. GSEA was used to analyze all the genes for differential analysis using c5.all.v7.symbols.GMT file. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the top ten cancer types with the highest incidence and mortality rates were finally selected to study the biological function of \textit{PRIM2}.

\textbf{PRIM2 and drug sensitivity analysis}

The National Cancer Institute (NCI)-60 database includes the expression levels of 22,379 genes in 60 human cancer cell lines and the data of 20,503 analyzed compounds (including 102 drugs approved by the U.S. Food and Drug Administration). The website is https://discover.nci.nih.gov/cellminer20. Pearson correlation was used to analyze the sensitivity of \textit{PRIM2} and the drugs. $p < 0.05$ was considered statistically significant.

\textbf{Correlation between \textit{PRIM2} and immunotherapy}

To investigate the correlation between \textit{PRIM2} and immunotherapy, the transcriptome data and clinical information were obtained from the IMvigor210 study. The survival difference and response to immunotherapy between the \textit{PRIM2} high and low expression group was calculated. Further, the correlation between the expression of \textit{PRIM2} and different immune response types and clinical subtypes was evaluated.

Peng Jiang et al. designed a novel computational framework called Tumor Immune Dysfunction and Rejection Score (TIDE), which uses transcriptional expression data to evaluate the ability of tumor immune escape 21. TIDE scores were calculated for each sample of 33 tumors, and the difference in TIDE scores between \textit{PRIM2} high and low expression groups was assessed.

\textbf{Statistical analysis}

Paired samples from TCGA were analyzed using a paired t-test. The combined analysis of GTEX and TCGA was carried out using t-test. The correlation between \textit{PRIM2} and tumor staging was carried out using the Kruskal-Wallis test. Pearson or Spearman’s rank test is used for correlation analyses. $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using \textit{R} software (version 4.1.0).

\textbf{Results}

\textbf{Expression of \textit{PRIM2} gene in pan-cancer}

The expression of \textit{PRIM2} in 18 tumors with matched samples retrieved from TCGA was analyzed (Fig. 1A). No significant difference in the expression of \textit{PRIM2} was observed in pancreatic cancer (PAAD) and prostate cancer (PRAD). Compared to paired adjacent normal tissues, a significant difference in \textit{PRIM2} expression was observed in tumor samples of 16 cancers like bladder urothelial carcinoma (BLCA), invasive breast carcinoma (BRCA), cholangiocarcinoma (CHOL), colon cancer (COAD),
esophageal cancer (ESCA), head and neck squamous cell carcinoma (HNSC), renal papillary cell cancer (KIRP), renal clear cell carcinoma (KIRC), hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), rectal adenocarcinoma (READ), gastric cancer (STAD), endometrial cancer (UCEC). Interestingly, tumor samples from renal chromophobe cell carcinoma (KICH), a thyroid cancer (THCA), had lower \textit{PRIM2} expression than paired adjacent samples. Because some tumors in TCGA, such as adrenal cortical carcinoma (ACC), brain low-grade glioma (LGG), ovarian serous cystic carcinoma (OV), etc., had no or few adjacent samples, the expression in normal tissue samples was retrieved from the GTEx database. As shown in Fig. 1B, the expression of \textit{PRIM2} was significantly different in most tumors, except for chromophobe (KICH), pheochromocytoma (PCPG), and paraganglioma sarcoma (SARC). In tumor samples for acute myeloid leukemia (AML) and renal chromophobe cell carcinoma (KICH), the expression of \textit{PRIM2} was significantly higher in tumor samples than in normal samples.

To fully understand the expression of \textit{PRIM2}, its expression was analyzed in samples retrieved from the GTEx database (Fig. 1C). The results reveal that the expression of \textit{PRIM2} was highest in prostate and skin tissue, while the expression in liver tissue was relatively low. In the CCLE, \textit{PRIM2} expression was relatively high in salivary cells but relatively low in kidney cells (Fig. 1D).

\textbf{Correlation between \textit{PRIM2}, clinical features, and its prognostic value}

The correlation between \textit{PRIM2} and tumor stage in different tumors was calculated (Fig. 2A-G). A significant difference in \textit{PRIM2} expression between any two of the four tumor stages was required to fit the criteria, and the results showed a significant correlation in seven tumors: BRCA, ESCA, KICH, KIRP, LIHC, LUAD, and THCA.

Cox regression analysis evaluated the correlation between the \textit{PRIM2} as a continuous variable and the overall survival. Kaplan-Meier analysis divided the \textit{PRIM2} levels into high and low expression groups based on the mean expression value of \textit{PRIM2}. The correlation between the two groups and OS was studied (Fig. 3A, Figure S1A-I). COX regression analysis showed that \textit{PRIM2} expression is correlated with the overall survival in ACC, KICH, KIRP, LGG, LIHC, PAAD, SARC, THYM, UCEC, and in THYM, hazard ratios (HR) were less than 1. Hence \textit{PRIM2} was considered a protective factor. On the contrary, for several other tumors, \textit{PRIM2} was regarded as an inhibitory factors. The Kaplan-Meier analysis further verified the results. As per the survival curve, the high expression of \textit{PRIM2} in THYM indicated a good prognosis, while the high \textit{PRIM2} expression in several other tumors indicated a poor prognosis. Cox regression and Kaplan-Meier analysis were used to evaluate the correlation between \textit{PRIM2} and DSS (Fig. 3B, Figure S2A-K), DFI (Fig. 3C, Figure S3A-E), and PFI (Fig. 3D, Figure S4A-K). The results show that high expression of \textit{PRIM2} in ACC, KICH, KIRC, KIRP, LGG, LIHC, PAAD, PCPG, SARC, and UCEC had a poor prognosis. In KIRC, OV, the high \textit{PRIM2} expression has a good prognosis based on DSS, and the results from COX regression analysis and Kaplan-Meier analysis were consistent (except for KIRC). High \textit{PRIM2} expression in KIRP, LIHC, LUSC, PAAD, and SARC had a poor prognosis based on DFI and Cox regression analysis. In ACC, KICH, KIRP, LGG, LIHC, MESO, PAAD, SARC, and UCEC, high expression of \textit{PRIM2} has a poor
prognosis, while in GBM, OV, high expression of PRIM2 has a better prognosis based on PFI and the Cox regression analysis.

**Correlation between PRIM2 with TMB and MSI**

TMB and MSI are the hotspots in research due to their association with immunotherapy 22–27. The correlation between PRIM2 and TMB (Fig. 4A) and MSI (Fig. 4B) in pan-cancer was studied. The results showed a statistically significant positive correlation between PRIM2 expression and TMB in SARC, STAD, ACC, LUAD, LGG, PAAD, MESO, BRCA, BLCA, OV, and LUSC tumors. Further, PRIM2 expression negatively correlated with TMB in THCA, PRAD, and THYM, and the correlation was statistically significant. A statistically significant positive correlation was observed between PRIM2 expression and MSI value in STAD and SARC, while in LGG and DLBC, PRIM2 expression negatively correlated with MSI, and the correlation was statistically significant.

**Correlation between PRIM2 and DNA methyltransferase family (DNMT1, DNMT3A, DNMT3B)**

DNA methylation plays an important role in biological processes where the gene expression can be altered without changing the DNA sequence 28. The correlation between PRIM2 and DNMT1, DNMT3A, and DNMT3B was studied in pan-cancer from the three databases GTEx (Fig. 5A), TCGA (Fig. 5B), and CCLE (Figure S5A). In the GTEx database, a positive correlation was observed between PRIM2 and DNMT1, DNMT3A, and DNMT3B in most normal tissues. Spleen positively correlated with DNMT1 (correlation value: 0.97), and kidney tissues positively correlated with DNMT3A and DNMT3B (correlations values: 0.97 and 0.92, respectively). Only a few tissues showed a negative correlation. For example, a negative correlation between the ovary and DNMT1 was observed. The prostate negatively correlated with DNMT1 and DNMT3A, and a negative correlation between skin and DNMT3B was observed. In the TCGA database, most tumors showed a positive correlation between PRIM2 and DNMT1, DNMT3A, and DNMT3B. A positive correlation was observed between DNMT1 and DNMT3B in LUAD (the correlation value: 0.85 and 0.79, respectively), and the most relevant correlation was observed between DNMT3A is MESO (the correlation is 0.78). Only DNMT1 is correlated with DNMT1, DNMT3A, and DNMT3B in cancer cell lines, and the correlations are 0.47, 0.06, and 0.15, respectively 29.

**Correlation between PRIM2 and immune microenvironment, immune cells, immune-related genes**

The tumor immune microenvironment includes immune cells and stromal cells, which play an important role in tumor development, invasion, and metastasis 29,30. PRIM2 has prognostic value in most tumors. Hence the correlation with tumor microenvironment was evaluated. As shown in Fig. 6A-S, a negative correlation was observed between PRIM2 with immune scores in most tumors, such as BRCA, CESC, ESCA, HNSC, KIRP, LUAD, LUSC, etc. Further, UCEC showed the least correlation value of -0.36. Tumors positively correlated with immune scores were BLCA, KIRC, and LGG, among which the highest correlation was observed with LGG.

The infiltration of immune cells can alter the prognosis of tumors 31. Different algorithms were used to explore the relationship between PRIM2 and different types of immune cells (Fig. 7A-D). CIBERSORT is a
deconvolution method. The results show that M1 macrophages and PRIM2 positively correlated in most tumor types, and the correlation was significant. The monocytes and PRIM2 negatively correlated with 13 tumor types. In LGG, PRIM2 correlated with 13 immune cell types. A significant positive correlation was observed with resting CD4 memory T cells, and a significant negative correlation was observed with memory B lymphocytes. In BRCA, LUAD, STAD, and THYM, PRIM2 correlated with 12 types of immune cells. In BRCA, a significant positive correlation was observed between PRIM2 and M1 type macrophages; a significant negative correlation was observed between PRIM2 and monocytes. In LUAD and STAD, a significant positive correlation was observed between PRIM2 and M1 type macrophages, and a significant negative correlation was observed between PRIM2 and quiescent mast cells. In THYM, a significant positive correlation was observed between PRIM2 and follicular helper T cells, and a significant negative correlation was observed between PRIM2 and M2 macrophages (Fig. 7A). xCell is a gene set enrichment method that can evaluate the abundance scores of 64 immune cells. As shown in Figure, a strong positive correlation between PRIM2 and Th2 cells was observed in various tumors. In THYM, a correlation was observed between PRIM2 and most immune cells, of which a significant positive correlation was observed with Th2 cells, and a significant negative correlation was observed with keratinocytes (Fig. 7B). Unlike xCell, ssGSEA is a gene set enrichment method and can evaluate the infiltration of 28 immune cells. ssGSEA results reveal a positive correlation between PRIM2 and activating CD4 T cells, memory B cells, and type 2 helper cells in most tumors. Furthermore, in LUSC, a correlation was observed between PRIM2 and most immune cells. Of which 24 immune cells correlated negatively with PRIM2, and three immune cells positively related with PRIM2. In KIRC, PRIM2 positively correlated with most immune cells (Fig. 7C). MCP-counter is used to quantify infiltrating immune cells like fibroblasts and epithelial cells. Figure 7D shows a positive correlation between PRIM2 and centrioles in most tumors and with most immune cells in UVM (Fig. 7D).

To fully understand the relationship between PRIM2 and immunity, the correlation between PRIM2 and immune-related cells was further analyzed. Immune-related genes analyzed were antigen presentation and processing, chemokines, chemokine receptors, HLA, and immune checkpoint (Fig. 8A-E). The heat map shows a correlation between PRIM2 and most antigen presentation and processing genes, chemokines, and cytokines in most tumors and positively correlated with various proteasome subunits. Further, a positive correlation was also observed between PRIM2 and immune checkpoints. In BLCA, KIRC, LIHC, PAAD, and UVM, a significant positive correlation between PRIM2 and all immune checkpoints was observed.

**Role of PRIM2 in different tumors**

To study the functions of PRIM2 in different tumors, GO enrichment analysis and GSEA were conducted on the top ten tumors with the highest morbidity and mortality. In LUAD, LIHC, STAD, BRCA, and PAAD, the correlation between PRIM2 and pathways associated with mitosis, nuclear division, organelle fission, chromosome separation, etc., were observed. In COAD, ESCA, READ, and CESC, the correlation between PRIM2 with DNA replication and repair were observed. In PRAD, PRIM2 correlated with only zinc ion homeostasis and copper ion detoxification (Fig. 9A). KEGG pathway analysis showed that in LUAD, LIHC,
STAD, BRCA, COAD, ESCA, PAAD, READ, PRIM2 positively regulated pathways associated with cell proliferation and division-related, such as DNA replication and cell cycle. PRAD and CESC, PRIM2 positively correlated with metabolic-related pathways, such as arachidonic acid metabolism, unsaturated fatty acid biosynthesis, etc. (Fig. 10A).

**Analysis of drug sensitivity of PRIM2**

The expression of PRIM2 is associated with the prognosis in most tumors, and immune cells and immune-related genes, the correlation between PRIM2 and drug sensitivity was analyzed. The results showed that the PRIM2 positively correlated with fenretinide (tumor preventive drugs; p < 0.01, r = 0.442, Fig. 11A), nelarabine (p = 0.004, r = 0.363, Fig. 11B), dexamethasone (p = 0.014, r = 0.317, Fig. 11C), curcumin (p = 0.022, r = 0.296, Fig. 11D), fluda, Rabine (p = 0.028, r = 0.283, Fig. 11E), and 6-mercaptopurine (p = 0.041, r = 0.268, Fig. 11F).

PRIM2 can be used as a potential target for immunotherapy

A comprehensive analysis of the IMvigor210 urothelial cancer data set and PRIM2 revealed that the survival rate was higher in the PRIM2 high expression group compared to the low expression group (p = 0.003, Fig. 12A). Compared to the low expression group, the PRIM2 high expression had a better response to PD-L1 immune check blockers (Fisher exact probability test, p = 0.004, Fig. 12B, Kruskal-Wallis test, p < 0.001, Fig. 12C). Further, patients with high expression of PRIM2 had a higher TMB (p = 0.004, Fig. 12D) and a high neoantigen negative (p < 0.001, Fig. 12F). The expression of PRIM2 in the inflammation group in the immunophenotype is also higher than that in the other two groups (p = 0.049, Fig. 12E), and these three indicators are closely related to immunotherapy.

Significant difference was observed in the TIDE score between the PRIM2 high and low expression groups in 25 tumors (Fig. 13A-Y). The TIDE score of the PRIM2 high expression group was low compared to the low expression group, indicating that the patients in the PRIM2 low expression group had more obvious immune escape.

**Discussion**

Pan-cancer analysis allows simultaneous evaluation of expression and the role of a gene in different tumors, which aids in developing therapy 32. DNA primase is a type of RNA polymerase which synthesizes short RNA primers required by DNA polymerase 33. DNA primase is a heterodimer composed of a small catalytic subunit (PRIM1) and a large auxiliary subunit (PRIM2). Studies have shown that the C-terminal of the large subunit plays a major role in primer synthesis 34. Our team has previously shown high expression of PRIM1 in liver cancer is closely related to pathological staging. Through a series of functional experiments, it has been shown that PRIM1 promotes the proliferation of cells and inhibits apoptosis in liver cancer 35. A similar role of PRIM1 has been observed in estrogen-related breast cancer 36. However, the mechanism of PRIM2 in cancer has not been thoroughly evaluated.
To the best of our knowledge, this study, for the first time, explores the expression, prognosis, and biological function of \textit{PRIM2} in pan-cancer and its correlation with immunity. Our results show the difference in the expression of \textit{PRIM2} in most tumors, irrespective of the data retrieved from TCGA database tumor paired with normal tissues and adjacent cancer tissues or from data retrieved from the GTEx database (cancer and normal tissues). This suggests \textit{PRIM2} may play a role in the development and tumorigenesis of some tumors. In BRCA, ESCA, KICH, KIRP, LIHC, LUAD, and THCA tumors, \textit{PRIM2} expression is related to the tumor stage. Interestingly higher levels of \textit{PRIM2} were observed at advanced stages in ESCA, KICH, and LUAD, suggesting that \textit{PRIM2} may be involved in tumor development. Further, the role of \textit{PRIM2} in prognosis was studied in 33 tumors, and the results reveal that the expression of \textit{PRIM2} is related to OS, DSS, DFI, and PFI in KIRP, LIHC, and PAAD tumors. High \textit{PRIM2} expression was associated with poor prognosis in patients. These results indicate oncogenic expression of \textit{PRIM2} in some tumors.

Mounting evidence has shown that tumor patients with high TMB respond better to immunotherapy \cite{37,38}. Our results reveal a positive correlation between \textit{PRIM2} and TMB in SARC, STAD, ACC, LUAD, LGG, PAAD, MESO, BRCA, BLCA, OV, and LUSC. The correlation was significant with SARC, while the negative correlation between \textit{PRIM2} and TMB was observed in THCA and PRAD, while the negative correlation was significant in THYM. We believe that \textit{PRIM2} could cooperate with TMB to predict tumor immunotherapy response. Studies have shown that high MSI has a higher tumor mutation burden and responds better to immunotherapy \cite{39,40}. Further, aberrant expression of \textit{PRIM2} positively correlated with MSI in STAD and SARC, and a negative correlation was observed between \textit{PRIM2} and MSI in THCA, LGG, and DLBC. These results suggest that the expression \textit{PRIM2} affects TMB and MSI and may alter immunotherapy response.

DNA methyltransferase can affect gene expression at the epigenetic level. \textit{DNMT1} regulates the methylation level of X-linked protein 1 (\textit{BEX1}) expressed in the brain, thereby altering \textit{BEX1} expression and ultimately regulating the characteristics of cancer stem cells in the liver \cite{41}. In acute myeloid leukemia (AML), about 25% of patients harbor mutations in \textit{DNMT3A}. DNMT3A-mediated CpG island hypermethylation leads to further deterioration of AML \cite{42}. Mutations in \textit{DNMT3B} cause centromeric instability, immune deficiency, and facial abnormalities, which lead to autosomal recessive genetic diseases \cite{43}. Our results show a positive correlation between \textit{PRIM2} expression in most normal tissues, tumor tissues, and tumor cell lines, and methyltransferase genes (\textit{DNMT1}, \textit{DNMT3A}, \textit{DNMT3B}). This suggests that methyltransferase genes may alter \textit{PRIM2} expression and influence tumor development and prognosis.

The tumor immune microenvironment is composed of immune and stromal cells \cite{44}. The occurrence of tumors is accompanied by the interaction of complex components in the immune microenvironment \cite{45}. The results reveal that \textit{PRIM2} correlated with immune cell scores of 19 types of tumors. The correlation was negative in most cases, suggesting that the higher \textit{PRIM2} expression lowers the immune cell scores, thus suggesting a poor prognosis in patients with high \textit{PRIM2} levels. Studies have shown that infiltrating cells mainly comprise tumor-associated macrophages and play an important role in helping tumor cells escape immune surveillance \cite{46–48}. The results reveal that using the CIBSERORT algorithm, \textit{PRIM2} and
M1 type macrophages show a positive correlation in most tumors. However, contradictory results were obtained using the xCell and ssGSEA algorithm, where a negative correlation was observed between PRIM2 and tumor-associated macrophages. This discrepancy could be due to different principles used by these algorithms. Interestingly, we also observed a positive correlation between PRIM2 with helper Th2 cells, activated CD4 T cells, and neutrophils in most tumors, indicative of complex regulation of tumor immune microenvironment. The subunit is the main component of the proteasome and plays an important role in the cell cycle, DNA replication, and signal transduction. Amongst all the subunits, proteasome 26S subunit ATPase 2 (PSMC2) and the proteasome 26S subunit ATPase 6 (PSMC6) are related to various tumors, such as breast cancer 50, gallbladder cancer 51, prostate cancer 52, lung adenocarcinoma 53. Non-ATPases 1 (PSMD1) and 3 (PSMD3) are also involved in chronic myeloid leukemia 54. Our results show that PRIM2 significantly correlates with proteasome subunit-related genes, suggesting that it may promote tumorigenesis through synergistic action with proteasome subunits and is positively correlated with most antigen presentation-related genes. PRIM2 also positively correlated with chemokines, chemokine receptors, and HLA-related genes. Interestingly, a positive correlation between PRIM2 and major immune checkpoint genes was observed in ACC, BLCA, BRCA, PAAD, and UVM. These results suggest that PRIM2 may help tumors evade immune surveillance.

GO and GSEA analysis of the top ten tumors with high mortality and incidence shows the involvement of PRIM2 in the cell cycle, DNA replication, chromatin replication, etc., suggesting that the aberrant expression of PRIM2 may promote the occurrence of tumors by altering various pathways.

Further NCI-60 database was used to study the correlation between PRIM2 and drug sensitivity. Results revealed that an increase in PRIM2 expression positively correlated with the drug sensitivity of fenretinide, nelarabine, dexamethasone, curcumin, fludarabine, and 6-mercaptopurine. Thereby indicating that PRIM2 can be used as a drug target.

The discovery of the PD-L1 immune checkpoint has provided insight into immunotherapy for solid tumors 55. However, some tumors still do not respond to immunotherapy. Our study reveals that the expression of PRIM2 correlates with the prognosis of patients treated with anti-PD-L1 and the response to anti-PD-L1 therapy. It is well established that tumor mutation burden and neoantigen load can alter immunotherapy response 56–58. Our results show that patients with high expression of PRIM2 have a better prognosis in anti-PD-L1 immunotherapy and have higher tumor mutation burden and neoantigen load. Thus, it is tempting to postulate that expression of PRIM2 sensitizes the tumor to immunotherapy.

This study comprehensively analyzes the PRIM2 expression in different tumors from multiple angles, dimensions, and prognosis. We have also established a correlation between PRIM2 with immune genes, immune cells, drug sensitivity, TMB, neoantigen load, and immunophenotypes, which makes PRIM2 potential targets for immunotherapy. The TIDE score was higher in PRIM2 low-expression group in most tumors, suggesting its role in immune escape. However, these discrepancies could be due to the complexity and diversity of the microenvironment in vivo. Hence, additional algorithms or experiments are required to understand the role of PRIM2 in cancer.
In short, the results reveal the clinical characteristics, prognosis, and correlation between immune cells and immune-related genes with abnormally expressed \textit{PRIM2} in various tumors. However, the samples used in this study were retrieved from publicly available databases, which may impact the results. The function and mechanism of \textit{PRIM2} need to be further verified \textit{in vivo}, \textit{in vitro}, and in clinical samples.

\section*{References}


**Figures**

![Figure 1](image_url)

**Figure 1**

PRIM2 expression levels in different data sets. A: Difference of PRIM2 expression in 18 tumors with paired data on cancer and paracancerous in TCGA. B: The difference in PRIM2 expression after combining GTEX normal tissues, TCGA tumors, and adjacent tissues. C: The expression level of PRIM2 in the GTEX dataset. D: PRIM2 expression levels in different tumor cell lines. *p<0.05, **p<0.01, ***p<0.001.
Figure 2

Figure 3

Cox analysis of PRIM2 in different tumors. A: The forest plot shows the Cox regression analysis to compare PRIM2 in different tumors and overall survival (OS). B: The forest plot shows the cox method to compare PRIM2 in different tumors and Disease-specific survival (DSS). C: The forest plot shows the Cox regression analysis to compare PRIM2 in different tumors and disease-free intervals (DFI). D: The forest plot shows the Cox regression analysis to compare PRIM2 in different tumors and progression-free interval (PFI).
Figure 4

Correlation between PRIM2 expression level, tumor mutation burden (TMB), and microsatellite instability (MSI) in different tumors. A: The relationship between PRIM2 and TMB. Blue dots represent no correlation, red dots represent correlation, and the number of dots represents how much correlation. B: The relationship between PRIM2 and MSI. Blue dots represent no correlation, red dots represent correlation, and the number of dots represents how much correlation.

Figure 5

The correlation between PRIM2 and DNA methyltransferase is in different data sets. A: The data from Genotype Tissue Expression (GTEx), A dot represents a type of normal tissue. B: In the Cancer Genome Atlas (TCGA), A dot represents a tumor.
Figure 6

Figure 7

The correlation between PRIM2 and immune cells in different tumors is based on four different methods of immune infiltration. A: CIBERSORT. B: xCell C: ssGSEA D: MCPcounter. Red represents positive correlation; purple expressed negative correlation. *p<0.05, **p<0.01, ***p<0.001.
Figure 8

Correlation between PRIM2 and immune-related genes in different tumors. *p< 0.05, **p < 0.01, ***p< 0.001, ****p < 0.0001.
Figure 9

PRIM2’s GO analysis is among the top 10 tumors in the latest morbidity and mortality. A: LUAD (Lung adenocarcinoma), LIHC (Liver hepatocellular carcinoma), STAD (Stomach adenocarcinoma), BRCA (Breast invasive carcinoma), COAD (Colon adenocarcinoma), ESCA (Esophageal carcinoma), PAAD (Pancreatic adenocarcinoma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), CESA (Cervical squamous cell carcinoma and endocervical adenocarcinoma)
Figure 10

Figure 11

Correlation analysis between *PRIM2* expression value and drug sensitivity. The method uses the Spearman rank’s correlation test; $p < 0.05$ is considered statistically significant.
Figure 12

Analysis of the correlation between PRIM2 and immunotherapy. A: Survival curve of PRIM2high and low expression group. B-C: Ratio of high and low PRIM2 expression group to immunotherapy response. CR, complete response; SD, stable disease; PR, partial response; PD, progressive disease. D-E: The relationship between high and low PRIM2 expression groups and tumor mutation burden and neoantigen burden.
Figure 13


**Supplementary Files**

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- FigureS1.tif
- FigureS2.tif
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