The importance of BUD13 in the prognosis of hepatocellular carcinoma revealed by pan-cancer analysis

Han He, Zhiyuan Qiu*

Department of Oncology, the Affiliated People’s Hospital of Jiangsu University, Zhenjiang, Jiangsu, China

Introduction

In both developed and developing nations, cancer has protruded as a major factor affecting morbidity and mortality (1). Worldwide, the leading cause of cancer mortality among various malignancies is liver hepatocellular carcinoma (LiHC) (2). Worldwide, hepatocellular carcinoma has the sixth and third highest incidence and mortality rates, respectively (3). With a 5-year survival rate of 18%, LiHC is the third most lethal cancer after pancreatic cancer and colorectal cancer. If diagnosed at an early stage, surgical excision can provide a better prognosis with a 5-year survival rate of more than 70% (4). The etiology and molecular mechanism of liver hepatocellular carcinoma are still unclear, and its development is a multifactorial and multistep complex process. Hepatocellular carcinoma is linked with a variety of risk factors, comprising non-alcoholic fatty liver, hepatitis B or C virus infection, chronic alcoholism and smoking, etc. (5, 6). In developing areas, such as China and India, chronic hepatitis B virus (HBV) infection and aflatoxin B1 (AFB1) consumption are the primary risk factors (7, 8). In developed regions, LIHC mainly develops from cirrhosis caused by hepatitis C virus (HCV) and non-alcoholic fatty liver disease (NAFLD) (9, 10). Since LIHC is a heterogeneous disease with widely varying clinical outcomes, efficient biomarkers are required for guiding treatment, anticipating relapse, and determining prognosis. To date, there are no widely accepted molecular markers of hepatocellular carcinoma aggressiveness. Over the past 40 years, levels of AFP have been utilized to diagnose hepatocellular carcinoma and predict its treatment response. In the landmark randomized controlled study of the Chinese hepatitis B population (n=18,816), the utilization of alpha-fetoprotein (AFP) and abdominal ultrasound every 6 months as a screening approach was claimed to reduce mortality by 30% (11). Even so, levels of AFP are affected by cancer stage and tumor size and are unreliable in clinical applications (6). Additionally, AFP lacks sensitivity and specificity to monitor or detect liver cancer effectively (12), so a new molecular marker is needed to guide clinical practice.

BUD13, also known as Cwc26 or ISAP71, is primarily located in the nucleoplasm and is involved in biological processes related to the splicing of intracellular RNA as an RNA binding protein (RBP). RNA splicing is a vital process involved in several aspects of cell proliferation, differentiation and survival, and taking in consideration the necessity of RNA splicing in gene regulation, modification in this pathway has been linked with various human cancers (13). To regulate gene expression in eukaryotic
cells, a sophisticated and extensive post-transcriptional machinery is utilized. Within these processes, splicing of precursor mRNA (pre-mRNA) has a crucial role in increasing cellular proteome complexity (14). Furthermore, RNA splicing is a very sophisticated and delicate process that is vulnerable to alteration throughout tumorigenesis. Somatic mutations of spliceosomal proteins or dysregulated expression of RBP splicing factors can result in misspliced mRNA transcripts in cancer cells, further promoting cancer cell development (15). Therefore, we can target malignant tumors by cancer cells misusing this vulnerable feature of RNA splicing.

In current study, the correlation between expression of BUD13 and clinical features was assessed in accordance with 33 cancers, including around 15,000 patients. Drug sensitivity analysis was then performed by mutation analysis, gene expression analysis, gene set enrichment analysis (GSEA), prognostic correlation analysis, immune infiltration, gene set variation analysis (GSVA) and LIHC dataset according to downloads from The Cancer Genome Atlas (TCGA). In compliance with our data, BUD13 has a vital prognostic role in hepatocellular carcinoma and warrants further investigation in clinical trials.

Materials and Methods

TCGA data acquisition and variance analysis
The TCGA database, the greatest accessible database of cancer genetic information, encompasses data comprising gene expression data, copy number variants, SNPs, etc. (https://portal.gdc.cancer.gov/). For further analysis, the SNP data and raw mRNA expression of 33 pan-cancer tumors were obtained. GTEx database was utilized for downloading gene expression data from various tissues (https://commonfund.nih.gov/GTEx), merged with data of TCGA, and adjusted to determine the variations in gene expression in various cancer species. Every tumor cell line's data was obtained from the CCLE database (https://portals.broadinstitute.org/ccle/), and data were analyzed for gene expression levels in these tumor tissues in accordance with tissue origin. Furthermore, the relationship between tumor stage and expression was investigated.

Prognostic correlation analysis
The Xena database was accessed for information on the overall survival (OS) and progression-free survival (PFS) of TCGA individuals for investigating the association between gene expression and patient prognosis. Survival analysis (p < 0.05) for each type of cancer was conducted utilizing the Kaplan-Meier method, and survival analysis were evaluated utilizing the "survivor" and "survminer" packages. Furthermore, the Cox analysis utilized the "surviv" and "forestplot" packages for exploring the association between gene expression and survival.

Immune cell infiltration analysis
RNA-seq data from 33 cancer sufferers in various subgroups were evaluated utilizing the CIBERSORT algorithm, and utilized to conclude the immune infiltrating cell's relative proportions and to correlate gene expression with immune cell composition. Additionally, the TISIDB website determined potential relationships between gene expression and immune modulators (chemokines, immunosuppressants, immunostimulatory factors and MHC molecules, etc.).

Drug sensitivity analysis
The Cellminer database depends on 60 cancerous cells specified by the National Cancer Institute's Center for Cancer Research (NCI), including the NCI-60 cell line being the most often utilized cancer cell sample population for examining anticancer medications. Among these investigations, downloading RNA-seq gene expression data and NCI-60 drug sensitivity data, then using correlation analysis to analyze the association between genes and susceptibility to common antitumor medicines. P < 0.05 was deemed significant.

GSVA enrichment analysis
Gene set variation analysis (GSVA) is a non-parametric non-supervised technique for estimating the transcriptomic gene sets enrichment. GSVA turns gene-level alterations into pathway-level changes via scoring the gene sets synthetically, and afterward identifies samples biological functions. Among these investigations, the molecular signatures database (v7.0) will be utilized for downloading gene sets, and the GSVA algorithm will be utilized for scoring each gene set for estimating the potential biological functional alterations of various samples.

GSEA enrichment analysis
Utilizing predefined gene sets, GSEA analysis ranks genes in accordance with their differential expression in two sample types and afterward determines if the predefined gene set is enriched at the bottom or top of this ranking table. Among these investigations, GSEA was conducted with the "clusterProfiler" and "enrichplot" packages to evaluate the probable molecular mechanisms behind the variations in prognosis among individuals with 33 tumor via comparing variations in signaling pathways among both high and low gene expression groups. In this investigation, we explored the probable molecular mechanisms behind the differences in prognosis among individuals with 33 tumors via comparing the variations in signaling pathways among both low and high expression groups.

TMB, MSI data analysis
All somatic gene coding errors, insertions, base substitutions, and deletions observed per million bases were defined as TMB. In this investigation, TMB was estimated by calculating the variants/exon length number and variants frequency for each tumor sample, dividing the protein-coding region total length by the nonsynonymous mutation sites. MSI values for every TCGA individual were acquired from previously reported studies (16).

Nomogram model construction
The nomogram is based on a multifactorial regression analysis, in compliance with gene expression and clinical symptoms, and then, the interrelationships among the variables in the prediction model are expressed utilizing scaled line segments that are drawn on the same plane at a specific scale. A multifactor regression model was constructed, and the predictive value was calculated by assigning a score to each value level of each influencing factor in compliance with contribution degree of each influencing factor in the model to the outcome variable (magnitude of the regression coefficient), and the individual scores were
then added up to get the total score.

Statistical analysis
R language was utilized for conducting all statistical analyses (version 4.0). Utilizing univariate survival analysis, Hazard Ratios (HRs) and 95% confidence intervals was estimated. For investigating patient survival in accordance with high or low gene expression levels, Kaplan-Meier was utilized. P<0.05 regarded statistically significant, and all statistical tests were two-sided.

Results
Pan-cancer expression analysis of BUD13 gene
BUD13 expression analysis in 33 human cancers was carried out through the TCGA and GTEx datasets usage. In compliance with results, the gene was substantially expressed in 23 tumors, comprising ACC, BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, LAML, LGG, LIHC, LUSC, OV, PAAD, PRAD, SKCM, STAD, TGCT, THCA, UCS (Fig1A-B). In the majority of normal tissues, BUD13 expression levels were low relative to cancer tissues. The BUD13 expression in several tumor cell lines in the CCLE expression profile (Fig1C). Moreover, BUD13 was associated with a variety of tumor stages, including ACC, LIHC, THCA, TGCT, SKCM, and KIRC (Fig1D-I).

Association between BUD13 expression and prognosis of cancer patients
The associations between BUD13 expression and cancer patient prognosis utilizing survival indicators comprising OS and PFI were assessed. According to our results, BUD13 expression was strongly correlated with OS in 7 cancer sufferers, comprising ACC, KIRC, LGG, LIHC, READ, THYM, and UCS tumors (Fig2A); in addition, according to KM-plot survival analysis results, BUD13 was related to poor OS in 2 cancers, comprising: ACC, LIHC (Fig2B). BUD13 expression was closely related to PFI in 6 cancer sufferers, comprising ACC, GBM, KIRC, LIHC, PRAD, UCS tumors (Fig2C), where BUD13 was related to poor PFIs, comprising ACC, LIHC, and PRAD according to Kaplan-Meier survival analysis results (Fig2D). The nomogram prediction model was constructed according to clinical symptoms and BUD13 gene expression, and their results by regression analysis were shown as column line plots, where logistic regression analysis results demonstrated that the gene expression of BUD13 contributed more to the model prediction efficacy in the LIHC sample (Fig2E); moreover, this investigation simultaneously plotted three-year and five-year correction curves. Moreover, three-year and five-year correction curves were plotted in this investigation, and the model effects were more consistent (Fig2F).
Pan-cancer expression and immune infiltration

The majority of tumor microenvironment comprised immune cells, tumor-associated fibroblasts, specific physicochemical features, numerous growth factors, inflammatory factors, extracellular matrix and cancerous cells, etc. The tumor microenvironment has a great impact on survival outcome, diagnosis, and clinical treatment sensitivity of tumors. According to our results, BUD13 expression was strongly related to immune infiltration, with 9 cancers considerably associated with T cells follicular helper cells, 13 cancers considerably associated with T cells CD8 cells, and 11 cancers considerably associated with T cells CD4 memory activated cells (Fig3A). In LIHC, further analysis of the TME was done, and the results showed that Immune_Checkpoint, TMEscoreA, Mismatch_Repair, Nucleotide_excision_repair, DNA_damage_response, DNA_replication, Base_excision_repair, Pan_F_TBR, EMT3, and TMEscoreB were considerably associated with LIHC (Fig3B).

Pan-cancer expression and key regulatory genes

Among these investigations, additional gene co-expression analysis was carried out for investigating the relation between BUD13 expression and 33 tumor immune-related genes. MHC, immunological activators, immunological suppressors, chemokines, and chemokine receptor proteins were among the genes analyzed. Almost all immune-related genes were substantially related to BUD13, according to the findings (Fig4A). Moreover, BUD13 was substantially associated with common tumor-related regulatory genes, including TNFA SIGNALING, BETA SIGNALING, TGF, iron death-related genes, hypoxia, DNA repair, autophagy genes, and pyroptosis (Fig4B).

Pan-cancer expression with TMB and MSI

TMB and MSI are novel biomarkers associated with immunotherapeutic response. Among these investigations, the association between BUD13 expression and TMB was examined. According to findings, the BUD13 expression level was significantly related to TMB across tumors, comprising in LUAD, THCA, HNSC, LUSC, COAD, STAD, LIHC, TGCT, and UCEC (Fig5A). In MSI, gene BUD13 was significantly different in BLCA, COAD, HNSC, SARC, UCEC, and PRAD (Fig5B).

Pan-cancer expression and drug sensitivity

Surgery combined with chemotherapy effectively treat early-stage tumors. We investigated the relationship between the BUD13 gene and common antitumor medications utilizing the Cellminer database and discovered that high BUD13 gene expression was expected to be related to tolerance to a variety of antitumor medications. Among
them, BUD13 was positively correlated with XK-469, Ifosfamide, 8-Chloro-adenosine, Karenitecin, Topotecan, 7-Ethyl-10-hydroxycamptothecin, LMP-400, and with Selumetinib, Trametinib, Cobimetinib (isomer 1), Vemurafenib, Dabrafenib, Tanespimycin, and Mithramycin drugs negatively (Fig 6).

**Pan-cancer expression and GSV A/GSEA**

For investigating the molecular mechanism of BUD13 gene in pan-cancer deeply, firstly, all tumors with GSV A were scored, and then in each tumor independently, the samples were categorized into two groups of high and low expression utilizing the median gene expression for comparing two groups. The findings revealed that in liver hepatocellular carcinoma, elevated BUD13 expression was mostly concentrated in G2M_CHECKPOINT, PI3K_AKT_MTOR_SIGNALING, E2F_TARGETS, UNFOLDED_PROTEIN_RESPONSE, DNA_REPAIR and other signaling pathways (Fig 7A). We also performed GSEA analysis in BUD13 and LIHC (Fig 7B).

**Discussion**

Among these investigations, a comprehensive pan-cancer analysis was used to expose BUD13 differences in cancer and paraneoplastic tissues by analyzing database data of different cancers and cancer cells, and the expression of BUD13 in different cancer species was analyzed in conjunction with relevant clinical data and bioinformatics databases. Our results showed that BUD13 is significantly expressed in various malignancies, and its expression level is strongly correlated with the progression, stage, and several tumor prognosis. Owing to TME's importance in malignancy, the association between BUD13 and immune cell infiltration requires additional analysis. We discovered an association between BUD13 expression and immune-related regulatory genes, cancer immunity, MSI and TMB. Then, GSVA and GSEA analyses were conducted for elucidating the underlying hepatocellular carcinoma mechanisms and exploring the correlation between BUD13 genes and common antitumor medications to provide relevant data for subsequent drug treatment options for hepatocellular carcinoma. Moreover, we developed a column line graph that can be utilized to assess the survival probability of hepatocellular carcinoma patients regarding clinical characteristics, including age, stage, and BUD13 expression. Our investigation revealed that BUD13 was significantly expressed in 23 malignancies and poorly expressed in 2 malignancies. Our finding of significantly increased expression of BUD13 in hepatocellular carcinoma suggests that BUD13 may have a crucial role in the hepatocellular carcinoma progression and development, opening up novel perspectives for antitumor therapy. It is well recognized that a critical analysis of parameters associated with survival and prognosis can assist patients and physicians in therapeutic decisions (17). Hence, we also investigated the relation between BUD13 expression and survival. Elevated BUD13 expression was linked with a bad prognosis and short survival periods in ACC, LIHC, and PRAD. Elevated BUD13 expression seems to be a protective factor for UCS, THYM, READ, KIRC, and according to our study. Nevertheless, the specific mechanism elucidation requires further study. Briefly, BUD13 pan-cancer analysis is of considerable value in identifying the differential expression and BUD13 role in many cancer types.

The tumor microenvironment (TME) is the living environment where tumor cells proliferate and metastasis in deep tissues, comprising surrounding blood vessels, immune cells, extracellular matrix (ECM), fibroblasts, signaling molecules, and has a crucial role in progression of tumor(18-21). Tumors and their surrounding microen-
environment are strongly linked and interact continually with each other. Tumors may influence the microenvironment via releasing extracellular signals, enhancing tumor angiogenesis, and promoting peripheral immunological tolerance, whereas immune cells in the microenvironment can influence cancer cell growth and evolution(22, 23). Immune cells are the body's most vital defensive mechanism. The immune system is composed of many immune cells that inhibit pathogen invasion or infection and eliminate cancerous or damaged cells(24, 25). T cells, MDCs, regulatory B cells (Breg), DCs, regulatory T cells (Treg), NK cells, and macrophages are among the immune cells occurring in the tumor microenvironment. Essential immunological effector cells in the immune system, tumor-infiltrating L lymphocytes could be categorized into CD8+ T cells (cytotoxic T cells) and CD4+ T cells (helper T cells). These cells are capable of secreting antitumor cytokines comprising interferon-γ (IFN-γ), tumor necrosis factor-β (TNF-β), interleukin-17 (IL-17), and interleukin-2 (IL-2) (26). Bregs and Tregs are immunosuppressive immune cells that release IL-10, IL-35, and transforming growth factor-β (TGF-β) to inhibit T lymphocyte's immunological response, hence preventing damage induced by T cell overactivation. Approximately 10% of peripheral lymphocytes are NK cells, which are widely distributed in lymph nodes, spleen, peripheral blood, and bone marrow. However, under the chemokine's induction, NK cells can migrate to areas of inflammation. NK cells' primary role is to induce cytotoxicity. When activated, NK cells can release IFN-γ, TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) for exerting antitumor effects (27).

However, most of the studies on BUD13 currently stay in the metabolic syndrome, for example, a considerable number of articles describe the association of BUD13 variants with hyperlipidemia, triglyceride abnormalities, coronary heart disease, etc. (28-30). The association of BUD13 with cancer has only been reported in relation to diffuse large B lymphoma, which positively upregulates fibronectin 1 (FN1) via the DBH-AS1/BUD13/FN1 axis and hence promoting the diffuse large B lymphoma proliferation, migration, and invasion (31). Hence, few studies investigating the correlation between BUD13 and immune cell infiltration in the tumor microenvironment. In our analysis, our findings revealed that BUD13 expression was in close relation to immune infiltration, with 9 cancers considerably associated with T cells follicular helper cells, 13 cancers considerably associated with T cells CD8 cells, and 11 cancers considerably associated with T cells CD4 memory activated cells. Additionally, our analysis indicated a correlation between BUD13 expression levels in hepatocellular carcinoma and four distinct kinds of immune infiltrating cells (B memory cells, follicular helper T cells, resting dendritic cells, and M2 type dendritic cells). No systematic molecular mechanism explaining the link between BUD13 and immune cells in hepatocellular carcinoma, which is creative and merits more study. Our study elucidates additional evidence that BUD13 has wider tumor applicability and verifies that BUD13 expression is strongly associated with immune cells and immune-related molecule biological processes in the majority of cancers. In addition, our study demonstrated that BUD13 was significantly co-expressed with almost all immune-related genes (chemokines, MHC, immune activators, immune suppressors and chemokine receptor proteins) and common tumor-related regulatory genes (autophagy-related genes, iron death-related genes, focal death-related genes, DNA repair genes, hypoxia-related genes). The previous results suggest that BUD13 may have a crucial role in TME, influencing patient's prognosis and might be implicated in cancer development, particularly LIHC.

The idea that MSI may lead to activation of proto-oncogenes or loss of oncopgenes and consequently to carcinogenesis dates back to 1993 when mismatch repair gene defects were found to be highly associated with hereditary nonpolyposis colorectal cancer (32-34). MSI is categorized as MSI-H, MSI-L, and MSS, and MSI also has been cited as a prognostic marker (35). Based on published studies, MSI is now considered an indicator to differentiate cancer types in COAD patients, and in addition, those with MSI-H in COAD show higher checkpoint inhibitor feedback and survival rates from early to advanced cancer stage (36). Programmed death ligand-1 (PD-L1) and tumor mutation burden (TMB) expression have protruded as the best biomarkers for immune checkpoint selection in a variety of tumors, including LUAD, COAD/READ, PRAD, and BRCA (37). In addition, a study found a significant correlation between blood TMB values and small cell lung cancer prognosis (38). In our study, both TMB and MSI in COAD were positively correlated with BUD13 expression, and significant differences were achieved for both MSI and TMB. This is congruent with Dzunic’s M et al. findings (36). In addition, BUD13 showed a significant positive correlation with both TMB and MSI in UCEC and COAD, suggesting that BUD13 may be a potential therapeutic target for the above diseases.

The Cellminer database was utilized to determine drug patterns and transcripts in the NCI-60 cell line in accordance with 60 cancerous cells specified by the National Cancer Institute (NCI) (39). In this study, we identified a total of up to 45 drugs that were associated with BUD13 expression, of which FDA-approved drugs showed a positive correlation in sensitivity with high BUD13 expression, and 7 drugs (Selumetinib, Trametinib, Cobimetinib (isomer1), Vemurafenib, Dabrafenib, Tanespimycin, Mitomycin) were negatively correlated with BUD13 expression. Our study suggests that the elevated BUD13 expression in cancerous cells may be deemed as a predictor of tumor cell sensitivity or resistance to antitumor drugs, providing efficient support for further clinical applications or basic research.

Next, we conducted GSEA and GSVA analyses on hepatocellular carcinoma patients to explore the potential biological mechanisms of BUD13 in hepatocellular carcinoma. Our enrichment analysis showed that BUD1 might affect the etiology or pathogenesis of hepatocellular carcinoma through G2M CHECKPOINT, PI3K AKT_MTOR_SIGNALING, E2F_TARGETS, UNFOLDED_PROTEIN_RE-SPONSE, DNA_REPAIR and other signaling pathways. According to findings, BUD13 is inextricably linked to cell cycle regulation, proliferation and differentiation at the cellular level, and transcription, translation, and repair of genetic material at the molecular level.

To my knowledge, the current investigation is the initial pan-cancer analysis of BUD13, indicating that BUD13 has a crucial role in tumorigenesis and progression and is anticipated to be an essential prognostic marker for certain
malignancies. This investigation solves the debate about whether BUD13 expression is high or low in various malignancies. Moreover, these study findings suggest that BUD13 plays a crucial role in tumor immunity, suggesting a novel direction for tumor research. Even so, this study still has drawbacks. Our investigation depended on bioinformatics analysis, and the findings would be more persuasive if paired with experimental validation, including immunohistochemistry or prospective studies of large clinical samples. Moreover, according to our findings, BUD-13 was a protective factor for a specific cancer subset whereas a risk factor for other cancers subset, whereas BUD-13 action mechanism in various malignancies requires additional exploration.

In summary, our pan-cancer analysis revealed the BUD13 characterization in tissues and cell lines. Furthermore, BUD13 expression is related to prognosis and risk of several malignancies. In compliance with this study’s findings, BUD13 expression is related to immune infiltration and is a potential marker for TME. Moreover, in several cancer types, BUD13 expression was related to TMB, MSI, and antitumor drug sensitivity. Multiple signaling pathways and biological processes in hepatocellular carcinoma may encompass BUD13, according to functional bioinformatics analysis. Eventually, a risk factor model was discovered for predicting OS in hepatocellular carcinoma according to BUD13 expression in combination with the underlying clinical features. In conclusion, this investigation findings may clarify the BUD13 role in tumorigenesis and progression.

Conflicts of interest
The authors confirm that there are no conflicts of interest.

References
3. International Agency for Research on Cancer


