A Pan-Cancer Analysis of the Immunological and Prognostic Role of BUB1 Mitotic Checkpoint Serine/Threonine Kinase B (BUB1B) in Human Tumors

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SUMMARY

Background: BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) is a member of the spindle assembly checkpoint family and is related to cancer disease progression, invasion, metastasis, and functional promotion of angiogenesis. Several studies have noted that the BUB1B gene is frequently upregulated in various types of cancers. However, the expression patterns of BUB1B across different cancer types and its diagnostic and prognostic potential have not been investigated from a pan-cancer perspective.

Methods: The Cancer Genome Atlas (TCGA) data were used to explore the diagnostic and prognostic immunological potential of BUB1B in 33 cancer types.

Results: BUB1B was almost universally upregulated across all cancers, with increased protein expression in at least six cancer types and an enhanced phosphorylation level of S670 in two cancer types. Furthermore, BUB1B expression was negatively associated with clinical progression and prognosis in most cancers. BUB1B expression was positively associated with tumor mutational burden and microsatellite instability in 17 and 7 cancer types, respectively, and there was a correlation between BUB1B expression and DNA methylation at multiple probes in 30 cancer types. Additionally, a positive relationship existed between BUB1B expression and the infiltration levels of Th2, Tcm, and T helper cells, whereas BUB1B showed a negative correlation with the infiltration levels of other immune cells in multiple cancers. Moreover, functions associated with cell cycle progression and ubiquitin-mediated proteolysis were involved in the functional mechanism of BUB1B.

Conclusions: Our pan-cancer study offers a comprehensive understanding of the role of BUB1B in tumorigenesis and tumor immunity across different types of cancer.

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INTRODUCTION
BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B), also known as BUBR1, has been extensively studied for nearly 30 years. Initially, it was noted for its role in the spindle checkpoint function [1]. Structurally, BUB1B contains two conserved domains: CD1 and CD2. CD1 directs kinetochore localization and binding to other BUB proteins, whereas CD2 encodes the contained kinase domain for the phosphorylation function [2]. Research on BUB1B has traditionally focused on normal mitotic progression, as it prevents cells from prematurely entering anaphase. Over the years, BUB1B has been found to play a remarkable number of regulatory roles at the cellular level in pathways such as DNA damage response [3], immunity [4], apoptosis [5], proliferation [6], and angiogenesis [7]. BUB1B functionality often complements classical tumor properties that lead to malignant proliferation, invasion, and immune system avoidance. Considering these qualities, BUB1B plays a major role in carcinogenesis [8]. Interestingly, BUB1B was also recently reported associated with the development of psoriasis [9], although further studies are required to validate this finding.

Dysregulation of BUB1B expression has been reported in numerous studies at the RNA and protein levels for various tumor types [10,11]. Additionally, BUB1B dysregulation is associated with poor prognosis—a trend noted in lung [12], renal [10], breast [13], pancreatic [14], and liver cancer [15]. In summary, research evidence suggests that BUB1B expression is altered in multiple human cancers and that its expression can impact patient prognosis. Therefore, we used data from The Cancer Genome Atlas (TCGA) to comprehensively examine the difference in the RNA expression of BUB1B in different types of cancer. By investigating data from 33 TCGA cancer types, we aimed to identify patterns of BUB1B dysregulation to investigate the potential molecular mechanism of BUB1B in the pathogenesis or clinical prognosis of different types of cancer.

MATERIALS AND METHODS
Gene expression analysis
In the TCGA data portal (https://tcga-data.nci.nih.gov), BUB1B transcript expression analysis is available for 33 tumor types. Gene expression data were utilized after deleting missing and duplicated results and then transforming the remaining data by log2(TPM+1). Analyses were conducted using R software (Version 4.0.5; https://www.r-project.org), and box plots were drawn using the R package “ggpubr.” The expression levels of the total protein or phosphoprotein (with phosphorylation at S670 and S543) in BUB1B (NP_001202.4) were then analyzed between the primary tumor and normal tissues using the UALCAN portal (http://ualcan.path.uab.edu/analysis-prot.html), an interactive web resource for analyzing cancer omics data.

Analysis of the relationships between BUB1B, prognosis, and clinical phenotype
Clinical phenotype and survival data were extracted for each sample obtained from TCGA. Three indicators were selected to study the relationship between the patient’s prognosis and BUB1B expression: overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI). Survival analyses were performed using the R packages “survival” and “survminer”. Additionally, to determine the pan-cancer relationship between BUB1B expression and survival, Cox analysis was conducted using the R packages “survival” and “forestplot.” Moreover, the correlation between BUB1B expression and clinical phenotypes, including tumor stage and age, was evaluated using the R-packages “limma” and “ggpubr.”

Correlation of BUB1B expression with tumor mutation burden, tumor microsatellite instability, and mismatch repair gene expression
The potential correlation between BUB1B expression, tumor mutation burden (TMB), and tumor microsatellite instability (MSI) was evaluated using the SangerBox website (http://sangerbox.com/Tool). The correlation analysis was performed using Spearman’s rank correlation test.

DNA mismatch repair, also known as MMR, is a multi-protein intracellular process for recognizing and repairing non-native DNA structures. The expression profile data of TCGA were used to determine the correlation between BUB1B expression and the expression level of MMR genes, including epithelial cell adhesion molecule (EPCAM), MutL homologous gene (MLH1), MutS homologous gene (MSH2), MSH6, and increased separation after meiosis (PMS2) in different cancers. The data were visualized through heat maps generated using the R packages “reshape2” and “RColorBrewer.”

Correlation of BUB1B expression with DNA methylation
The DNA methylation levels of BUB1B in multiple probes were obtained for different tumors using the illumine human methylation 450 platform. The correlation between BUB1B expression and gene methylation of multiple probes was evaluated using Pearson’s correlation test. Next, the correlation between BUB1B methylation and prognosis was evaluated via Kaplan–Meier survival analysis using the MethSurv online tool (https://biit.cs.ut.ee/methSurv/) [16].

W. Huang et al.
Relationship between BUB1B expression and immunity

We used the R package “GSVA” to conduct a Single Sample Gene Set Enrichment Analysis (ssGESA) to explore the association between BUB1B expression and the infiltration enrichment of 24 common immune cells, including dendritic cell (DC), activated DCs (aDCs), immature DCs (iDCs), plasmacytoid DC (pDC), macrophages, mast cells, neutrophils, B cells, CD8 T cells, cytotoxic cells, eosinophils, natural killer (NK) cells, NK 56bright cells, NK 56dim cells, T central memory (Tcm), T follicular helper (Thf), T effector memory (Tem), T gamma delta (Tgd), type 1 Th cells (Th1), type 2 Th cells (Th2), type 17 Th cells (Th17), T cells, T helper cells, and regulatory T cells (Treg). Then, the relationship between BUB1B and immune infiltration was estimated using Pearson’s analysis and the Wilcoxon rank sum test.

Additionally, the correlation between co-expressed BUB1B and immune-related genes, including genes encoded chemokine receptors, chemokines, immunosuppressive, major histocompatibility, and immune activation proteins, were analyzed using R software, and the R package “ggplot2” was used to visualize the results.

BUB1B-related gene enrichment analysis

The STRING website was used to construct a protein-protein interaction network (https://string-db.org/). Then, the top 100 BUB1B-correlated targeting genes among the datasets of all TCGA tumors and normal tissues were obtained using GEPIA2 (http://geopia2.cancer-pku.cn).

A Venn diagram analysis was conducted for the BUB1B-binding and interacting genes using the Venn Diagram R package. We combined two datasets to perform a Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis and gene ontology (GO) enrichment analysis by using the R package “clusterProfiler” and the org.Hs.evg.db annotation package (ver 3.10.0). The R package “ggplot2” was used to visualize the enriched pathways.

RESULTS

Gene expression analysis data

To investigate the BUB1B mRNA expression patterns, data from TCGA database were analyzed across various cancer types and then compared to the data on matched normal tissues in TCGA database. As shown in Figure 1A, BUB1B transcription was significantly higher in adenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal papillary cell carcinoma (KIRC), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinoma (UCS). However, we did not obtain a significant difference for sarcoma (SARC) and non-tumor; in addition, reduced BUB1B expression was observed in testicular germ cell tumors (TGCT) and myeloid leukemia (LAML). In addition, statistical tests of mesothelioma (MEMO) and uveal melanoma (UVM) were unavailable due to a lack of sufficient data. Next, we found that BUB1B total protein is highly expressed in LIHC, GBM, HNSC, LUAD, UCEC, and BRCA (Figure 1B). Furthermore, the S670 of BUB1B exhibits a higher phosphorylation level in LUAD and HNSC, followed by an increased phosphorylation level of S543 in HNSC (Figure 1C).

Prognostic value of BUB1B in cancers

We further investigated the association between BUB1B expression and the prognosis of patients with different tumors, including OS, DSS, and PFI. Cox proportional hazards model analysis showed that highly expressed BUB1B was associated with a poor prognosis for cancers of LIHC, stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinoma (UCS). Furthermore, Kaplan–Meier survival analysis also demonstrated that patients with high levels of BUB1B experienced shorter OS in ACC, BLCA, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PAAD, SARC, and UCEC, whereas low BUB1B expression was related to poor OS prognosis for THYM and READ (Figure S1A).

Consistent with OS data, high BUB1B expression was significantly associated with shorter DSS in TCGA cases of LIHC (p = 0.013), KIRC (p = 0.039), LUAD (p < 0.001), BLCA (p = 0.021), ACC (p < 0.001), KIRP (p = 0.005), LGG (p < 0.001), MESO (p < 0.001), PAAD (p = 0.001), SARC (p = 0.001), and UCEC (p = 0.016), except for READ (p = 0.044) and THYM (p = 0.016; Figure 2A). Similarly, Kaplan–Meier survival analysis also demonstrated that patients with high levels of BUB1B experienced shorter OS in ACC, BLCA, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PAAD, SARC, and UCEC, whereas low BUB1B expression was related to poor OS prognosis for THYM and READ (Figure S1A).

We also demonstrate that patients with high BUB1B expression exhibited a significantly shorter PFI compared to patients with low BUB1B expression in LIHC (p < 0.001), KIRC (p = 0.001), LUAD (p < 0.001), BLCA.
Figure 1. The expression level of the BUB1B gene in different tumors.

A - BUB1B mRNA expression in 33 types of tumors. B - The total protein level of BUB1B between the normal tissues and primary tissues of LIHC, GBM, HNSC, LUAD, UCEC, and BRCA was analyzed using the CPTAC dataset. C - The expression level of BUB1B phosphoprotein (NP_001202.4, S670, and S543 sites) between the normal tissues and primary tissues of LUAD and HNSC was analyzed using UALCAN. * - p < 0.05, ** - p < 0.01, *** - p < 0.001.

Kaplan-Meier analysis showed that individuals with high BUB1B expression had poorer outcomes in ACC, BLCA, KIR, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PCPG, PRAD, SARC, THCA, UCEC, and UVM, whereas patients with GBM and high BUB1B expression had longer PFI (Figure S1C).
Correlation of BUB1B expression with advanced stages of cancer

Next, we examined the relevance of the tumor stage and found that BUB1B expression is associated with more advanced stages of various cancers, including ACC, BRCA, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, and UCEC. Strikingly, the most significant differences in BUB1B expression were between Stage I and Stage IV tumors. As shown in Figure 3A, BUB1B expression increased from Stage I to Stage IV in ACC, KICH, KIRC, KIRP, and LUAD; from Stage I to Stage III in KIRP, LIHC, LUAD, and UCEC; and from Stage I to Stage II in BRCA and LUSC. It is worth noting that low BUB1B expression was observed in SKCM when the pathological stage progressed from Stage I to Stage II and III in SKCM. Further analysis showed that younger patients had higher BUB1B expression levels compared with older patients in BRCA, ESCA, HNSC, KIRP, LIHC, THYM (Figure 3B). Moreover, male patients had predominant higher BUB1B expression than female patients in HNSC, LUAD, LUSC and LAML, while the opposite was observed in KIRP and SARC (Figure 3C).

Correlation of BUB1B expression levels with tumor mutation burden, tumor microsatellite instability, and mismatch repair genes

Subsequently, we analyzed the correlation between BUB1B expression and TMB/MSI across all tumors in TCGA data. As shown in Figure 4A, we observed that BUB1B was positively correlated with TMB for LGG (R = 0.34; p < 0.001), LUAD (R = 0.40; p < 0.001), COAD (R = 0.22; p < 0.001), LIHC, KIRC, COAD, LUSC, GBR, and THYM (R = 0.27; p < 0.001), STAD (R = 0.36; p < 0.001), KIRC (R = 0.11; p = 0.043), READ (R = 0.22; p = 0.038), PAAD (R = 0.39; p < 0.001),
Figure 3. Expression level of BUB1B in different cancer types in different pathological stages.

A - Based on TCGA data, the expression levels of BUB1B were analyzed at the main pathological stages (Stage I, II, III and IV) of ACC, BRCA, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, UCEC, and SKCM. Association between patient age (B), gender (C) and BUB1B expression.

PCPG (R = 0.15; p = 0.043), SKCM (R = 0.20; p = 0.043), ACC (R = 0.51; p < 0.001), KICH (R = 0.36; p = 0.002), and CHOL (R = 0.37; p = 0.003), but negatively correlated with TMB in THYM (R = -0.54; p < 0.001). BUB1B expression was also positively correlated with the MSI of LUAD (R = 0.33; p < 0.001), COAD (R = 0.18; p = 0.003), SARC (R = 0.29; p < 0.001), LIHC (R = 0.33; p < 0.001), LUSC (R = 0.31; p < 0.001), and BLCA (R = 0.10; p = 0.047; Figure 4B). The importance of TMB/MSI in predicting the response of immune checkpoint inhibitors of various cancer types has been widely reported [17]. We investigated whether BUB1B is also associated with MMR genes, including MSH2, MSH6, MLH1, PMS2, and EPCAM. We found a positive relationship between MMR gene expression and BUB1B levels in most tumor types, whereas LGG and THYM showed a negative association between BUB1B and EPCAM (Figure 4C).

Association between BUB1B DNA methylation and prognosis in pan-cancer

Specific gene DNA methylation is related to the stages of cancer progression and outcomes [18]. As shown in Figure S3, BUB1B was significantly hypermethylated in several cancer types for the TSS1500-N_shore-cg24584161 probe and significantly hypomethylated for other probes. In addition, we observed a significant negative correlation between BUB1B gene expression and DNA methylation in LIHC, KIRC, LUAD, CHOL, DLBC, MESO, OV, PAAD, PCPG, STAD, TGCT, and UCS, whereas a positive association was observed in
Figure 4. Correlation between BUB1B expression and tumor mutational burden (TMB), microsatellite instability (MSI), and mismatch repair (MMR).

We explored the potential correlation between BUB1B expression and TMB (A) and MSI (B) based on the different tumor types in TCGA data. C - Heatmap illustrating the correlation between BUB1B expression and MMR genes. The intensity of the grayscale color indicates the absolute value of correlation strength between variables. * - p < 0.05, ** - p < 0.01.

KIRP, GBM, THCA, THYM, and UVM (Figure 5A). Next, we assessed the association of BUB1B methylation levels at multiple probes with survival outcomes in multiple tumor types (Figure 5B). Interestingly, we found that TSS1500-N_shore-cg24584161 is significantly hypermethylated in KIRC, KIRP, MESO, LGG, STAD, UCEC, PAAD, LAML, and CESC, which indicates adverse prognosis although no differences in SARC, BLCA, and LIHC were found. However, compared with normal tissues, we observed a low methylation level of BUB1B in tumor tissues for other probes, which indicates a poorer prognosis. These results suggest that aberrant methylation is associated with changes in BUB1B expression and its effect on prognosis in multiple cancers.

Relationship between BUB1B expression and the infiltration levels of different immune cells
As prominent components of the tumor microenvironment, tumor-infiltration immune cells play a key role in tumor initiation, progression, and metastasis [19]. Our data demonstrate that BUB1B expression was significantly positively associated with the infiltration levels of Th2 and T helper cells in most types of tumors (Figure 6) but had a negative relationship with the infiltration levels of other immune cells.

To reveal the functional relationships between BUB1B expression and immune-related genes in 33 tumors, we conducted co-expression analyses of BUB1B with genes encoded immunosuppressive, chemokine receptors, major histocompatibility complex, chemokines, and immune activation proteins. Our resulting heatmap indicates that these immune-related genes were co-expressed with BUB1B and showed a significant positive association with BUB1B in six cancer types (LIHC, KIRC, LGG, PRAD, THCA, and UVM) but a significant negative association with BUB1B in three cancer types (GBM, LUSC, and SARC; Figure S4 A - E).
Figure 5. Association between BUB1B DNA methylation and gene expression on prognosis in different cancer types.

A - The visualization between the methylation level and BUB1B expression across all types of cancers in TCGA data. * - p < 0.05, ** - p < 0.01.

B - Forest plot of the survival analysis of BUB1B methylation at multiple probes for certain tumor types, including KIRC, KIRP, MESO, LGG, STAD, UCEC, PAAD, LAML, CESC, SARC, BLCA, and LIHC.
Enrichment analysis of BUB1B-related partners

To investigate the potential molecular mechanism of BUB1B in tumorigenesis, we conducted a series of pathway enrichment analyses to screen out the targeting BUB1B-binding proteins and the BUB1B expression-correlated genes. We used GEPIA2 to combine all tumor expression data of TCGA and obtained the top 100 genes that correlated with BUB1B expression. A total of 50 BUB1B-binding proteins were generated using the STRING tool, which is supported by experimental evidence (Figure 7A). As shown in Figure S5A, the BUB1B expression level was positively correlated with that of ARHGAP11A (Rho GTPase activating protein 11A; R = 0.73), BUB1 (BUB1 mitotic checkpoint serine/threonine kinase; R = 0.75), KIF11 (kinesin family member 11; R = 0.73), NUSAP1 (nucleolar and spindle associated protein 1; R = 0.75), and OIP5 (Opa interacting protein 5; R = 0.73). The resulting heatmap also shows a positive correlation between BUB1B and the abovementioned five genes in all detailed cancer types (Figure S5B). Moreover, we found six intersection genes, BUB1, CASC5 (Kinetochore scaffold 1), TTK (TTK protein kinase), CENPE (Centromere protein E), PLK1 (Polo-like kinase 1), and ZWINT (ZW10 interacting kinetochore protein), based on the intersection analysis between BUB1B-binding proteins and BUB1B expression-correlated genes (Figure 7B). Furthermore, we performed KEGG and GO enrichment analyses by combining two datasets. The KEGG data indicate that “Cell cycle” and “Ubiquitin-mediated proteolysis” might be involved in the effect of BUB1B on tumor pathogenesis (Figure 7C). The GO enrichment analysis data further suggest that most of these genes are linked to mitogenic pathways, such as microtubule binding, tubulin binding, condensed chromosomes, mitotic nuclear division, etc. (Figure 7D-F).
**DISCUSSION**

The observation of BUB1B upregulation across various cancer types is consistent with prior knowledge of gene function facilitating tumor progression by promoting proliferation and causing defects in the spindle assembly checkpoint and genomic instability. Upregulated in 28 of the 33 cancer types analyzed herein, BUB1B has a gene expression fold change of 5.5 in OV, which is consistent with prior studies on OV that have also indicated that BUB1B could predict a more aggressive phenotype of OV and correlate negatively with patient survival [20]. However, TCGA data did not show any significant associations between BUB1B and survival in OV. Although upregulated in other cancer types, the degree of upregulation in CHOL is truly remarkable, with a fold change greater than 10. Part of the reason for this level of upregulation could be ascribed to the small sample size or the intrinsic expression of BUB1B in CHOL versus other tissues. Additionally, CPTAC data indicate that TCGA RNA-Seq data is in line with the altered protein levels in LUAD, GBM, HNSC, LUAD, UCEC, and BRCA, but not in OV, KIRC, and PAAD (data not shown). This leads us to assume that the elevated RNA levels of BUB1B may be common, but they may not be associated with actual protein expression or responses to certain types of cancer; however, further experimental validation is required. Our data also indicate a high phosphorylation level at the S670 of BUB1B LUAD and HNSC compared with the normal control. Huang et al. reported that the cell expression of the BUB1B S670A mutant displayed chromosome attachment defects [21]. Chromosome instability is commonly seen in cancer cells and is related to tumor progression and poor prognosis. If the high BUB1B phosphorylation level of S670 is not a byproduct of dysregulated sig-

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**Figure 7. BUB1B-related gene enrichment analysis.**

A - The available experimentally determined BUB1B-binding proteins were obtained using the STRING tool. B - An intersection analysis of BUB1B binding and correlated genes was conducted. C - Based on the BUB1B binding and interacting genes, KEGG pathway analysis was performed. The cnetplots for the molecular function (D), cellular component (E), and biological process (F) in the GO analyses are also shown.
naling without functional significance in tumor cells, BUB1B phosphorylation at the S670 site might play a significant role in cell cycle regulation in tumorigenesis. Survival in cancer can be improved by early diagnosis and treatment. Current cancer screening tests are often costly, invasive, or uncomfortable, and other tests, such as fecal occult blood tests for colon cancer diagnosis, have lower sensitivity. Thus, the identification of novel biomarkers for cancer screening will hopefully lead to relatively non-invasive, highly sensitive, and cost-effective diagnostics. BUB1B offers theoretical potential for achieving these properties. Sixteen of the 33 cancer types contained BUB1B with an AUC > 0.9 (Figure S2). However, although previous studies have shown that BUB1B overexpression is associated with poor prognosis in tumors, including ovarian [20] and glioma [22], the current clinical big data-based evidence cannot support the role of BUB1B expression in the clinical prognosis of these tumors. In view of the different volumes of data in between, we postulate that the discrepancy may be due to different data processing processes or updated survival information. Therefore, we performed a Cox regression survival analysis in OncoLnc (http://www.oncolnc.org/) but failed to observe a statistical correlation (data not shown). In addition, Liu et al. [23] and Zhuang et al. [24] reported that high expression of BUB1B is correlated with poorer prognoses in patients with hepatocellular cancer. Consistent with these findings, our results show a positive correlation between high BUB1B expression and poor OS, DSS, and PFI in LHC. Similarly, we also observed a positive correlation between high BUB1B and poor prognosis of OS, DSS, and PFI in patients with PAAD and LUAD, which is in agreement with recent reports [25].

Several studies have reported that high expression of BUB1B is correlated with advanced pathological stages and disease progression in bladder cancer [26], liver cancer [27], and glioblastoma [28]. Unexpectedly, we found that BUB1B expression was positively correlated cancer stage in many cancer types, especially in the later stages of tumor growth. In addition, we observed significant age- and gender-related changes in BUB1B expression in 6 cancer types. Although these results indicate that BUB1B could be a potential biomarker for cancer diagnosis, confirmation of upregulation in more easily accessed biofluids remains to be determined, as high gene expression levels do not always correlate with overexpression of protein within a biofluid, such as serum, at a high enough level to be acted upon. If serum detection is achievable, ubiquitously upregulated BUB1B could serve as a pan-cancer marker that can identify the presence of cancer, but not the specific location of the cancer.

Over the past decade, cancer immunotherapy has made impressive strides. TMB is an index related to immunotherapy of different types and can predict prognosis after immunotherapy in pan-cancer patients [29]. Moreover, high TMB is associated with prolonged progression-free and overall survival after immunotherapy, possibly due to its high neoantigen burden [30]. Similarly, tumors with high MSI may contain abundant new antigens that can also offer an opportunity to identify patients who may benefit from immunotherapy [31], and MSI is characterized by the size variation of microsatellites in tumor DNA compared with matching normal DNA [32]. In the present study, we demonstrated that BUB1B expression is associated with TMB in 16 cancer types and with MSI in 7 cancer types. Considering that TMB/MSI occurs frequently in tumor cells harboring MMR mutations, we performed correlation analysis and found that BUB1B correlates positively with MMR genes across multiple cancer types. Therefore, our results suggest that patients with high BUB1B expression could be sensitive to immunotherapy, and further research on the integration of TMB/MSI and BUB1B expression may provide a new biomarker for the prognosis of immunotherapy. The relationship between DNA methylation and gene expression is complex, and studies have shown both positive and negative correlations between genomic DNA methylation and gene expression [33]. We observed a low methylation level of BUB1B at 92% CpG sites (except for cg24584161) in 25 cancer types (Figure S3) and a significant negative correlation between DNA methylation and gene expression at multiple CpG sites, which might, at least partially, contribute to the aberrant expression of BUB1B in human cancers. Notably, both BUB1B hypermethylation at cg24584161 and hypomethylation at other sites are associated with poor prognosis in multiple cancer types, which suggests that BUB1B methylation may serve as a potential prognostic biomarker in cancer patients; however, further investigation is needed.

Immune cells of the adaptive and innate immune systems that infiltrate the tumor microenvironment have been shown to play an important role in tumor progression and affect the clinical outcomes of cancer patients [34]. Activation of the adaption immune response is generally dependent on innate immune recognition of the antigen and the appropriate differentiation and maturation of lymphocytes. However, cancer cells can escape immune surveillance by deflecting antigen presentation, upregulating negative regulatory pathways, and recruiting immunosuppressive cell populations [35], leading to the abrogation of antitumor immune responses. NK cells are prototypical innate lymphoid cells that directly eradicate tumor cells through cytotoxic granules and cooperate with other immune cells through proinflammatory cytokines and chemokines [36]. As an essential component of adaptive immunity, T cells have beenthe core pillar of immunotherapy and are noted for their specificity toward antigen recognition and potent tumor-killing ability [37]. In this study, we observed a statistically negative correlation between BUB1B expression and the infiltration levels of innate immune cells, including NK cells, eosinophils, and phagocytic cells (mast cells, neutrophils, macrophages, and dendritic cells) in most cancer types. In addition, BUB1B cor-
relates negatively with the infiltration of B lymphocytes but positively with that of some T-cell subsets, including Tcm, T helper, and Th2 cells, consistent with recently published findings [38]. Further, we found a significant positive correlation between BUB1B expression and genes encoded immunosuppressive, chemokine receptors, MHC, chemokines, and immune activation proteins in multiple cancer types. These results suggest that BUB1B may serve as guidance for using immunotherapy in patients with different types of immune infiltration and deserves further exploration. Furthermore, we integrated the information on BUB1B expression-related genes and BUB1B-binding components across different tumor types for enrichment analyses. Our results indicate that BUB1B can potentially impact the etiology or pathogenesis of cancers by functioning in the cell cycle, ubiquitin-mediated proteolysis, and mitogenic pathways. This discovery is in line with recent studies that have reported that BUB1B is responsible for chromosomal instability in multiple myeloma [39] and breast cancer [11].

Overall, this manuscript explored the significance of BUB1B expression in comparison to normal tissue expression and observed how BUB1B levels are associated with clinical stage, survival difference, TMB, MSI, and immune cell infiltration. Despite our findings, this study has some limitations. One limitation is that, based on the information from TCGA, it was not feasible for us to correlate how a tumor’s microenvironment and supporting cells contribute to BUB1B expression. Another limitation is that we did not perform any analyses that correlate BUB1B expression to the demographic characteristics of tumor histological subtypes. Despite these limitations, the findings of this manuscript are relevant. The information gained from our pan-cancer analyses supports that BUB1B is almost ubiquitously upregulated across different cancer types, and we believe that the most actionable point is to investigate the minimally invasive diagnostic capabilities of BUB1B based on the information presented in this manuscript. Moreover, functional studies or further exploration of BUB1B as a potential pan-cancer biomarker for diagnosis, prognosis, and response therapy may prove extremely beneficial for the medical community.

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Data Availability Statement:
The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

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All data analyzed in the present study are available in the TCGA database. No ethics approval was required for the purpose of this study.

Declaration of Interest:
The authors declare no competing interests.

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Additional material can be found online at: http://supplementary.clin-lab-publications.com/230632/