Am J Transl Res 2024;16(2):400-414
www.ajtr.org /ISSN:1943-8141/AJTR0153401

Original Article

Bone morphogenetic protein 1: a prognostic indicator and potential biomarker in three cancer types

Ming Wang[1], Chunmei Liu[1], Yingjie Wang[1], Muhammad Jamil[2], Majid Alhomrani[3,4], Abdulhakeem S Alamri[3,4], Walaa F Alsanie[3,4], Abdulaziz Alsharif[3,4], Mubarik Ali[5], Norina Jabeen[6]

[1]Department of Oncology, Hebei Yanda Hospital, Langfang 065200, Hebei, China; [2]PARC Arid Zone Research Centre, Dera Ismail Khan 29050, Pakistan; [3]College of Applied Medical Sciences, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; [4]Research Centre for Health Sciences, Taif University, Taif 26571, Saudi Arabia; [5]Animal Science Institute, National Agricultural Research Center, Islamabad 54000, Pakistan; [6]Department of Rural Sociology, University of Agriculture Faisalabad, Faisalabad 38040, Punjab, Pakistan

Received September 13, 2023; Accepted January 10, 2024; Epub February 15, 2024; Published February 28, 2024

Abstract: Background: Bone morphogenetic protein 1 (BMP1) is a metalloprotease that plays a role in activating both transforming growth factor-β (TGF-β) and BMP signaling pathways. TGF-β has been identified as a factor initiating and facilitating cancer development. Consequently, we propose the hypothesis that dysregulation of BMP1 could potentially contribute to the onset and advancement of human cancers. Methods: In this research, we aimed to analyze BMP1 expression level and the associated clinical outcomes across various cancers using online cancer OMICS databases, advanced Bioinformatics tools, and molecular analyses. Results: The outcomes of our web server-based expression analysis indicated an up-regulation of BMP1 in a majority of the human cancers examined. External validation using clinical samples also showed higher expression of BMP1. Moreover, heightened BMP1 expression exhibited a noteworthy correlation with reduced overall survival (OS) duration in Bladder Cancer (BLCA), Kidney Renal Clear Cell Carcinoma (KIRC), and Lung Adenocarcinoma (LUAD) patients. This suggests a substantial involvement of the BMP1 gene in the development and progression of these three types of cancers. The major signaling pathways related with BMP1 enriched genes were "ECM-receptor interaction, Amoebiasis, Focal adhesion, Protein digestion and absorption, progesterone-mediated, PI3K-Akt signaling pathway, and platelet activation". Moreover, we also explored some interesting correlations among BMP1 expression and its DNA promoter methylation level, CD8+ T immune cells level, and genetic variations. Conclusion: In conclusion, our study has provided some solid basis for BMP1 to be used as a reliable common biomarker for BLCA, KIRC, and LUAD patients.

Keywords: Bone morphogenetic protein 1, analysis, biomarker, cancer

Introduction

Cancer is a multifactorial disease that can originate in any part of the body due to abnormal cell division [1, 2]. In 2023, 1,958,310 new cancer cases and 609,820 cancer deaths are projected to occur in the United States [3]. Despite the substantial improvements in cancer treatment methods, still it is the 2nd leading cause of death worldwide after cardiovascular diseases [4]. Therefore, early and accurate diagnosis and prediction of cancer outcomes are critical to patients, physicians, and researchers for its effective treatment. Because this will help inform about patient illness and to design future treatment strategies [5-7]. For example, an earlier study based on the Australian population has ensured cancer treatment success up to 85% with the help of early detection [8]. Therefore, it is urgently required to discover precise, strong, and reliable diagnostic and prognostic biomarkers that could facilitate early diagnosis and prediction of the prognosis of cancer patients.

Bone Morphogenetic Protein 1 (BMP1) is a member of the astacin family, characterized by three primary domains: an astacin-like Zinc metalloproteinase domain, an epidermal growth factor (EGF)-like domain, and three Com-

https://doi.org/10.62347/PWZA6242

plement-Uegf-BMP-1 (CUB) domains [9]. The protein encoded by BMP1 is important for the formation of extracellular matrix [9]. *Wozney et al.* revealed that BMP1 binds with other BMP members of the TGF-β superfamily to stimulate these members, whereas its CUB domain facilitates the protein-protein interactions [10]. Within the extracellular matrix, BMP1 performs the cleavage of TGF-β, releasing TGF-β complexes. These complexes are subsequently targeted by various metalloproteinases, including MMP2, resulting in the release of free TGF. Therefore, the TGF-β signaling pathway and BMP pathways activation are dependent on the BMP1 activity [11]. Previously, BMP1 dysregulation has been documented in gastric cancer [12]. However, systemic pan-cancer research on BMP1 expression in distinct other types of human cancers using data mining, advance Bioinformatics tools, and molecular analyses is still lacking. Recently, advanced Bioinformatics tools, accurate RNA sequencing techniques, and molecular studies exploring genetic basis have speed up the discovery of potential candidate genes involved in cancer development and progression [13-16].

In this study, we conducted a brief examination of BMP1 expression variations and investigated its correlation with the prognosis of diverse human cancers. To this purpose, we utilized well-established databases including UALCAN, TIMER, cBioPortal, Kaplan-Meier Plotter, GEPIA, and CTD, and molecular experiments.

Materials and methods

BMP1 expression analysis

UALCAN web server (http://ualcan.path.uab.edu/) provides easy access for researchers to analyze OMICS data of more than 31 cancer subtypes. Structurally, UALCAN is based on the PERL-CGI framework and uses JavaScript and CSS to provide higher-quality graphics. Altogether, UALCAN assists the researchers to analyze and understand the gene expression levels of gene(s) of interest in individual TCGA cancer subtype [17]. In this study, we acquired BMP1 mRNA and protein expression data, along with various clinicopathological features, from the TCGA pan-cancer view using the UALCAN web server. Additionally, we utilized UALCAN to evaluate the BMP1 promoter methylation level across different cancer subtypes. For statistical purpose, a student's t-test was employed and a *p*-value (<0.05) was considered as statistically significant.

Kaplan-Meier plotter and GEPIA

In this study, we assessed the clinical prognostic value of BMP1 across various cancer subtypes using online tools such as Kaplan-Meier (KM) plotter, GEPIA, and GENT2. These tools were built to measure the effect of 54 thousand genes and proteins on the survival of 21 types of cancer patients [18, 19]. The GEO and TCGA databases are among the data sources of the KM plotter. A *p*-value (<0.05) was considered as statistically significant.

Verification of BMP1 genes expressions

Next, we also verified BMP1 expression in BLCA, KIRC, and LUAD cell lines, and tissues relative to normal tissues using GENT2 online databases [20]. These databases are newly developed for customized and integrated multi-omics analyses of gene(s) of interest across various TCGA datasets. For defining the differential expression of the BMP1 gene, a P<0.05 value was used as a cutoff criterion.

The cBioportal database

cBioPortal (http://www.cbioportal.org) is an easy to use source resource of cancer OMICS data. It assists the researchers to analyze multidimensional genetic variations in the gene(s) and pathway(s) of interest across multiple TCGA datasets [21]. In our work, cBioPortal was used to investigate the BMP1-associated genetic alterations across defined cancer types.

Protein-protein interaction (PPI) and pathway enrichment

In our work, we obtained a PPI network of the BMP1 enriched genes from the STRING web server [22] and visualized it using Cytoscape software [23]. Later on, the KEGG pathway was performed via DAVID tool (http://david.ncifcrf.gov/summary.jsp) [24]. A *p*-value <0.05 was considered as significant.

BMP1 and immune cells

TIMER (https://cistrome.shinyapps.io/timer/) is an online database that predicts the levels of various immune cells in distinct cancer types

[25]. In our work, we used this database to explore the Spearman correlation between BMP1 expression and CD8+ T immune cells across different cancers. A *p*-value <0.05 was considered as significant.

BMP1 gene-drug interaction network analysis

The Comparative Toxicogenomics Database (CDT) [26] was used in our work to construct the gene-drug interaction network for exploring BMP1-associated chemotherapeutic drugs.

RT-qPCR analysis

A total of 18 LUAD tissue samples, along with corresponding adjacent controls, were sourced from Nishtar Hospital in Multan, Pakistan, to verify the mRNA expression pattern of BMP1. Ethical approval was obtained from the Pakistan Agriculture Research Center's ethical committee. TRIzol™ (Takara Biotechnology Co., Ltd.) and additional reagents such as chloroform, isopropyl alcohol, and 75% alcohol were utilized for total RNA extraction from patients' tissues, comprising nine samples from individuals with GBM and nine normal tissues.

RT-qPCR was executed on the CFX Connect Real-Time PCR Detection System (Bio-Rad, Shanghai, China), integrating amplification and detection steps. Reactions utilized the TB Green Premix Ex Taq II PCR kit (TaKaRa, Dalian, China). All assays were conducted with three biological replicates. Each qPCR reaction was performed in a 20 µL volume comprising 10 µL SYBR Green Master Mix, 0.8 µL of each primer, 2 µL of cDNA sample, and 6.4 µL RNase- and DNase-free water. The primer sequences were as follows: GAPDH-F: 5'-CAGGAGGCATTGCTGATGAT-3'; GAPDH-R: 5'-GAAGGCTGGGGCTCATTT-3'; BMP1-F: 5'-CCAATGGCTACTCTGCTCACATG-3'; BMP1-R: 5'-AAGCCATCTCGGACCTCCACAT-3'.

Results

BMP1 expression across multiple human cancers and normal controls

Using the UALCAN web server, we examined the distinct expression patterns of BMP1 in 24 types of cancer tissues and their corresponding paired controls. Notably, a significant (P<0.05) down-regulation of BMP1 was observed in only two types of cancers-Kidney Chromophobe (KICH) and Uterine Corpus Endometrial Carcinoma (UCEC). Conversely, significant overexpression of BMP1 was detected in 22 other cancer types, including Bladder cancer (BLCA), Kidney Renal Clear Cell Carcinoma (KIRC), Lung Adenocarcinoma (LUAD), and several others (**Figure 1**).

BMP1 expression and its prognostic potential

We investigated the correlation between BMP1 expression and Overall Survival (OS) in patients with diverse cancer types using KM plotter, GEPIA, and GENT2 databases. The analysis results revealed a significant (P<0.05) association between BMP1 overexpression and reduced OS in patients with BLCA, KIRC, and LUAD, among the 24 analyzed cancer subtypes (**Figure 2**). These findings emphasize the substantial involvement of the BMP1 gene in the development and progression of BLCA, KIRC, and LUAD. As a result, the subsequent part of our study will primarily focus on exploring the distinctive role of BMP1 in these three cancers.

Verification of BMP1 genes expression

To validate BMP1 gene expression in patients with BLCA, KIRC, and LUAD from another cohort, GENT2 facilitated the detection of BMP1 gene expression levels in BLCA, KIRC, and LUAD tissues, as well as cell lines, in comparison to normal cancer tissues and cell lines. Concurrently, UALCAN assisted in confirming BMP1 expression at the protein level across BLCA, KIRC, and LUAD tissues paired with controls. The analysis results indicated a significant (P<0.05) up-regulation of BMP1 at both mRNA and protein levels in BLCA, KIRC, and LUAD samples, as well as cell lines, relative to controls (**Figure 3**).

Associations among BMP1 expression level and diverse clinical variables

In this study, we utilized the UALCAN web server to examine the associations between BMP1 expression and various clinical variables in BLCA, KIRC, and LUAD patients. Our findings revealed a significant (P<0.05) increase in BMP1 expression across different cancer stages, patient races, ages, genders, and nodal

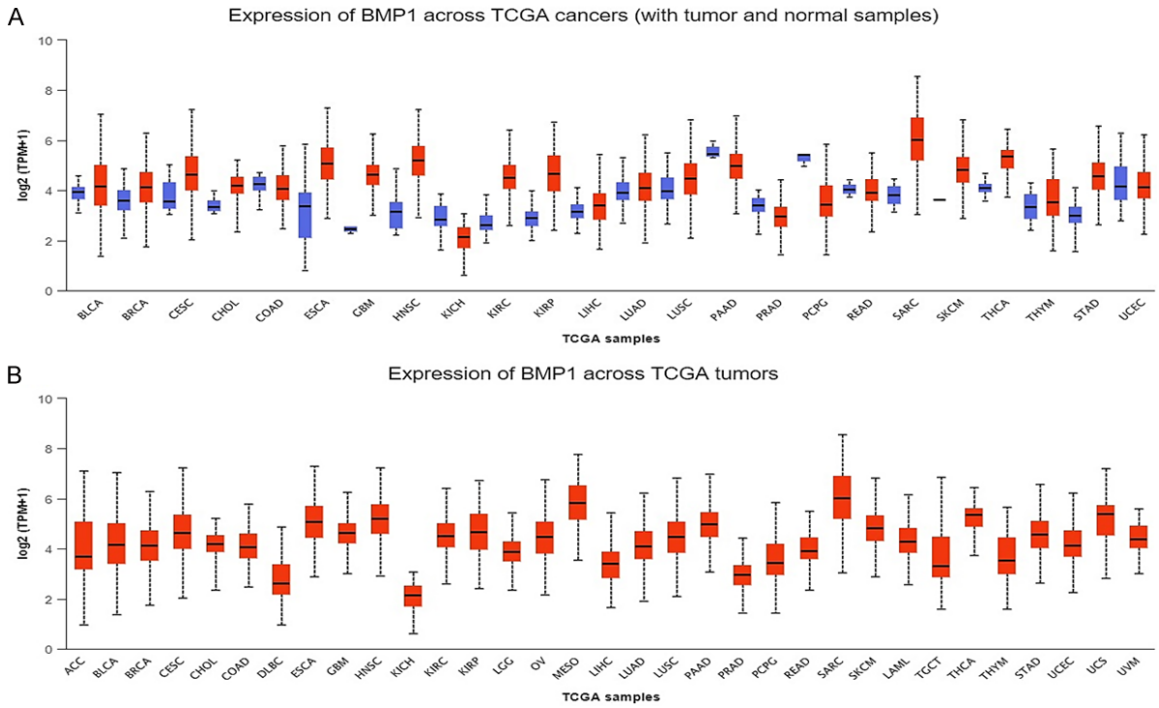


Figure 1. This figure presents a pan-cancer analysis of BMP1 expression, providing insights into its differential expression patterns. (A) illustrates BMP1 expression across cancerous samples paired with their respective normal controls, while (B) focuses on BMP1 expression exclusively in cancerous samples without normal controls. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1.

metastasis statuses of BLCA, KIRC, and LUAD patients (**Figures 4**-**6**). In summary, these results suggest that BMP1 overexpression could serve as a potential biomarker, transcending the heterogeneity observed in BLCA, KIRC, and LUAD patients.

DNA promoter methylation level of BMP1

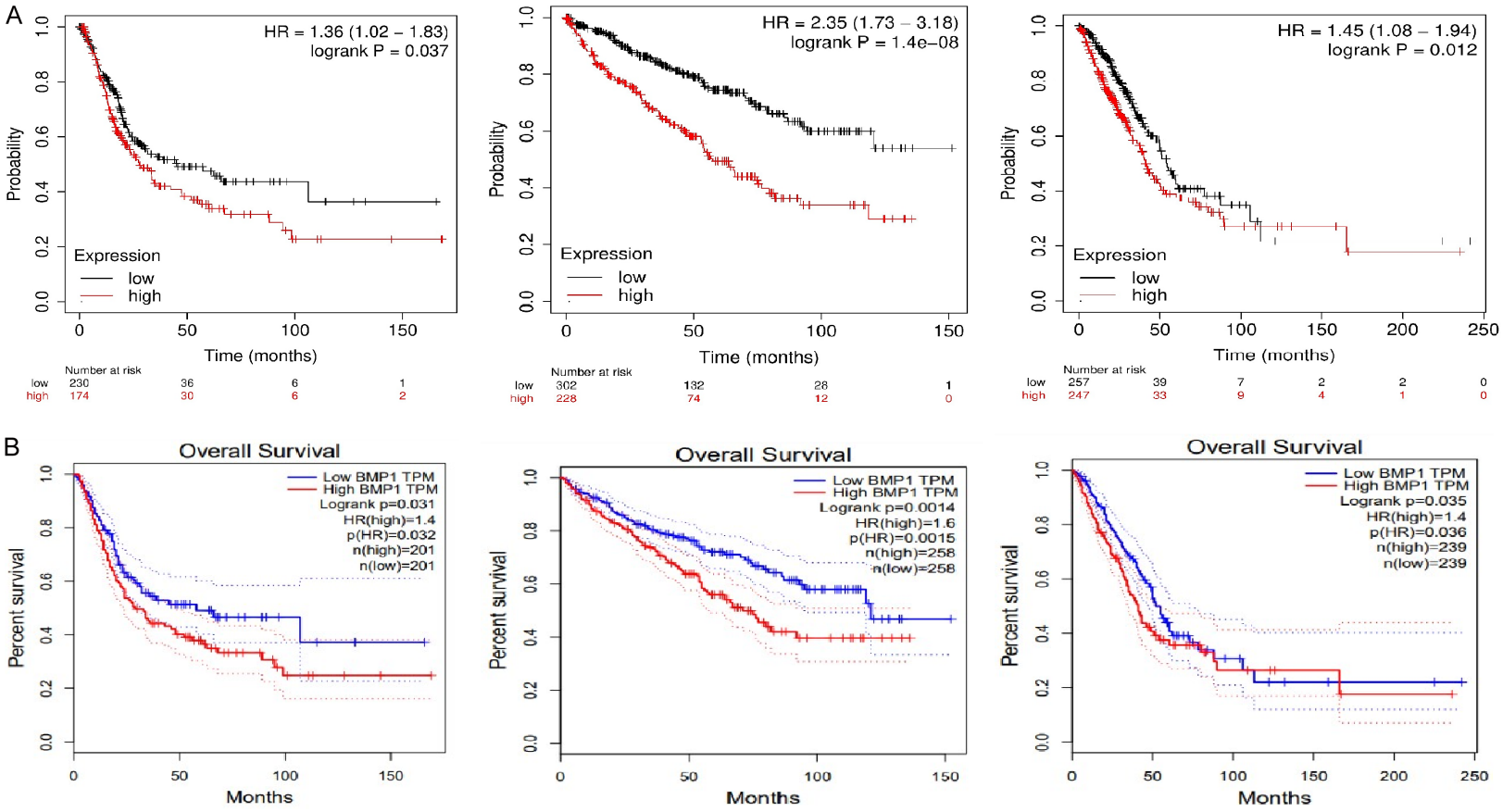
DNA promoter methylation is a crucial mechanism implicated in gene silencing and genetic imprinting [27]. Anomalous methylation patterns in the CpG regions within CpG Islands have been associated with cancers. Consequently, we conducted a promoter methylation analysis of BMP1 in BLCA, KIRC, and LUAD patients using the UALCAN web server. The results revealed significant (P<0.05) hypermethylation of BMP1 in BLCA, KIRC, and LUAD patients compared to their respective paired controls (**Figure 7**).

Genetic alteration in BMP1

For the analysis of genetic alterations and copy number variations (CNVs) in the BMP1 gene in BLCA, KIRC, and LUAD, we employed the cBioPortal web server. The BMP1 gene exhibited alterations in 7% of examined BLCA and LUAD samples and 2% of KIRC samples, primarily manifesting as deep deletion genetic abnormalities. In summary, our findings suggest that BMP1 overexpression in BLCA, KIRC, and LUAD may not be directly associated with genetic mutations and CNVs, despite the presence of these genetic alterations in a minimal proportion of the analyzed BLCA, KIRC, and LUAD samples (**Figure 8**).

PPI network and pathway enrichment of BMP1

We obtained a Protein-Protein Interaction (PPI) network of 11 BMP1-enriched genes using STRING (**Figure 9A**). Utilizing these BMP1-enriched genes as a foundation, we delved into the underlying signaling pathways contributing to the initiation and progression of BLCA, KIRC, and LUAD. To achieve this, the DAVID tool was employed, revealing diverse signaling pathways associated with BMP1-enriched genes, including "ECM-receptor interaction, Amoebiasis, Focal adhesion, Protein digestion and


A
HR = 1.36 (1.02 – 1.83)
logrank P = 0.037
Probability
Expression
low
high
Time (months)
Number at risk
low 230 36 6 1
high 174 30 6 2
HR = 2.35 (1.73 – 3.18)
logrank P = 1.4e–08
Probability
Expression
low
high
Time (months)
Number at risk
low 302 132 28 1
high 228 74 12 0
HR = 1.45 (1.08 – 1.94)
logrank P = 0.012
Probability
Expression
low
high
Time (months)
Number at risk
low 257 39 7 2 2 0
high 247 33 9 4 1 0
B
Overall Survival
Low BMP1 TPM
High BMP1 TPM
Logrank p=0.031
HR(high)=1.4
p(HR)=0.032
n(high)=201
n(low)=201
Percent survival
Months
Overall Survival
Low BMP1 TPM
High BMP1 TPM
Logrank p=0.0014
HR(high)=1.6
p(HR)=0.0015
n(high)=258
n(low)=258
Percent survival
Months
Overall Survival
Low BMP1 TPM
High BMP1 TPM
Logrank p=0.035
HR(high)=1.4
p(HR)=0.036
n(high)=239
n(low)=239
Percent survival
Months

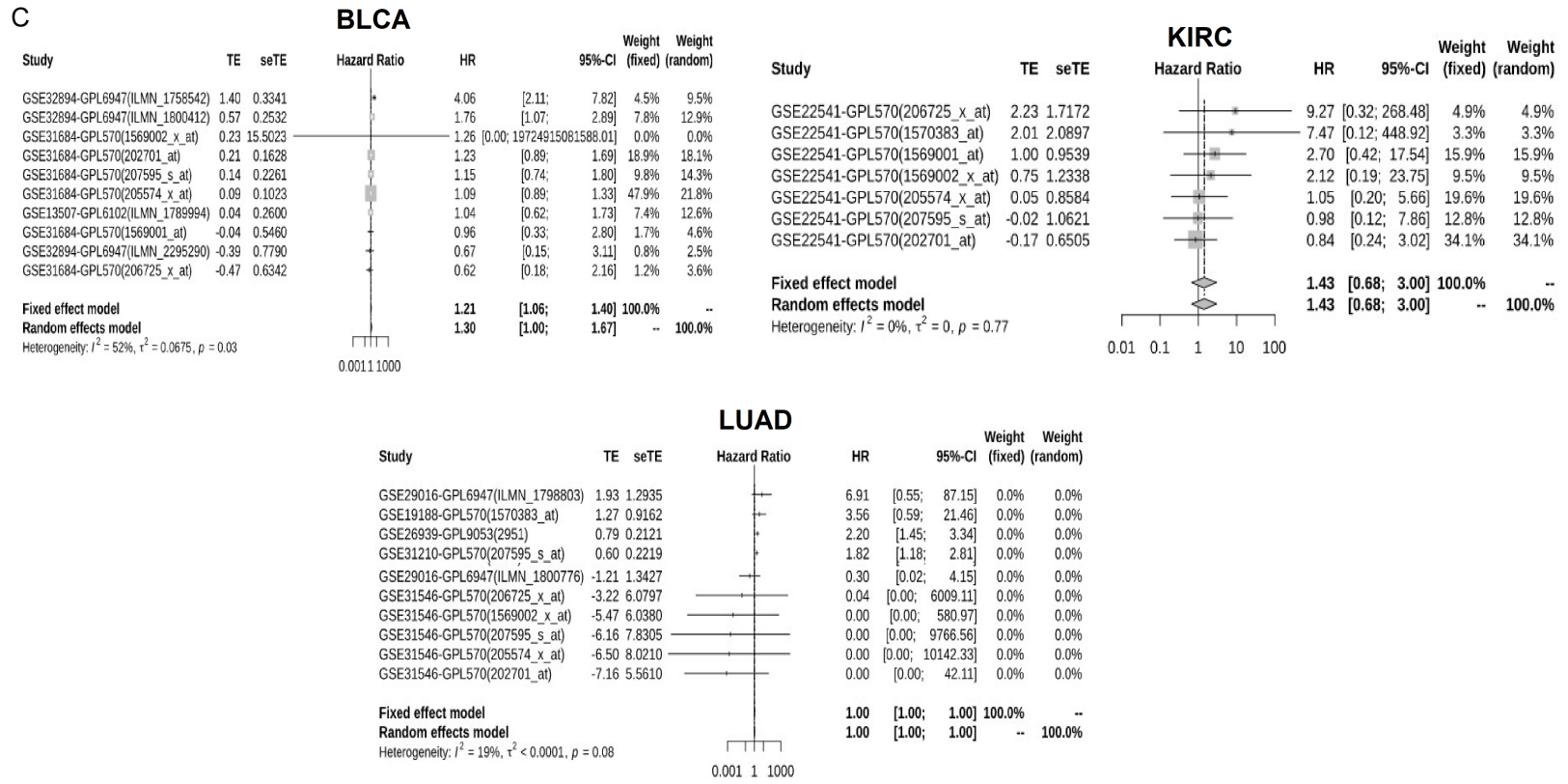


Figure 2. Association between BMP1 expression and overall survival (OS) in patients with BLCA, KIRC, and LUAD. (A) Features OS analysis through Kaplan-Meier (KM) plotting, while (B) Presents OS analysis via GEPIA (Gene Expression Profiling Interactive Analysis). (C) Consolidates OS meta-analysis through GENT2 (Gene Expression Database of Normal and Tumor tissues 2). A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Bladder Cancer = BLCA, Kidney Renal Clear Cell Carcinoma = KIRC, Lung Adenocarcinoma = LUAD.

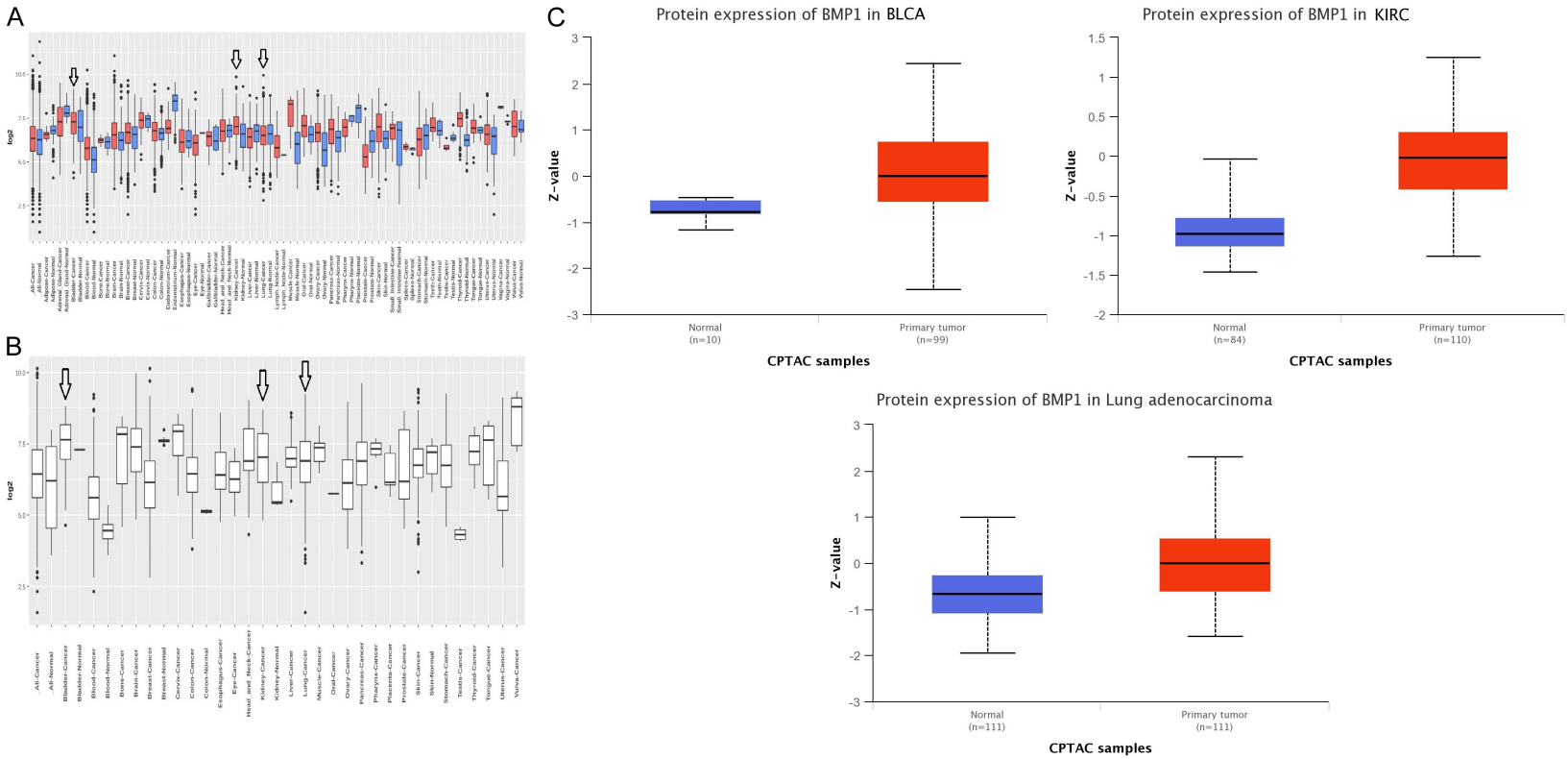


Figure 3. Expression pattern of the BMP1 across BLCA, KIRC, and LUAD samples and cell lines. A. Provides GENT2-based expression data for BMP1 in BLCA, KIRC, and LUAD tissue samples, shedding light on its expression profiles in clinical specimens. B. Extends the analysis to GENT2-based expression of BMP1 in BLCA, KIRC, and LUAD cell lines, offering insights into its expression dynamics within cell line models. C. Delves into UALCAN-based protein expression data for BMP1 in BLCA, KIRC, and LUAD tissue samples, providing an additional layer of information on the protein-level expression of BMP1 in these cancer types. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Bladder Cancer = BLCA, Kidney Renal Clear Cell Carcinoma = KIRC, Lung Adenocarcinoma = LUAD.

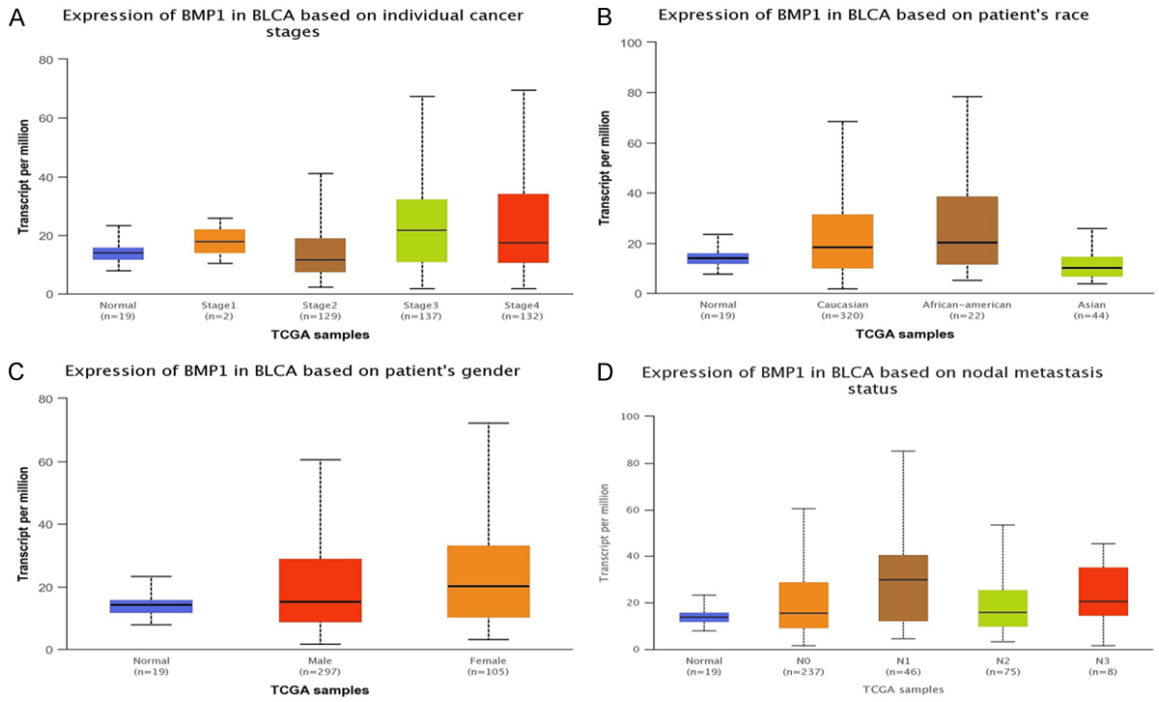


Figure 4. BMP1 expression across diverse clinicopathological parameters within BLCA. A. Dissects BMP1 expression in relation to different cancer stages, providing insights into its potential relevance at distinct disease progression points. B. Delves into BMP1 expression across different patient races, shedding light on potential racial disparities. C. Examines BMP1 expression in connection with different patient genders, unraveling any gender-associated variations. D. Investigates BMP1 expression concerning different nodal metastasis statuses, offering valuable information on its potential implications in metastatic processes. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Bladder Cancer = BLCA.

absorption, progesterone-mediated, PI3K-Akt signaling pathway, and platelet activation" (**Figure 9A**, **9B**; **Table 1**).

BMP1 and immune cell levels

CD8+ T immune cells play a pivotal role in the immune response against cancer [28]. Consequently, in our investigation, we examined the Pearson correlation between CD8+ T immune cells and BMP1 expression levels in BLCA, KIRC, and LUAD using the TIMER database. The findings indicated a notable (P<0.05) negative correlation between BMP1 expression and the infiltrating level of CD8+ T immune cells across BLCA, KIRC, and LUAD samples (**Figure 10**).

BMP1-associated drugs

To investigate potential drugs targeting BMP1, we employed the Comparative Toxicogenomics Database (CTD), and the outcomes were visualized using Cytoscape software. The comprehensive analysis suggested that BMP1 expression could be modulated by various drugs. For example, bacitracin and zoledronic acid drugs are capable of enhancing the expression of BMP1 while valporic acid, colifibrate, and sunitinib are capable of decreasing BMP1 expression level (**Figure 11**).

Validation of BMP1 expression on clinical samples

Importantly, to reinforce the robustness of our findings, we conducted additional validation of BMP1 gene expression using RT-qPCR on clinical samples. The relative expression level of the BMP1 gene was determined using RT-qPCR. BMP1 revealed statistically significant up-regulation in the LUAD sample group as compared to the control group (**Figure 12**).

Discussion

In the current study, we analyzed BMP1 significance as a diagnostic and prognostic biomarker of multiple cancers using a detailed methodology. According to data from multiple databas-

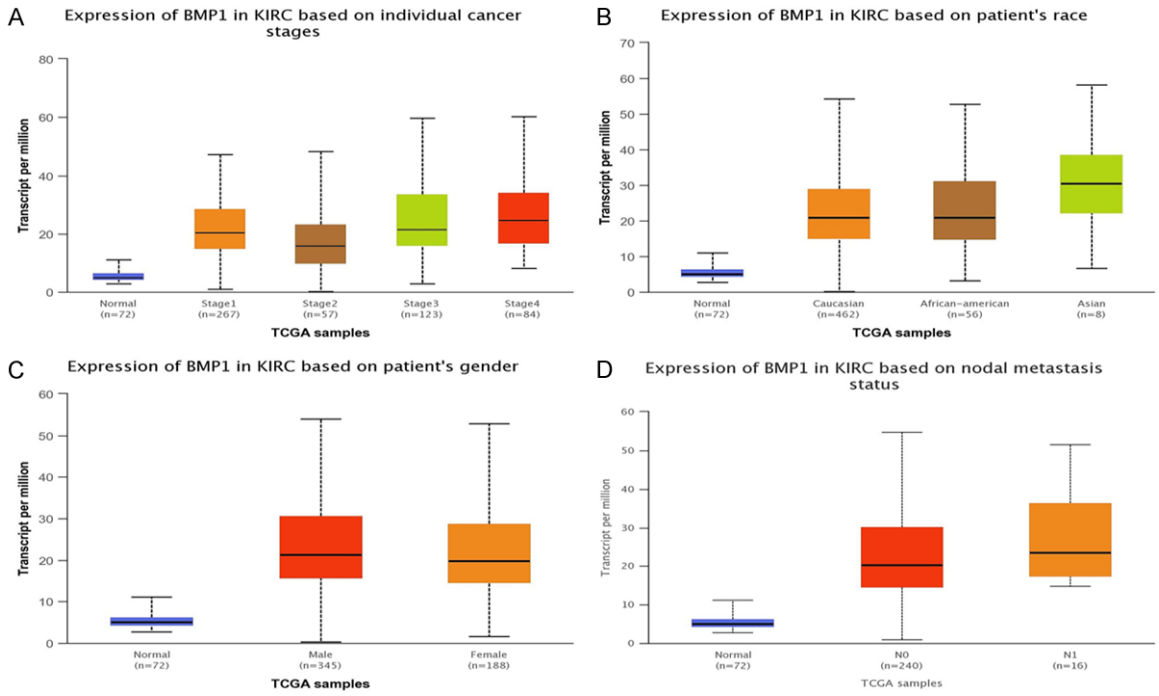


Figure 5. BMP1 expression across diverse clinicopathological parameters within KIRC. A. Dissects BMP1 expression in relation to different cancer stages, providing insights into its potential relevance at distinct disease progression points. B. Delves into BMP1 expression across different patient races, shedding light on potential racial disparities. C. Examines BMP1 expression in connection with different patient genders, unraveling any gender-associated variations. D. Investigates BMP1 expression concerning different nodal metastasis statuses, offering valuable information on its potential implications in metastatic processes. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Kidney Renal Clear Cell Carcinoma = KIRC.

es, we observed a noteworthy up-regulation of BMP1 in 22 distinct cancer types, and conversely, its down-regulation in 2 other types of cancers. We noted the detrimental impact of elevated BMP1 levels on the OS of cancer patients compared to lower BMP1 levels. The OS analysis of BMP1 expression across these cancers revealed a negative correlation, indicating that its up-regulation was associated with a shorter OS duration for BLCA, KIRC, and LUAD patients, with an overall Hazard Ratio (HR) greater than 1. An earlier study has revealed that higher BMP1 expression is associated with poor prognosis in gastric cancer patients [12]. Collectively, these findings substantiate the notion that BMP1 may play a pivotal role in the onset and progression of BLCA, KIRC, and LUAD. Consequently, our primary focus in this study centers on these three cancers. Furthermore, we noted that elevated BMP1 expression is significantly correlated with various clinical variables in BLCA, KIRC, and LUAD patients, encompassing individual cancer stage, patient's race, age, gender, and nodal metastasis status.

There are basically four different causes of cancer initiation including genomic, transcriptomic, proteomic, and epigenetic alterations [29, 30]. Majorly, any variation in the genomic region can either suppress or initiate the oncogenic effects [31]. For an examination of the genetic mutation status and copy number variations (CNVs) of BMP1 in BLCA, KIRC, and LUAD, we employed the cBioPortal web server. In the samples analyzed, the BMP1 gene exhibited alterations in 7% of BLCA and LUAD cases, and 2% of KIRC samples, primarily characterized by deep deletion genetic abnormalities. As a result, we postulate that genetic mutations and CNVs are less likely to be contributory factors in the regulation of BMP1 expression across BLCA, LUAD, and KIRC.

Subsequently, we examined the relationship between BMP1 promoter methylation levels

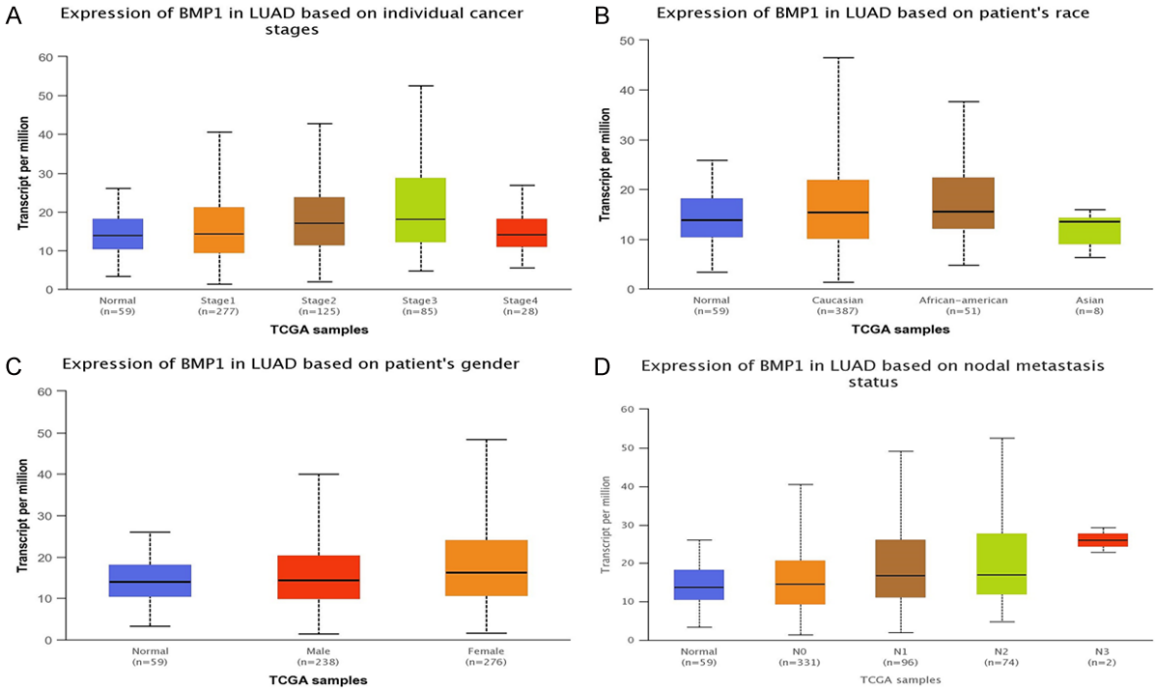


Figure 6. BMP1 expression across diverse clinicopathological parameters within LUAD. A. Dissects BMP1 expression in relation to different cancer stages, providing insights into its potential relevance at distinct disease progression points. B. Delves into BMP1 expression across different patient races, shedding light on potential racial disparities. C. Examines BMP1 expression in connection with different patient genders, unraveling any gender-associated variations. D. Investigates BMP1 expression concerning different nodal metastasis statuses, offering valuable information on its potential implications in metastatic processes. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Lung Adenocarcinoma = LUAD.

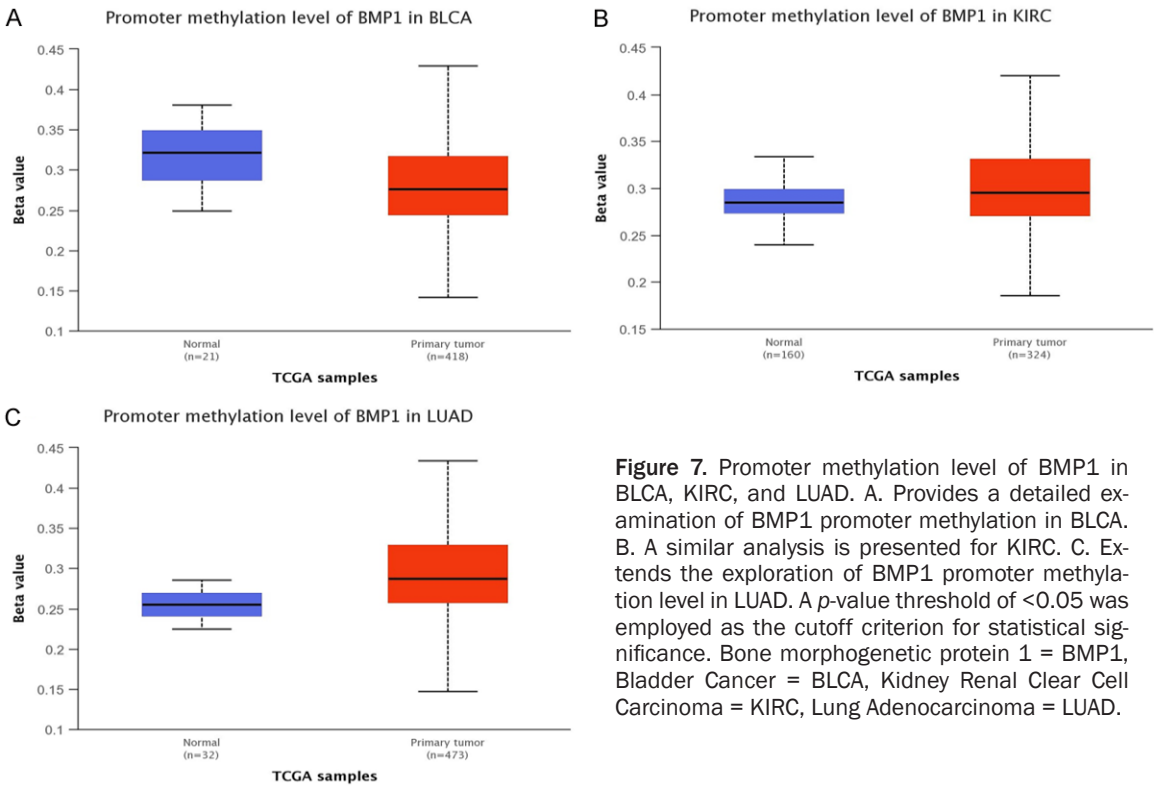


Figure 7. Promoter methylation level of BMP1 in BLCA, KIRC, and LUAD. A. Provides a detailed examination of BMP1 promoter methylation in BLCA. B. A similar analysis is presented for KIRC. C. Extends the exploration of BMP1 promoter methylation level in LUAD. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Bladder Cancer = BLCA, Kidney Renal Clear Cell Carcinoma = KIRC, Lung Adenocarcinoma = LUAD.

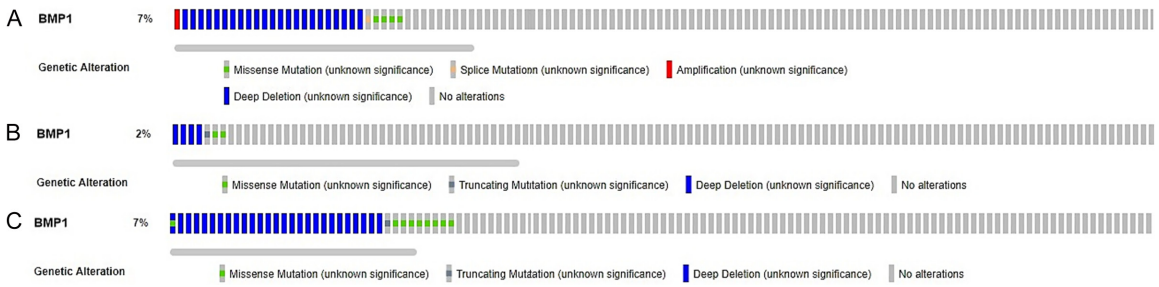


Figure 8. Investigating genetic alterations and copy number variations (CNVs) affecting BMP1 in the cancer genome atlas (TCGA) BLCA, KIRC, and LUAD datasets. A. BMP1 genetic alterations are scrutinized in BLCA. B. The image showed similar analysis in KIRC. C. The exploration of genetic alterations is extended in LUAD. Bone morphogenetic protein 1 = BMP1, Bladder Cancer = BLCA, Kidney Renal Clear Cell Carcinoma = KIRC, Lung Adenocarcinoma = LUAD.

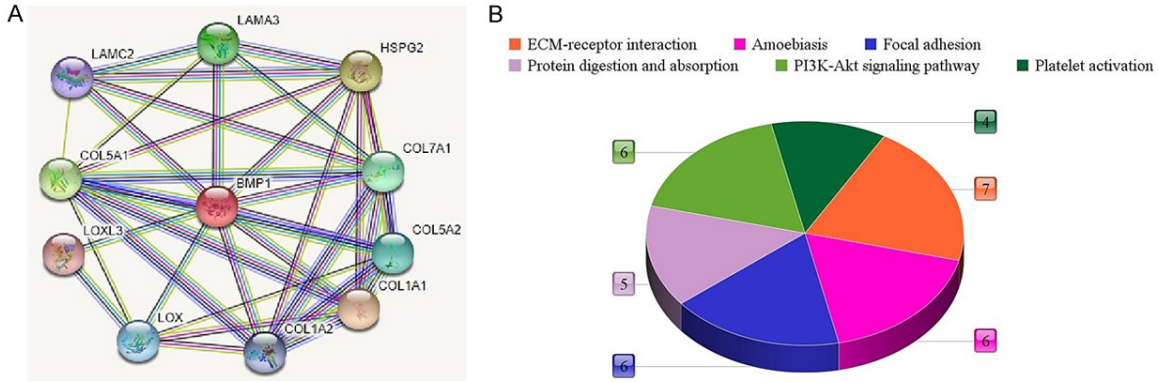


Figure 9. A PPI network and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of the BMP1 enriched genes. A. A PPI network of BMP1 enriched genes. B. KEGG pathway analysis of the BMP1 enriched genes. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1.

Table 1. Detail of BMP1 enriched genes pathway analysis

Pathway ID	Pathway Name	Gene count	*P*-value	Gene name
Hsa04512	ECM-receptor interaction	7	<0.05	COL1A1, COL1A2, COL5A1, LAMA3, COL5A2, LAMC2, HSPG2
Hsa05146	Amoebiasis	6	<0.05	COL1A1, COL1A2, COL5A1, LAMA3, COL5A2, LAMC2
Hsa04510	Focal adhesion	6	<0.05	COL1A1, COL1A2, COL5A1, LAMA3, COL5A2, LAMC2
Hsa04974	Protein digestion and absorption	5	<0.05	COL1A1, COL1A2, COL5A1, COL7A1, COL5A2
Hsa04151	PI3K-Akt signaling pathway	6	<0.05	COL1A1, COL1A2, COL5A1, LAMA3, COL5A2, LAMC2
Hsa04611	Platelet activation	4	<0.05	COL1A1, COL1A2, COL5A1, COL5A2

Bone morphogenetic protein 1 = BMP1.

and its expression in BLCA, KIRC, and LUAD. Our findings unveiled a notable positive correlation; challenging the conventional belief that hypermethylation is typically negatively associated with gene expression. It's important to note that this does not always conform to the general trend, as several studies in the medical literature have identified positive correlations between hypermethylation and gene expression in diverse cancer types [32, 33].

Currently, various BLCA-related biomarkers are being used for its early diagnosis and predicting the prognosis. These biomarkers include NTRK3 [34], P4HB [35], KIF2C, BUB1B, KIF11, CDCA5, KIFC1, CDCA8, AURKA, KIF18B, NEK2, KPNA2, NCAPG, NUSAP1, and RACGAP1 [36]. For the early diagnosis and predicting the prognosis of KIRC, different studies have identified multiple key genes such as ACAA1, ACADSB, ALDH6A1, AUH, HADH, PCCA [37],

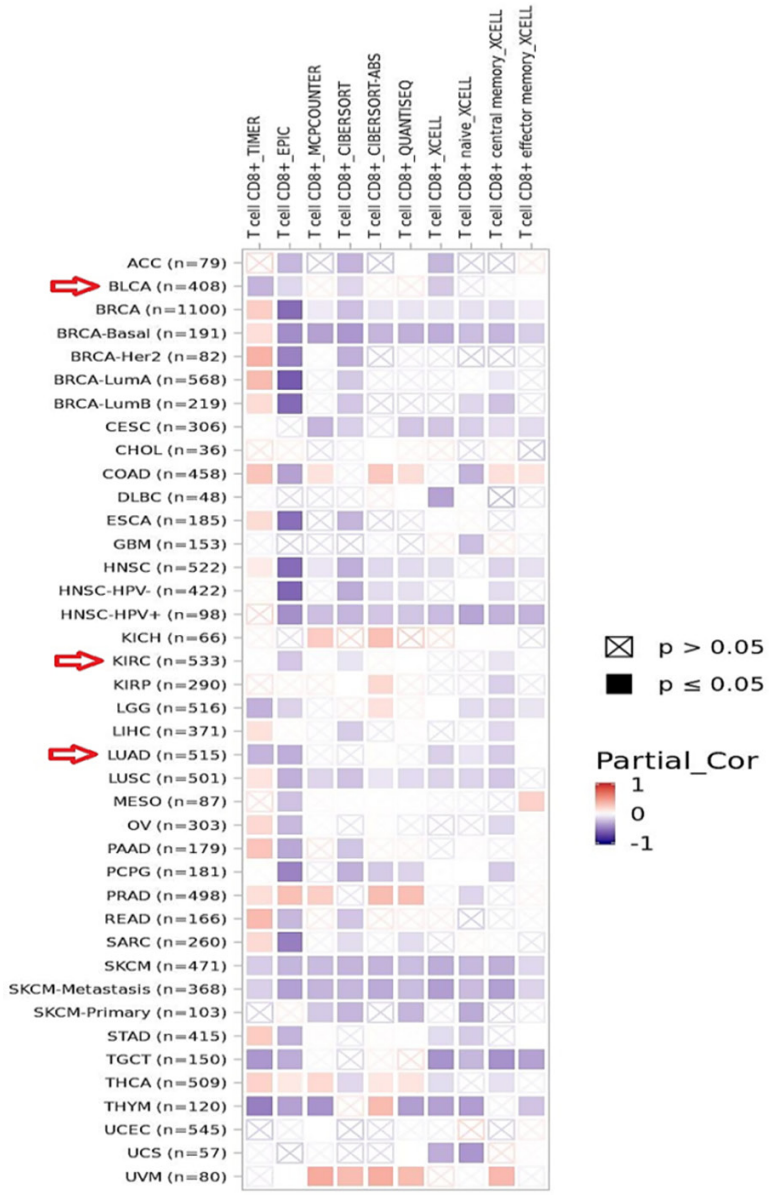


Figure 10. TIMER based Spearman correlational analysis between BMP1 expression and CD8+ T immune cells level in BLCA, KIRC, and LUAD. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Bladder Cancer = BLCA, Kidney Renal Clear Cell Carcinoma = KIRC, Lung Adenocarcinoma = LUAD.

AGXT, PTGER3, SLC12A3 [38], and Linc00342 [39]. Similarly, for the early diagnosis and predicting the prognosis of LUAD, the roles of 9 miRNAs (has-mir-486-1, has-mir-486-2, has-mir-153, has-mir-210, has-mir-9-1, has-mir-9-2, has-mir-9-3, has-mir-577, and has-mir-4732) and 15 genes (EDN1, FOS, IL6, MMP9, CDH1, BIRC5, CDK1, CDKN3, VWF, CD34, UBE2C, AURKA, EGR1, CDKN2A, and CCNB2) are already well described [40, 41].

Nevertheless, none of these, or any other biomarkers, have been universally applied in BLCA, KIRC, and LUAD patients with varied clinicopathological features. In this current study, we highlighted the significant (P<0.05) up-regulation of BMP1 expression in BLCA, KIRC, and LUAD patients across diverse clinicopathological features, including cancer stages, patient race, gender, age, and nodal metastasis status, as compared to normal controls. Moreover, the survival analysis of the BMP1 gene indicated its up-regulation as a prognostic biomarker for BLCA, KIRC, and LUAD. In summary, based on our findings, we posit that elevated BMP1 expression could potentially serve as a novel diagnostic and prognostic tool for BLCA, KIRC, and LUAD patients.

Furthermore, based on the fact that tumor infiltrating immune cells, especially CD8+ T immune cells, contributes to the tumor initiation, development, and progression [42], we found out the correlations among BMP1 expression level and CD8+ T immune cells infiltration via TIMER database. Results of the analysis have shown the notable negative correlations among BMP1 expression and CD8+ T immune cells infiltration across BLCA, KIRC, and LUAD. Therefore, we also speculated that BMP1 may initiate tumor development by negatively regulating the CD8+ T immune cells immune cells within tumor cells.

We explored that the BMP1-associated genes were linked with some diverse underpinning molecular pathways, such as “ECM-receptor interaction, Amoebiasis, Focal adhesion, Protein digestion and absorption, progesterone-

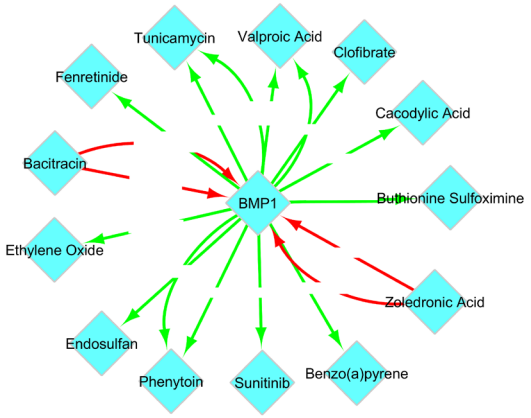


Figure 11. Gene-drug interaction network of the BMP1 and chemotherapeutic drugs. Red arrows: chemotherapeutic drugs increase the expression of BMP1; green arrows: chemotherapeutic drugs decrease the expression of BMP1. The numbers of arrows between chemotherapeutic drugs and key genes in this network represent the supported numbers of literatures by previous reports. Bone morphogenetic protein 1 = BMP1.

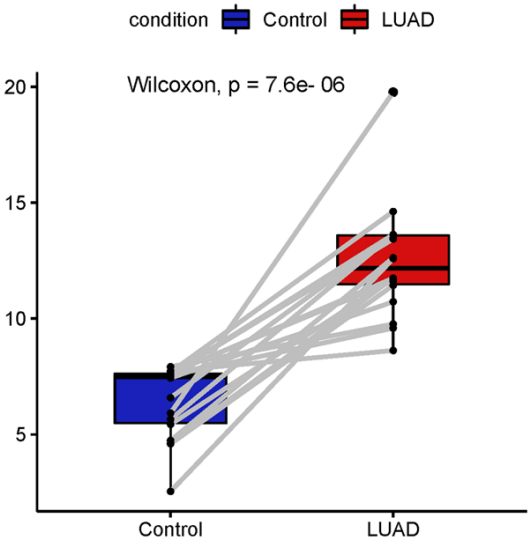


Figure 12. The quantitative analysis results of BMP1 gene expression in LUAD tissue sample and adjacent control tissue sample groups. A *p*-value threshold of <0.05 was employed as the cutoff criterion for statistical significance. Bone morphogenetic protein 1 = BMP1, Lung Adenocarcinoma = LUAD.

mediated, PI3K-Akt signaling pathway, and platelet activation". It was reported earlier that any disturbance in these pathways can initiate cancer by facilitating cell proliferation and chromosomal abnormalities [43]. Finally, we investigated key drugs targeting the BMP1 gene that could be valuable in formulating novel treatment approaches for BLCA, KIRC, and LUAD.

Conclusion

In this study, our aim was to identify molecular biomarkers crucial for cancer initiation and progression, aiming for effective cancer treatment strategies that minimize risks for patients and reduce the potential side effects of overtreatment. To assess the BMP1 gene's suitability as a potential diagnostic and prognostic biomarker in cancer development, we examined various aspects, including BMP1 transcriptomic expression levels, DNA methylation levels, mutational status, enriched genes, prognostic capabilities, and gene-drug network interactions. Our findings indicated a significant up-regulation of BMP1 across BLCA, KIRC, and LUAD patients, correlating with reduced overall survival durations. Importantly, our results unveiled the signaling pathways associated with BMP1 that contribute to the initiation and progression of BLCA, KIRC, and LUAD. Consequently, these pathways could be targeted therapeutically to impede the development of BLCA, KIRC, and LUAD. In summary, BMP1 emerges as a potentially common novel diagnostic and prognostic biomarker, as well as a therapeutic target for patients with BLCA, KIRC, and LUAD.

Acknowledgements

The researchers would like to acknowledge Deanship of Graduate studies and Scientific Research, Taif University for funding this work.

Disclosure of conflict of interest

None.

Address correspondence to: Chunmei Liu, Department of Oncology, Hebei Yanda Hospital, Langfang 065200, Hebei, China. E-mail: cantu37139346658@163.com; Muhammad Jamil, PARC Arid Zone Research Centre, Dera Ismail Khan 29050, Pakistan. E-mail: jamilmatrah@parc.gov.pk

References

[1] Ullah L, Hameed Y, Ejaz S, Raashid A, Iqbal J, Ullah I and Ejaz SA. Detection of novel infiltrating ductal carcinoma-associated BReast CAncer gene 2 mutations which alter the deoxyribonucleic acid-binding ability of BReast CAncer

gene 2 protein. J Cancer Res Ther 2020; 16: 1402-1407.
[2] Zhang L, Sahar AM, Li C, Chaudhary A, Yousaf I, Saeedah MA, Mubarak A, Haris M, Nawaz M, Reem MA, Ramadan FA, Mostafa AAM, Feng W and Hameed Y. A detailed multi-omics analysis of GNB2 gene in human cancers. Braz J Biol 2022; 84: e260169.
[3] Finley LWS. What is cancer metabolism? Cell 2023; 186: 1670-1688.
[4] Bray F, Laversanne M, Weiderpass E and Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer 2021; 127: 3029-3030.
[5] Sial N, Saeed S, Ahmad M, Hameed Y, Rehman A, Abbas M, Asif R, Ahmed H, Hussain MS, Rehman JU, Atif M and Khan MR. Multi-omics analysis identified TMED2 as a shared potential biomarker in six subtypes of human cancer. Int J Gen Med 2021; 14: 7025-7042.
[6] Usman M, Okla MK, Asif HM, AbdElgayed G, Muccee F, Ghazanfar S, Ahmad M, Iqbal MJ, Sahar AM, Khaliq G, Shoaib R, Zaheer H and Hameed Y. A pan-cancer analysis of GINS complex subunit 4 to identify its potential role as a biomarker in multiple human cancers. Am J Cancer Res 2022; 12: 986-1008.
[7] Sial N, Ahmad M, Hussain MS, Iqbal MJ, Hameed Y, Khan M, Abbas M, Asif R, Rehman JU, Atif M, Khan MR, Hameed Z, Saeed H, Tanveer R, Saeed S, Sharif A and Asif HM. CTHRC1 expression is a novel shared diagnostic and prognostic biomarker of survival in six different human cancer subtypes. Sci Rep 2021; 11: 19873.
[8] Weigel MT and Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. Endocr Relat Cancer 2010; 17: R245-R262.
[9] Sharma T, Kapoor A and Mandal CC. Duality of bone morphogenetic proteins in cancer: a comprehensive analysis. J Cell Physiol 2022; 237: 3127-3163.
[10] Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM and Wang EA. Novel regulators of bone formation: molecular clones and activities. Science 1988; 242: 1528-1534.
[11] Costantini A, Guasto A and Cormier-Daire V. TGF-β and BMP signaling pathways in skeletal dysplasia with short and tall stature. Annu Rev Genomics Hum Genet 2023; 24: 225-253.
[12] Rafi JH, Jafar T, Pathan MT, Reza R, Islam S, Sourna IJ, Alam R, Samad A and Ahammad F. High expression of bone morphogenetic protein 1 (BMP1) is associated with a poor survival rate in human gastric cancer, a dataset approaches. Genomics 2021; 113: 1141-1154.
[13] Ahmad M, Hameed Y, Khan M, Usman M, Rehman A, Abid U, Asif R, Ahmed H, Hussain MS, Rehman JU, Asif HM, Arshad R, Atif M, Hadi A, Sarfraz U and Khurshid U. Up-regulation of GINS1 highlighted a good diagnostic and prognostic potential of survival in three different subtypes of human cancer. Braz J Biol 2021; 84: e250575.
[14] Usman M, Hameed Y, Ahmad M, Iqbal MJ, Maryam A, Mazhar A, Naz S, Tanveer R, Saeed H, Bint-E-Fatima, Ashraf A, Hadi A, Hameed Z, Tariq E and Aslam AS. SHMT2 is associated with tumor purity, CD8+ T immune cells infiltration, and a novel therapeutic target in four different human cancers. Curr Mol Med 2023; 23: 161-176.
[15] Usman M, Hameed Y, Ahmad M, Jalil Ur Rehman, Ahmed H, Hussain MS, Asif R, Murtaza MG, Jawad MT and Iqbal MJ. Breast cancer risk and human papillomavirus infection: a Bradford Hill criteria based evaluation. Infect Disord Drug Targets 2022; 22: e200122200389.
[16] Hameed Y, Usman M, Liang S and Ejaz S. Novel diagnostic and prognostic biomarkers of colorectal cancer: capable to overcome the heterogeneity-specific barrier and valid for global applications. PLoS One 2021; 16: e0256020.
[17] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.
[18] Maciejczyk A, Szelachowska J, Czapiga B, Matkowski R, Hałoń A, Györffy B and Surowiak P. Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. J Histochem Cytochem 2013; 61: 330-339.
[19] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.
[20] Park SJ, Yoon BH, Kim SK and Kim SY. GENT2: an updated gene expression database for normal and tumor tissues. BMC Med Genomics 2019; 12 Suppl 5: 101.
[21] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-404.
[22] von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P and Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res 2003; 31: 258-261.

[23] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
[24] Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA. The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007; 8: R183.
[25] Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020; 48: W509-W514.
[26] Davis AP, Grondin CJ, Johnson RJ, Sciaky D, Wiegers J, Wiegers TC and Mattingly CJ. Comparative toxicogenomics database (CTD): update 2021. Nucleic Acids Res 2021; 49: D1138-D1143.
[27] Luo X, Hu Y, Shen J, Liu X, Wang T, Li L and Li J. Integrative analysis of DNA methylation and gene expression reveals key molecular signatures in acute myocardial infarction. Clin Epigenetics 2022; 14: 46.
[28] van der Leun AM, Thommen DS and Schumacher TN. CD8(+) T cell states in human cancer: insights from single-cell analysis. Nat Rev Cancer 2020; 20: 218-232.
[29] Ray SK and Mukherjee S. Assimilating epigenetics and transcriptomics for the identification of prognostic novel biomarkers and imminent targets in colorectal carcinoma with therapeutic potential. Curr Mol Med 2023; 23: 784-798.
[30] Sharma S, Kelly TK and Jones PA. Epigenetics in cancer. Carcinogenesis 2010; 31: 27-36.
[31] Ha DP, Huang B, Wang H, Rangel DF, Van Krieken R, Liu Z, Samanta S, Neamati N and Lee AS. Targeting GRP78 suppresses oncogenic KRAS protein expression and reduces viability of cancer cells bearing various KRAS mutations. Neoplasia 2022; 33: 100837.
[32] Rauluseviciute I, Drabløs F and Rye MB. DNA hypermethylation associated with upregulated gene expression in prostate cancer demonstrates the diversity of epigenetic regulation. BMC Med Genomics 2020; 13: 6.
[33] Arechederra M, Daian F, Yim A, Bazai SK, Richelme S, Dono R, Saurin AJ, Habermann BH and Maina F. Hypermethylation of gene body CpG islands predicts high dosage of functional oncogenes in liver cancer. Nat Commun 2018; 9: 3164.
[34] Zhang Z, Yu Y, Zhang P, Ma G, Zhang M, Liang Y, Jiao W and Niu H. Identification of NTRK3 as a potential prognostic biomarker associated with tumor mutation burden and immune infiltration in bladder cancer. BMC Cancer 2021; 21: 458.
[35] Wu Y, Peng Y, Guan B, He A, Yang K, He S, Gong Y, Li X and Zhou L. P4HB: a novel diagnostic and prognostic biomarker for bladder carcinoma. Oncol Lett 2021; 21: 95.
[36] Pan S, Zhan Y, Chen X, Wu B and Liu B. Identification of biomarkers for controlling cancer stem cell characteristics in bladder cancer by network analysis of transcriptome data stemness indices. Front Oncol 2019; 9: 613.
[37] Zhang B, Wu Q, Wang Z, Xu R, Hu X, Sun Y, Wang Q, Ju F, Ren S, Zhang C, Qin L, Ma Q and Zhou YL. The promising novel biomarkers and candidate small molecule drugs in kidney renal clear cell carcinoma: evidence from bioinformatics analysis of high-throughput data. Mol Genet Genomic Med 2019; 7: e607.
[38] Cui H, Shan H, Miao MZ, Jiang Z, Meng Y, Chen R, Zhang L and Liu Y. Identification of the key genes and pathways involved in the tumorigenesis and prognosis of kidney renal clear cell carcinoma. Sci Rep 2020; 10: 4271.
[39] Liu C, Xu Y, Wu X and Zou Q. Clinical significance of Linc00342 expression in the peripheral blood lymphocytes of patients with chronic kidney disease. Int J Nephrol Renovasc Dis 2019; 12: 251-256.
[40] Ren ZP, Hou XB, Tian XD, Guo JT, Zhang LB, Xue ZQ, Deng JQ, Zhang SW, Pan JY and Chu XY. Identification of nine microRNAs as potential biomarkers for lung adenocarcinoma. FEBS Open Bio 2019; 9: 315-327.
[41] Ni KW and Sun GZ. The identification of key biomarkers in patients with lung adenocarcinoma based on bioinformatics. Math Biosci Eng 2019; 16: 7671-7687.
[42] Kitamura T, Qian BZ and Pollard JW. Immune cell promotion of metastasis. Nat Rev Immunol 2015; 15: 73-86.
[43] Xie Y, Su N, Yang J, Tan Q, Huang S, Jin M, Ni Z, Zhang B, Zhang D, Luo F, Chen H, Sun X, Feng JQ, Qi H and Chen L. FGF/FGFR signaling in health and disease. Signal Transduct Target Ther 2020; 5: 181.