ACCSCIENCE PUBLISHING

Gene & Protein in Disease

ORIGINAL RESEARCH ARTICLE

A pan-cancer analysis of high mobility group box 1 and its role in human tumorigenesis

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Abstract

Understanding the specific and co-driving mechanisms of carcinogenesis in human tumors is indispensable for cancer research and can guide the development of effective treatment methods for tumors. High mobility group box 1 (HMGB1) participates in a variety of physiological processes of the body and has an inseparable relationship with tumors. In this study, The Cancer Genome Atlas, Gene Expression Omnibus database, Human Protein Atlas, and bioinformatic tools were used to conduct pan-cancer analysis of HMGB1 in various cancers so as to elucidate its role in human tumorigenesis. We analyzed and evaluated the expression of *HMGB1* in tumors, and discovered that overexpression of *HMGB1* usually indicated poor overall survival of adrenocortical carcinoma ($P < 0.01$) and lung adenocarcinoma (LUAD) ($P < 0.05$). High *HMGB1* expression is also associated with unfavorable disease-free survival for patients with adrenocortical carcinoma ($P < 0.001$), cervical squamous cell carcinoma and endocervical adenocarcinoma ($P < 0.01$), head and neck squamous cell carcinoma ($P < 0.05$), LUAD ($P < 0.05$), and sarcoma ($P < 0.05$). The potential mechanism of HMGB1-mediated tumorigenesis is also discussed. In conclusion, our pan-cancer analysis offers a comprehensive description of the carcinogenic roles of HMGB1 in a variety of human cancers.

Keywords: High mobility group box 1; Cancer; Survival; Prognosis

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Citation: Long W, Li J, Shi H, *et al.*, 2023, A pan-cancer analysis of high mobility group box 1 and its role in human tumorigenesis. *Gene Protein Dis*, 2(1):301.
https://doi.org/10.36922/gpd.301

Received: December 15, 2022
Accepted: March 1, 2023
Published Online: March 16, 2023

1. Introduction

In view of the complexity of tumorigenesis, it is vital to conduct a pan-cancer analysis of the significant genes to evaluate their relevance to possible molecular mechanisms and clinical prognosis. Cancer genome repositories and databases, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO), offer access to many tumor-associated functional genomic datasets that can be used for pan-cancer analysis[1-3].

Situated on chromosome 13q12, the high mobility group box 1 (*HMGB1*) gene encodes and produces the HMGB1 protein[4], which is a highly conserved nuclear protein belonging to the group of nonhistone chromatin-related protein. It was first extracted from the bovine thymus in 1973[5]. HMGB1 consists of two DNA-binding

HMG-box domains (N-terminal A and center B), which constitute the non-specific DNA binding region of HMGB1 and an acidic C-terminal tail[6]. In general, HMGB1 exists in the nucleus and helps maintain the stability of the nucleus as a DNA chaperone. It plays a key role in DNA replication, V(D)J recombination, transcription and chromatin remodeling. However, the expression of HMGB1 has also been found in mitochondria, cytosol, and cell surface membranes, and the protein can be released into the extracellular space[7]. Cytoplasmic HMGB1 participates in immune responses by regulating mitochondrial function, inhibiting apoptosis, and increasing autophagy[8]. On the membrane, HMGB1 can activate platelets, promote neurite growth and axonal sprouting, and induce cell migration. HMGB1 can be secreted into the extracellular environment as a cytokine, and participates in many immune reactions by promoting the maturation and activation of immune cells and the production of cytokine[9]. In addition, extracellular HMGB1 is able to interact with chemokines to advance immune responses[10]. In a word, HMGB1, as a multifunctional protein, plays different biological roles under different circumstances, and more investigations are required to illustrate the potential effects. In pan-cancer analysis, to probe the expression profile of HMGB1 in different tumor types, databases (TGCA and GEO) were used. Except for comparing HMGB1 expression profiles in different tumor types, a number of factors such as survival status, genetic alteration, protein phosphorylation, and related cellular pathways were also taken into account. This comprehensive analysis uncovers potential molecular mechanism of HMGB1 in the pathogenesis and clinical prognosis of a variety of human cancers.

2. Materials and methods

2.1. Gene expression analysis

To observe *HMGB1* expression differences between tumor and adjacent normal tissues, we put HMGB1 into the "Gene_DE" section of tumor immune estimation resource, version2 (TIMER2) web (http://timer.cistrome.org/). With regard to some tumors without corresponding adjacent normal tissues as a control (e.g., TCGA-Mesothelioma [MESO], TCGA-Sarcoma [SARC]), the "Expression analysis-Box plot" plate of the Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2) web server (http://gepia2.cancer-pku.cn/#analysis)[11] was used to obtain box plots of the Genotype-Tissue Expression (GTEx) database, with log2FC (fold change) cutoff = 1, *P*-value cutoff = 0.01, and "Match TCGA normal and GTEx data." HEPIA2 was applied to analyze *HMGB1* expression in different pathological stages of all TCGA cancers. The expression data transformed by log2 (TPM [Transcripts per million] + 1) were used for violin or box plots.

The UALCAN (http://ualcan.path.uab.edu/analysis-prot.html) was used to analyze cancer omics data, allowing us to analyze the protein expression of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) dataset[12]. We investigated the expression level of HMGB1, either total protein or phosphoprotein, between primary tumor or normal tissues.

2.2. Survival analysis

To gain significant map data for overall survival (OS) and disease-free survival (DFS) of *HMGB1* in overall TCGA tumors, GEPIA2 was used in the analysis. Cutoff-low (50%) and cutoff-high (50%) values were used as expression thresholds to separate low and high expression cohorts[11]. The log-rank test was used in the hypothesis testing. Through the "Survival Analysis" plate of GEPIA2, survival plots were obtained.

2.3. Genetic variation analysis

Using the cBioPortal (https://www.cbioportal.org/) web[13-15], we collected the protein structure data, including variation frequency, mutated site information, mutation type, three-dimensional (3D) structure, and copy number alteration (CNA) in overall TCGA tumors. We also obtained survival data, including progression-free survival, OS, disease-specific survival, and DFS differences for the overall TCGA cancer types, regardless of whether there is *HMGB1* genetic variation.

2.4. Immune infiltration analysis

TIMER2 was used to observe the relationship between *HMGB1*expression and immune infiltrates among TCGA tumors. Cancer-associated fibroblast was selected for detailed analysis. The EPIC, MCPCOUNTER, XCELL, and TIDE algorithms were used for estimations. The data was visualized as heatmaps and scatter plots.

2.5. HMGB1 enrichment analysis

STRING (https://string-db.org/) web was used for protein-protein interaction network analysis. We set the following main parameters: minimum required interaction score ("low confidence [0.150]"), meaning of network edges ("evidence"), maximum number of interactors to be displayed ("no more than 50 interactors" in first shell), and active interaction sources ("experiments").

On the basis of the datasets of all TCGA tumors and normal tissues, GEPIA2 was used to obtain the top 100 *HMGB1*-related genes. We performed a pairwise Pearson's correlation analysis between *HMGB1* and selected

genes. The *P*-values and correlation coefficient (R) were calculated and displayed in figures. In addition, TIMER2 was applied to obtain heatmap data of selected genes, including *P*-values and partial correlation (cor) in the purity-adjusted Spearman's rank correlation test.

Jvenn[16] was used for cross-analysis to compare the HMGB1-binding and interacted genes. We integrated these two sets of data for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The "tidyr" and "ggplot2" R packages were applied to show the enrichment pathway. Besides, the "clusterProfiler" R packages were applied to conduct Gene Ontology (GO) enrichment analysis. The R language software (R-4.1.0, 64-bit; https://www.r-project.org/) was applied to this analysis. Two-tailed $P < 0.05$ was considered statistically significant[17]. A simple list of methods and corresponding tools is shown in Table 1.

3. Results

3.1. Gene expression analysis

TIMER2 was used to study *HMGB1* expression among different TCGA tumor types. The results demonstrated that *HMGB1* expression was significantly higher in cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), stomach adenocarcinoma (STAD), liver hepatocellular carcinoma (LIHC), rectum adenocarcinoma (READ) ($P < 0.001$), bladder urothelial carcinoma (BLCA), and glioblastoma multiforme (GBM) ($P < 0.01$) than in the control group. Obviously, there were also some tumor types showing undifferentiated expression (e.g., kidney renal clear cell carcinoma [KIRC], cervical squamous cell carcinoma and endocervical adenocarcinoma [CESC], thyroid carcinoma [THCA], and pheochromocytoma and paraganglioma [PCPG]).

Table 1. Methods and corresponding tools

Methods	Tools
Gene expression analysis	TIMER2, GEPIA2, UALCAN, TCGA, GTEx, CPTAC
Survival analysis	GEPIA2, TCGA, GEO
Genetic variation analysis	cBioPortal, TCGA
Immune infiltration analysis	TIMER2, TCGA
Gene enrichment analysis	STRING, TCGA, GEPIA2, TIMER2, Jvenn, R language

TCGA: The Cancer Genome Atlas; TIMER2: Tumor immune estimation resource, version 2; GTEx: Genotype-tissue expression; CPTAC: Clinical Proteomic Tumor Analysis Consortium; GEPIA2: Gene Expression Profiling Interactive Analysis, version 2; GEO: Gene Expression Omnibus; Jvenn: An interactive Venn diagram viewer.

However, compared with the control group, *HMGB1* expression in kidney chromophobe (KICH), lung adenocarcinoma (LUAD) ($P < 0.001$), and prostate adenocarcinoma (PRAD) ($P < 0.05$) were relatively low (Figure 1A).

We used the GTEx dataset to obtain normal tissue data as a control, and further evaluated the *HMGB1* expression differences between the tumor and normal tissues. The results showed that *HMGB1* was highly expressed in CHOL, COAD, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), GBM, brain lower grade glioma (LGG), READ, pancreatic adenocarcinoma (PAAD), STAD, and thymoma (THYM) (Figure 1B, $P < 0.05$).

In addition to transcription, CPTAC was used to evaluate HMGB1 at the protein level. As displayed in Figure 1C, we found that the total protein expression of HMGB1 in COAD, GBM, LIHC, ovarian cancer (OV) was significantly higher than that in normal tissues (all $P < 0.001$). However, the HMGB1 protein expression in the breast cancer (BRCA) ($P < 0.01$), uterine corpus endometrial carcinoma (UCEC), LUAD, and PAAD ($P < 0.001$) was decreased.

By using GEPIA2 tool, we probed the relationship between *HMGB1* expression and tumor pathological stage, including adrenocortical carcinoma (ACC), LIHC, skin cutaneous melanoma (SKCM), and THCA (Figure 1D, all $P < 0.05$).

3.2. Survival analysis

On the basis of the expression level of *HMGB1*, cancer cases were divided into low expression group and high expression group. Then, we used TCGA and GEO datasets to explore the correlation between *HMGB1* expression and prognosis of patients with different tumor types. As displayed in Figure 2A, the high expression of *HMGB1* was related to poor OS in ACC ($P < 0.01$) and LUAD ($P < 0.05$) cancers. On the contrary, the low expression of *HMGB1* was related to poor OS in KIRC ($P < 0.05$) and THYM ($P < 0.05$). DFS analysis displayed that the high expression of *HMGB1* was associated with the poor prognosis in ACC ($P < 0.001$), CESC ($P < 0.01$), HNSC ($P < 0.05$), LUAD ($P < 0.05$), and SARC ($P < 0.05$) (Figure 2B).

3.3. Genetic variation analysis

Cancer in humans occurs as a result of an accumulation of genetic changes. We researched the *HMGB1* genetic variations among different tumor samples in the TCGA list. From our analysis, we found that the frequency of *HMGB1* variation (>4%) was the highest in DLBC tumors, mainly including "mutation" and "deep deletion" types. Colorectal cases have the highest frequency in the

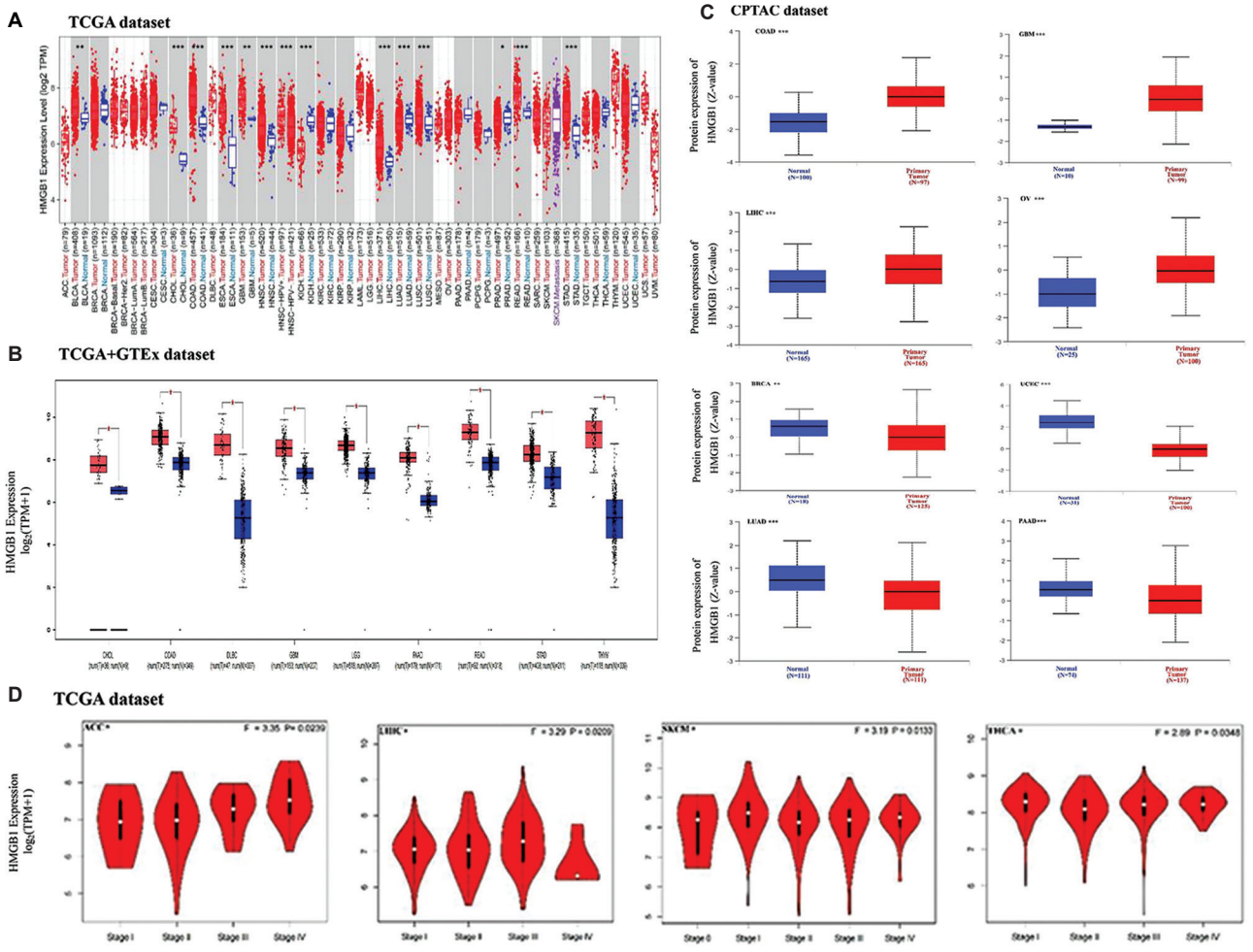

Figure 1. Expression and protein level of HMGB1 in human tumors. (A) Expression level of *HMGB1* in TCGA tumors versus adjacent tissues (if available) as visualized by TIMER2. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. (B) Box plot representation of *HMGB1* expression level comparison in CHOL, COAD, DLBC, GBM, LGG, PAAD, READ, STAD, and THYM (TCGA project) relative to the corresponding normal tissues (GTEx database). *$P < 0.05$. (C) Total protein level of HMGB1 in normal tissue and primary COAD, GBM, LIHC, OV, BRCA, UCEC, LUAD, and PAAD. Protein data was extracted and analyzed using CPTAC. **$P < 0.01$; ***$P < 0.001$. (D) Stage-dependent expression level of *HMGB1*. Major pathological stages (stage Ⅰ, stage Ⅱ, stage Ⅲ, and stage Ⅳ) of ACC, LIHC, SKCM, and THCA were assessed and compared using TCGA data. Expression levels are expressed in Log 2 (TPM + 1).
HMGB1: High mobility group box 1; TCGA: The Cancer Genome Atlas; TIMER2: Tumor immune estimation resource, version 2; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBC: Diffuse large B-cell lymphoma; GBM: Glioblastoma multiforme; LGG: Lower grade glioma; PAAD: Pancreatic adenocarcinoma; READ: Rectum adenocarcinoma; STAD: Stomach adenocarcinoma; THYM: Thymoma; GTEx: Genotype-tissue expression; LIHC: Liver hepatocellular carcinoma; SKCM: Skin cutaneous melanoma; THCA: Thyroid carcinoma; BRCA: Breast cancer; UCEC: Uterine corpus endometrial carcinoma; LUAD: Lung adenocarcinoma; CPTAC: Clinical Proteomic Tumor Analysis Consortium.

"amplification" type of CNA, with a frequency of about ~4% (Figure 3A). It is worthwhile noting that all ACC cases with genetic variation (about ~1% frequency) had *HMGB1* copy number deletion (Figure 3A). Figure 3B shows the types, sites and number of cases of *HMGB1* gene variation. We have not distinguished the main types of genetic changes, and their locations appear to be sporadic, with some belonging to the HMG-box domain. For example, a truncating mutation, R163*/Q alteration, in the HMG-box domain, was only detected in 2 cases of UCEC and 1 case of GBM. The R163*/Q site is in the 3D structure of HMGB1 protein (Figure 3C). Besides, we searched the underlying association between certain genetic variations of *HMGB1* and the clinical survival prognosis of patients with different cancer types. We studied and collected information of various tumor types, and the data in Figure 3D showed that UCEC patients with *HMGB1* variation had better prognosis in terms of progression-free survival (PFS) ($P < 0.05$) than those without *HMGB1* variations. However, no significant differences were observed in OS ($P = 0.0505$), disease-specific survival (DSS) ($P > 0.05$), and DFS ($P > 0.05$).

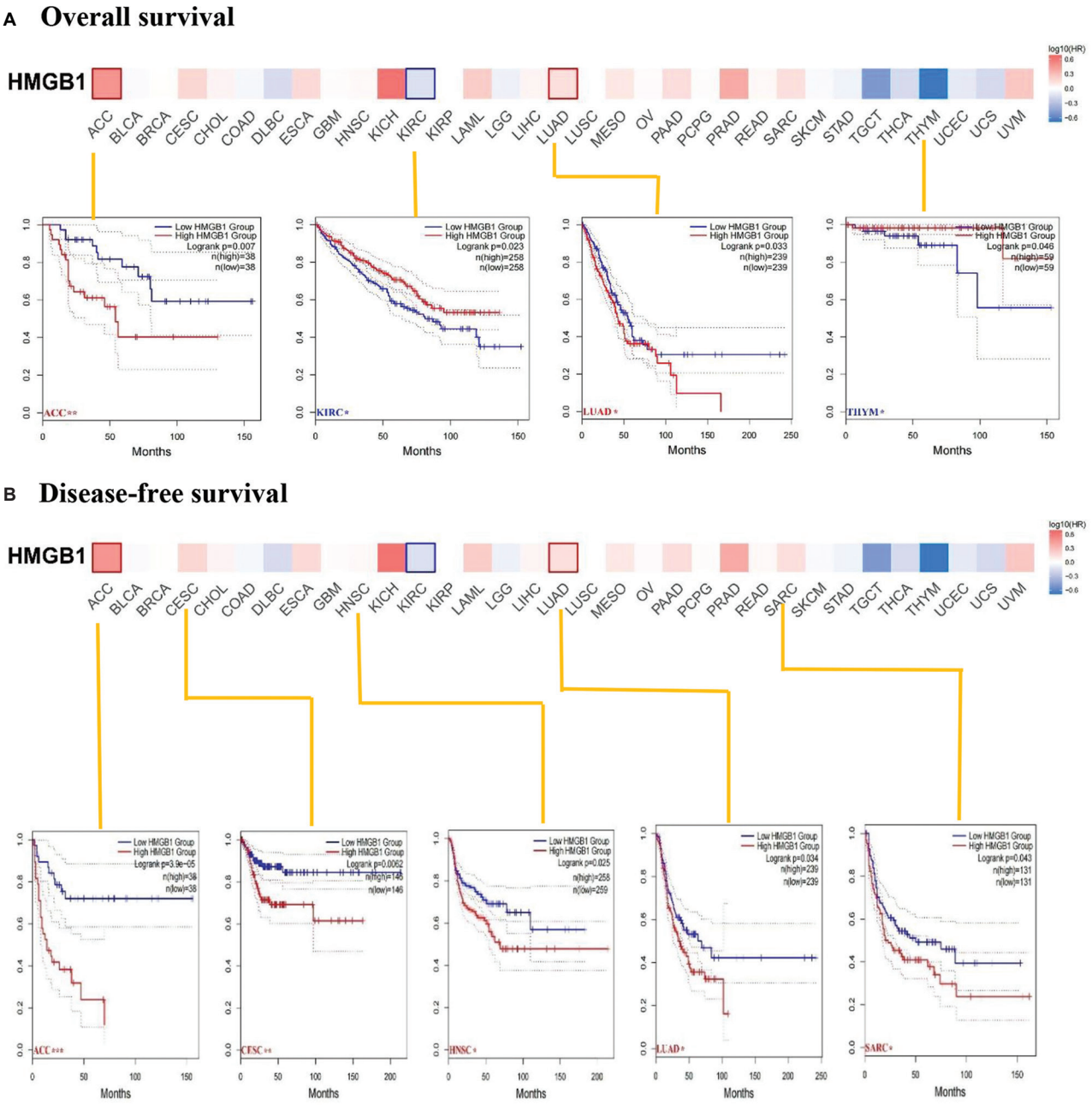

Figure 2. Correlation between the expression level of *HMGB1* and patient survival in TCGA tumors. GEPIA2 was used to evaluate the relationship between *HMGB1* gene expression and overall survival (A) and disease-free survival (B) in all TCGA tumors. Positive results of survival diagram and Kaplan–Meier curves are presented.
HMGB1: High mobility group box 1; TCGA: The Cancer Genome Atlas; GEPIA2: Gene Expression Profiling Interactive Analysis, version 2.

3.4. Protein phosphorylation analysis

Phosphorylation is one of the key processes for protein to exert biological effects, and phosphorylation state (e.g., phosphorylation and dephosphorylation) is known to be critical in the occurrence of tumor. Therefore, the differences of HMGB1 phosphorylation levels between normal tissues and primary tumor tissues were compared. CPTAC dataset was used to analyze three kinds of tumors (BRCA, lung cancer and UCEC) in details. As shown in Figure 4A, we observed that S35 phosphorylation level of HMGB1 in primary tumor tissues of BRCA, lung cancer and UCEC was significantly reduced. At the same time, we found that the S100 phosphorylation level of BRCA and lung cancer was low (Figure 4B–D).

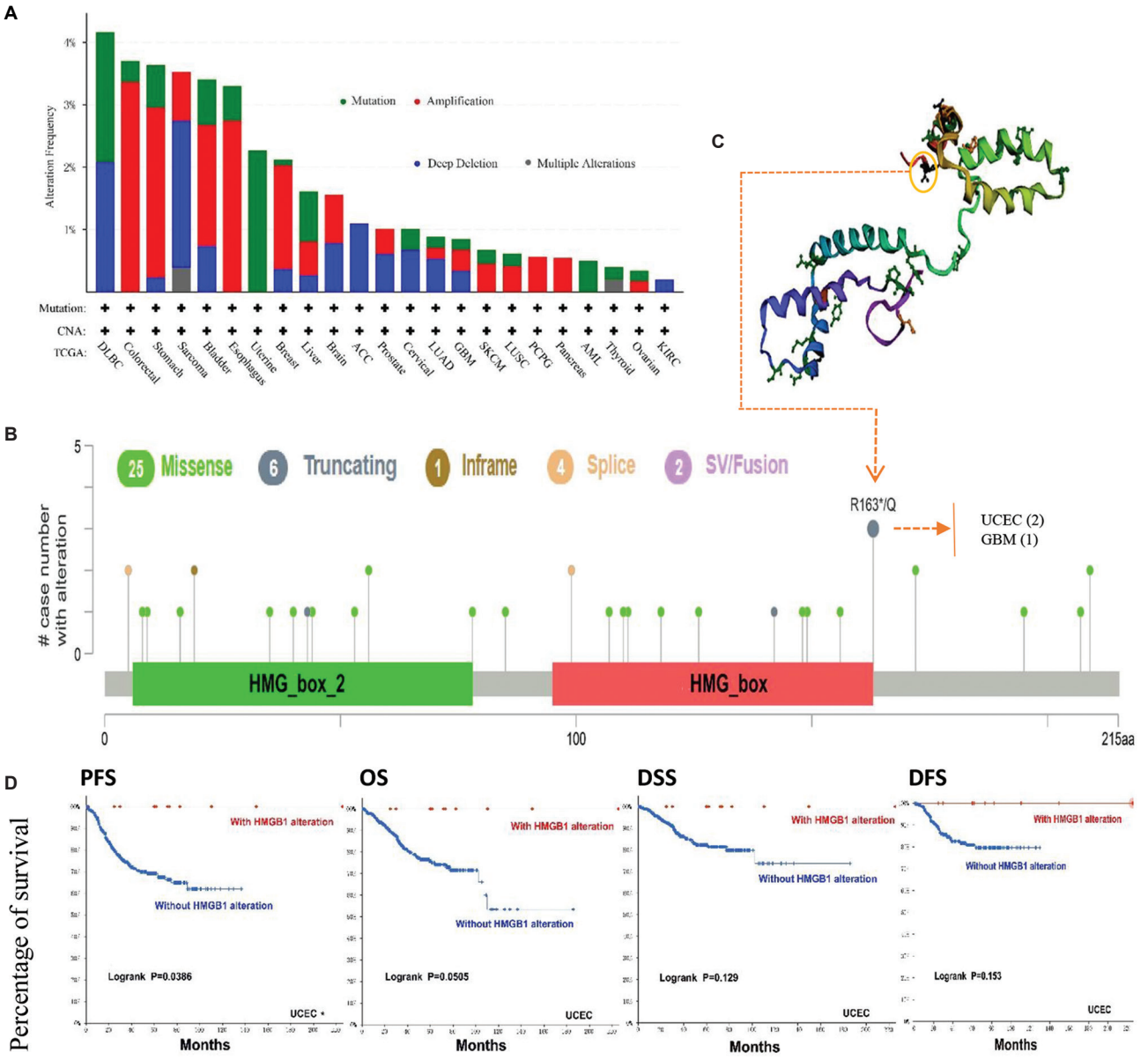

Figure 3. Mutation status of *HMGB1* in TCGA tumors. Mutation status of *HMGB1* in TCGA tumors was analyzed using the cBioPortal tool. The alteration frequency with mutation type (A) and mutation site (B) is displayed. (C) Mutation site (R163*/Q) within the HMG-box domain is shown in the 3D structure of *HMGB1*. (D) Analysis of the correlation between mutation status and PFS, OS, DSS, and DFS of UCEC using the cBioPortal tool.
HMGB1: High mobility group box 1; TCGA: The Cancer Genome Atlas; PFS: Progression-free survival, OS: Overall survival, DSS: Disease-specific survival; DFS: Disease-free survival; UCEC: Uterine corpus endometrial carcinoma.

3.5. Immune infiltration analysis

As a significant part of tumor microenvironment, tumor-infiltrating immune cells are closely related to the development, occurrence and metastasis of tumors[18-20]. Cancer-associated fibroblasts in tumor microenvironment matrix can take part in regulating the function of various tumor infiltrating immune cells[21-23]. In this study, EPIC, MCPCOUNTER, XCELL, and TIDE algorithms were used to seek the potential relationship between different infiltration levels of immune cells and *HMGB1* gene expression across different TCGA tumor types. After analysis, we found that *HMGB1* expression was positively related to the estimated infiltration value of cancer-associated fibroblasts of BRCA-LumA, HNSC-HPV$^-$, MESO, and testicular germ cell tumors (TGCT), but THYM was negatively correlated (Figure 5). The scatterplots of the above-mentioned tumors are shown in Figure 5.

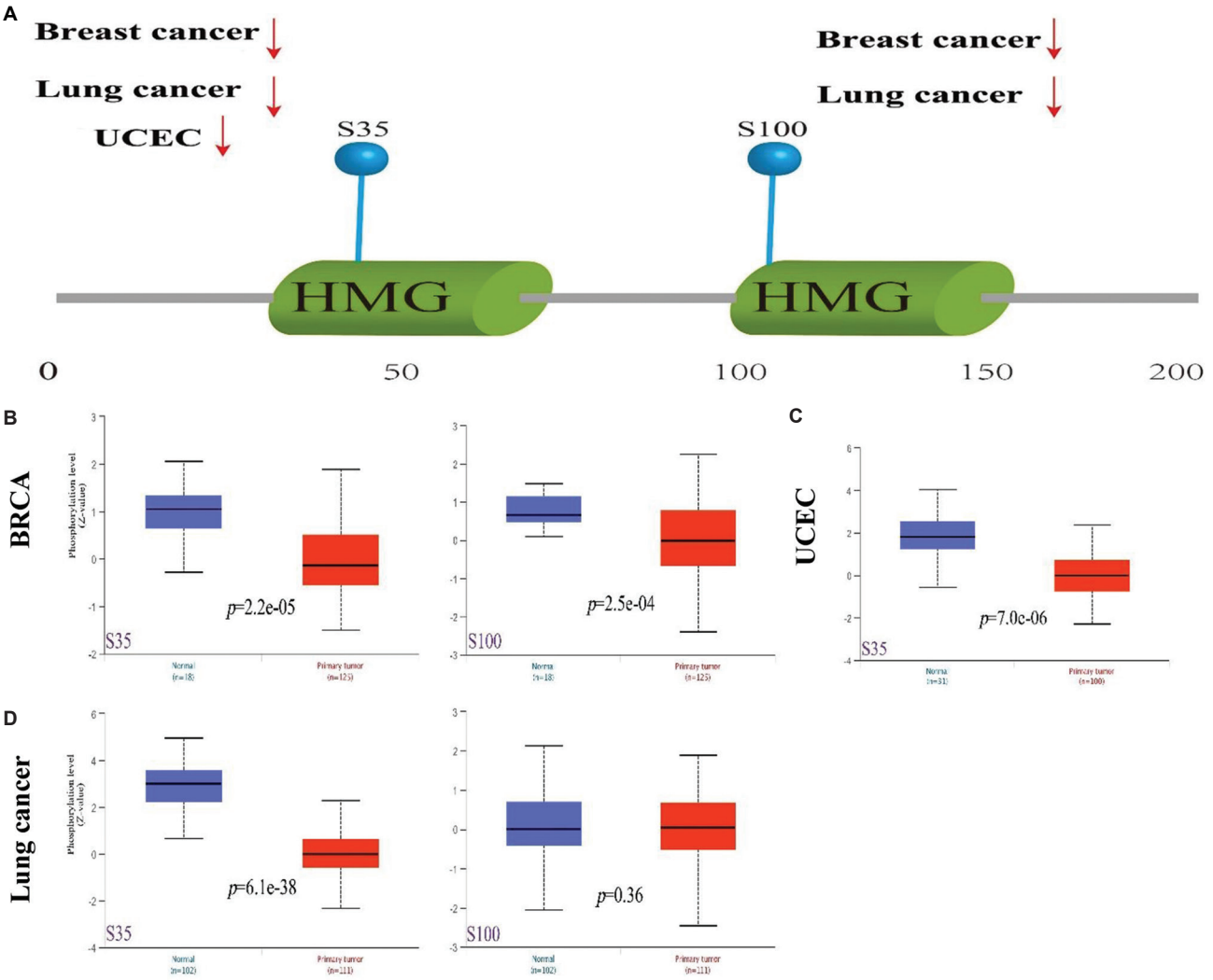

Figure 4. Tumor-associated protein phosphorylation of HMGB1. Comparison of HMGB1 phosphorylated protein levels (S35 and S100 sites) in normal and selected primary tumor tissues. (A) Phosphorylated protein sites detected in HMGB1 based on the CPTAC dataset are shown in the figure. (B–D) Box plots representation of HMGB1 phosphorylated protein levels in BRCA, UCEC and lung cancer.
HMGB1: High mobility group box 1; UCEC: Uterine corpus endometrial carcinoma; CPTAC: Clinical Proteomic Tumor Analysis Consortium; BRCA: Breast cancer.

3.6. HMGB1 enrichment analysis

To further research the molecular mechanism of *HMGB1* gene in the occurrence and development of tumor, the HMGB1-interacting proteins and *HMGB1* expression-related genes were screened by enrichment analyses. STRING was used to get a total of 50 HMGB1 interacting proteins which were verified in the experiments. The relationships of these proteins are displayed in Figure 6A. GEPIA2 was applied to combine all tumor expression data of TCGA to obtain the top 100 genes that are related to *HMGB1* expression. The expression level of *HMGB1* was positively related to that of heterogeneous nuclear ribonucleoproteins A2/B1 (*HNRNPA2B1*) (R = 0.68), heterogeneous nuclear ribonucleoprotein D0 (*HNRNPD*) (R=0.61), heterogeneous nuclear ribonucleoprotein R (*HNRNPR*) (R = 0.63), KH domain-containing, RNA-binding, signal transduction-associated protein 1 (*KHDRBS1*) (R = 0.61), replication factor C subunit 3 (*RFC3*) (R = 0.63), and structure-specific recognition protein 1 (*SSRP1*) (R = 0.55) genes (all $P < 0.001$, Figure 6B). The corresponding heatmap indicated that *HMGB1* was positively correlated with the above six genes in most cancer types (Figure 6C). The interaction analysis of the above groups displayed that there were four common members, namely, SSRP1, SRSF1, SUPT16H, and high mobility group box 2 (HMGB2) (Figure 6D).

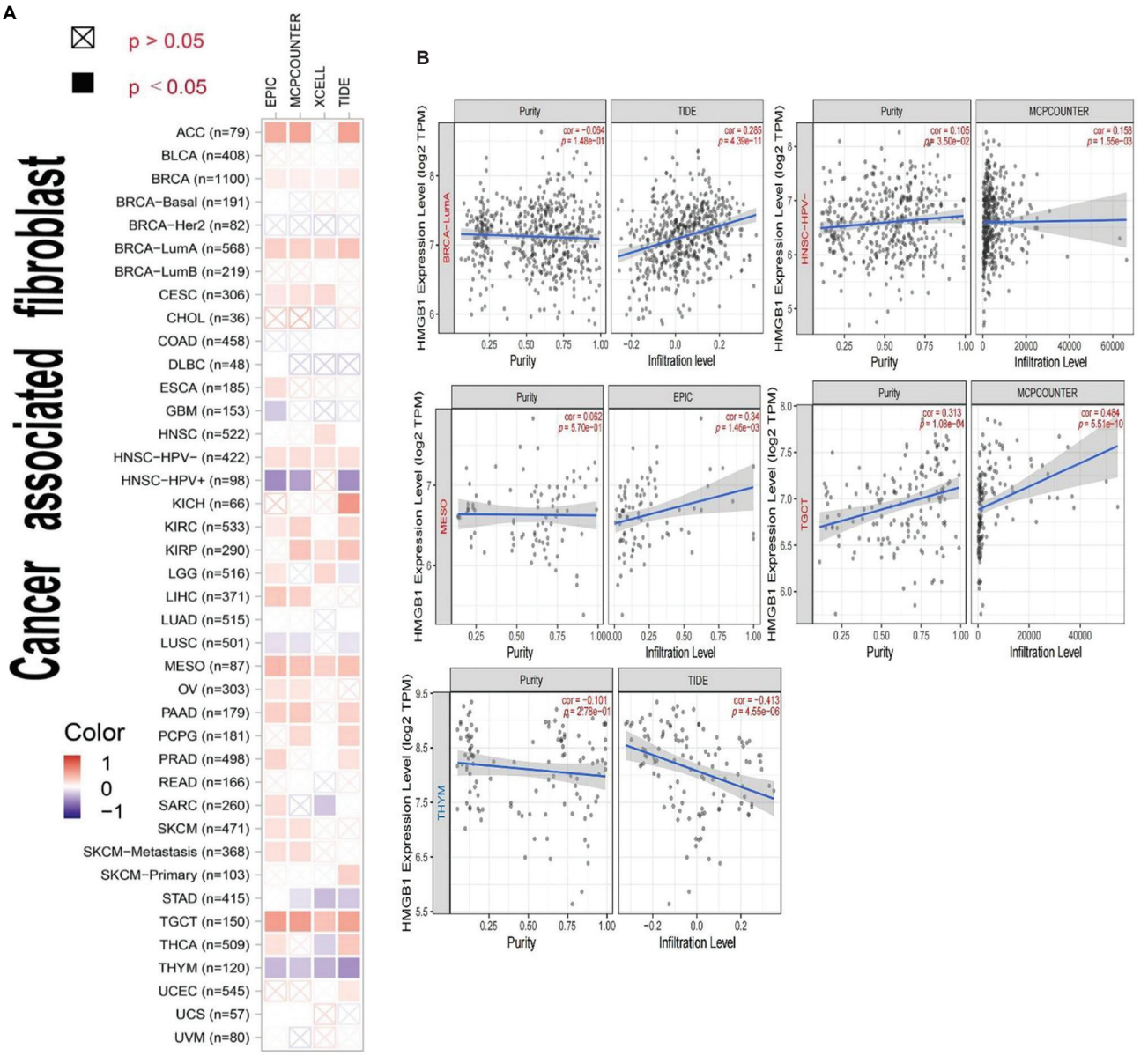

Figure 5. Correlation between *HMGB1* expression level and infiltration of cancer-associated fibroblasts. (A) EPIC, MCPCOUNTER, XCELL, and TIDE algorithms were applied to analyze the correlation between the level of cancer-associated infiltration of fibroblasts and the level of *HMGB1* gene expression in all TCGA tumors. Red color indicates a positive correlation (0–1), and blue color represents a negative correlation (−1–0). $P < 0.05$ is considered statistically significant. Values with no statistically significant correlation are marked with crosses. (B) The associated results of scatter plots of BRCA-LumA, HNSC-HPV-, MESO, TGCT, and THYM are listed.

HMGB1: High mobility group box 1; TCGA: The Cancer Genome Atlas; BRCA: Breast cancer; HNSC: Head and neck squamous cell carcinoma; MESO: Mesothelioma; TGCT: Testicular germ cell tumors; THYM: Thymoma.

KEGG and GO enrichment analyses were used to analyze the above two datasets which we combined. In Figure 6E, KEGG data revealed that "spliceosome" and viral carcinogenesis maybe involved in the influence of HMGB1 on the pathogenesis of tumors. GO enrichment analysis data showed that most of these genes are related to DNA state, such as catalytic activity acting on DNA, chromatin DNA binding, single-stranded DNA binding, telomeric DNA binding, and helicase activity (Figure 6F). A simple list of expression/mutation of HMGB1 among different tumor types and its function is shown in Table 2.

4. Discussion

Many studies have shown that HMGB1 has many biological functions across different cell types and

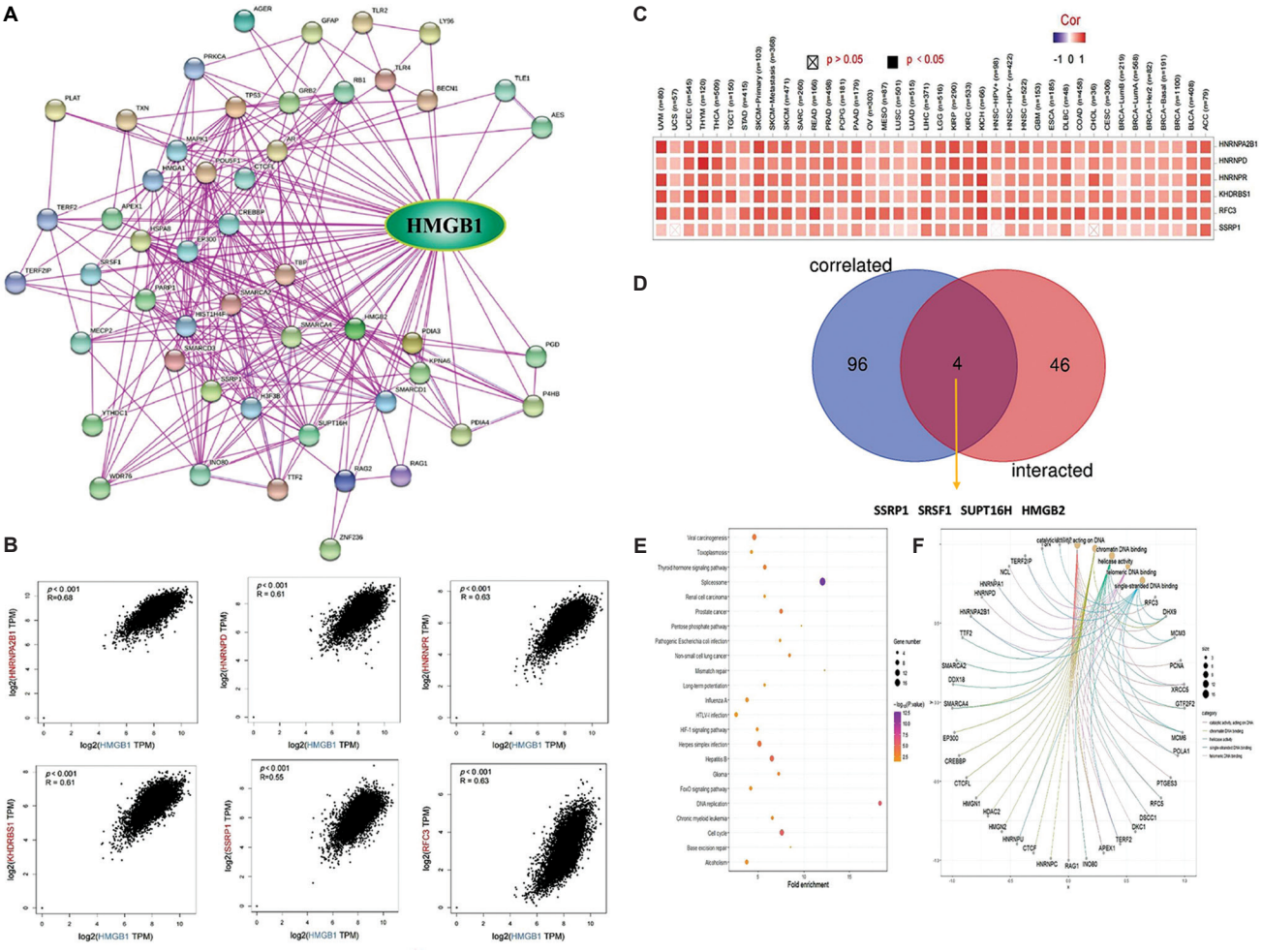

Figure 6. HMGB1 enrichment and pathway analysis. (A) STRING protein network diagram of experimentally determined HMGB1-binding proteins. Colored nodes represent the individual proteins identified. (B) GEPIA2 was used to determine the expression correlation between representative genes (*HNRNPA2B1*, *HNRNPD*, *HNRNPR*, *KHDRBS1*, *RFC3*, and *SSRP1*) of the top *HMGB1*-related genes and *HMGB1* in TCGA tumors. (C) The expression-related data between *HMGB1* and *HNRNPA2B1*, *HNRNPD*, *HNRNPR*, *KHDRBS1*, *RFC3,* and *SSRP1* in TCGA tumors are shown by heatmap. (D) The common members found by the interaction analysis of the HMGB1-binding and correlated genes. (E and F) KEGG and GO analyses based on the HMGB1-binding and interacted genes.

HMGB1: High mobility group box 1; TCGA: The Cancer Genome Atlas; GEPIA2: Gene Expression Profiling Interactive Analysis, version 2; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Ontology; *HNRNPA2B1*: Heterogeneous nuclear ribonucleoproteins A2/B1; *HNRNPD***:** Heterogeneous nuclear ribonucleoprotein D0; *HNRNPR*: Heterogeneous nuclear ribonucleoprotein R; *KHDRBS1*: KH domain-containing, RNA-binding, signal transduction-associated protein 1; *RFC3*: Replication factor C subunit 3; *SSRP1*: Structure-specific recognition protein 1.

plays a critical role in many diseases such as cancer and inflammatory diseases[24-27]. Studies have shown that HMGB1 is a highly conservative protein in different species, and its functions in the nucleus are very complex, including stabilizing nucleosome formation, promoting the DNA bending, and increasing DNA transcription, repair, replication, etc[28,29]. Although studies have reported the functions of HMGB1 in many physiological processes, whether HMGB1 is involved in the pathogenesis of different tumor types remains unclear. Therefore, we conducted a pan-cancer analysis of HMGB1. The methods include exploring HMGB1 gene expression in 33 different tumors based on TCGA data, collecting and integrating protein and phosphor-protein data, as well as gene mutations, and other molecular characteristics by making use of GEO and CPTAC databases.

From our results, the expression level of *HMGB1* in CHOL, ESCA, HNSC, LIHC, STAD, LUSC, COAD, READ, BLCA, and GBM tumor tissues is higher than that of control tissues, but low expression was observed in KICH, LUAD, and PRAD. These results are similar to those found in other studies on STAD, COAD, LIHC, and LUAD[30-33]. The differential expression of *HMGB1*

Table 2. Expression/mutation of HMGB1 among different tumor types and its function

Cancer types	Gene expression	Survival	Genetic variation	Protein phosphorylation	Immune infiltration
CHOL	↑				
COAD	↑				
ESCA	↑				
HNSC	↑	↑			↑
LIHC	↑				
LUSC	↑				
READ	↑				
STAD	↑				
BLCA	↑				
GBM	↑				
KIRC	--	↓			
THCA	--				
PCPG	--				
CESC	--	↑			
KICH	↓				
LUAD	↓	↑		↓	
PRAD	↓				
DLBC	↑				
LGG	↑				
PAAD	↑				
THYM	↑	↓			↓
OV	↑				
BRCA	↓			↓	↑
UCEC	↓		↓	↓	
ACC		↑			
SARC		↑			
MESO					↑
TGCT					↑

↑: Tumor-promoting effect; -- : Insignificant correlation; ↓: Tumor-suppressing effect.
CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; ESCA: Esophageal carcinoma; HNSC: Head and neck squamous cell carcinoma; LUSC: Lung squamous cell carcinoma; STAD: Stomach adenocarcinoma; LIHC: Liver hepatocellular carcinoma; READ: Rectum adenocarcinoma; BLCA: Bladder urothelial carcinoma; GBM: Glioblastoma multiforme; KIRC: Kidney renal clear cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; THCA: Thyroid carcinoma; PCPG: Pheochromocytoma and paraganglioma; KICH: Kidney chromophobe; LUAD: Lung adenocarcinoma PRAD: Prostate adenocarcinoma; DLBC: Diffuse large B-cell lymphoma; LGG: Lower grade glioma PAAD: Pancreatic adenocarcinoma; THYM: Thymoma; OV: Ovarian cancer; BRCA: Breast cancer; ACC: Adrenocortical carcinoma; UCEC: Uterine corpus endometrial carcinoma; MESO: Mesothelioma; SARC: Sarcoma; TGCT: Testicular germ cell tumors

in different tumors may suggest different potential mechanisms and functions. We also found that high *HMGB1* expression may predict the poor OS for patients with ACC and LUAD. These results indicate that *HMGB1* can be used as a biomarker to predict the prognosis of tumor patients.

We discovered that the low expression of *HMGB1* was related to poor OS in some TCGA tumors, such as KIRC and THYM. This is similar to what other studies have found[34]. However, Huang *et al.* revealed that HMGB1 overexpression was significantly correlated with poor prognosis of patients with BLCA[35]. These findings may suggest that HMGB1 expression is not the same across various kinds of tumors. Based on the characteristics of each tumor, high or low expression of HMGB1 may provide a useful reference for the clinical treatment of tumor patients.

As for lung cancer, our research found that high expression of HMGB1 was correlated with poor OS ($P < 0.05$), and poor DFS ($P < 0.05$) in LUAD, but not in LUSC. In fact, many studies have shown that HMGB1 can promote the occurrence, proliferation, and metastasis of lung cancer[36,37], but the molecular mechanism and signaling pathway involving HMGB1 still need to be further clarified.

Compared with the corresponding control tissues, the total protein expression level of HMGB1 in BRCA tumor tissues was relatively lower. Studies have shown that HMGB1 has a close relationship with BRCA. The majority of the BRCA studies have reported that HMGB1 can promote the migration and invasion of BRCA[38,39]. MiR-142-3p can enhance the chemosensitivity of BRCA and inhibit autophagy by targeting *HMGB1*[40]. Our findings are contradictory to the literature reports. We consider that these may be linked to the dual role of HMGB1 in tumors. These results also indicate that HMGB1 may play a crucial role in the treatment of BRCA.

In addition to ACC and LUAD, our survival analysis data also indicated that the high expression of *HMGB1* in CESC, HNSC, and SARC was associated with poor DFS. Li *et al.* and Xu *et al.* suggested that HMGB1 expression was a predictor of shorter OS and DFS in patients with cervical cancer[41,42]. Liu *et al.* also indicated that HMGB1 was upregulated in human HNSC and overexpression of HMGB1 was significantly related to the malignant progression and poor survival rate of HNSC patients, suggesting that HMGB1 might be a potential therapeutic target of HNSC[43].

In this study, our results showed that *HMGB1* expression was positively correlated with the level of cancer-associated fibroblast immune infiltration in certain tumors, including BRCA-LumA, TGCT, HNSC-HPV$^-$, and MESO. Nevertheless, the relationship between *HMGB1* expression and THYM was reversed.

5. Conclusions

Our pan-cancer analysis of *HMGB1* demonstrates that the expression of *HMGB1* is associated with clinical prognosis, protein phosphorylation and immune cell infiltration in various human tumors. These findings are very helpful to decipher the role of HMGB1 in tumorigenesis and to further uncover the functions of HMGB1 in tumors.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets in this study are available in online repositories, TCGA datasets (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), GTEx datasets (https://www.genome.go-v/Funded-Programs-Projects/Genotype-Tissue-Expression-Project), and GEO databases (https://www.ncbi.nlm.nih.gov/geo/).

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