Multimolecular Characteristics and Role of BRCA1 Interacting Protein C-Terminal Helicase 1 (BRIP1) in Human Tumors: A Pan-Cancer Analysis

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Abstract

Background

The aberrant expression of BRIP1 was associated with several cancers, the panoramic picture of BRIP1 in human tumors is unclear. The purpose of this study is to explore the panoramic picture of expression of BRIP1 which was associated with several cancers.

Methods

Based on the data from TCGA, we utilized online databases to systematically analyze the multimolecular characteristics of BRIP1 in 33 human tumors.

Results

We observed prognosis-related differential BRIP1 expression between various carcinomas and the corresponding normal tissues. “Basal transcription factors”, “Homologous recombination”, “Nucleotide excision repair”, and DNA metabolism pathways may play a role in the functional mechanisms of BRIP1. Patients with uterine corpus endometrial carcinoma presented with the highest alteration frequency of BRIP1 (near 10%). Single nucleotide and copy number variation of BRIP1 were noticed in multiple cancer, and the expression of BRIP1 is significantly regulated by copy number variation in breast invasive carcinoma and lung squamous cell carcinoma. BRIP1 expression was negatively correlated to the methylation level in many human tumors, and the expression was associated with the activation of apoptosis, cell cycle, and DNA damage response, and inhibition of hormone ER and RNS/MARK signaling pathways. Moreover, a positive correlation was observed between BRIP1 expression and the immune infiltration level of cancer-associated fibroblasts and CD8 + T cells in lung adenocarcinoma.

Conclusion

Our pan-cancer analysis of BRIP1 provide valuable resource for understanding the characteristics of BRIP1 across human cancers.

Background

Over the past decade, the incidence and mortality of tumors have continued to rise. A recent study based on data of 38 cancers from 185 countries estimated that there were more that 19 million new cancer cases in 2020 worldwide, and nearly half of them died because of cancer(1). To figure out the molecular mechanisms of tumor carcinogenesis, development, prognosis, and factors that influence the treatment efficiency, many studies had been taken; influences such as tumor microenvironment (TME)(2), mutations(3), epigenetics(4), and immune microenvironment(5) had been identified.

As one of *DEAH* helicase family members, BRCA1 Interacting Protein C-THelicase 1 (BRIP1), also known as BRCA1-associated C-terminal helicase-1 (BACH1), is consisted of 20 exons and located at 17q23 chromosome(6). BRIP1 was found linked to Fanconi Anemia (FA), which is an autosomal recessive genetic disease, and characterized by cancer susceptibility, bone marrow failure, and multiple physical abnormalities(7). Since mutations of BRIP1 were noticed in patients of Fanconi Anemia who belongs to the complementation group J, BRIP1 was also named FANCJ(8). With respect to the physiology of BRIP1, direct interaction between BRCA1 and BRIP1 was detected, and such interaction is facilitated by an important domain of BRCA1 (BRCT), which is essential for establishing the G2 cell-cycle when responding to DNA damage(9). Besides, PALB2 (FANCN), BRCA2, and BRIP1 are three of the crucial genes that worked commonly in the FA-BRCA pathway, acted in the downstream of FANCD2, the details of these interactions were shown in Figure S1. Meanwhile, the direct contribution of BRIP1's helicase domains in DNA repairing sites was suggested(10). Recently, the association of BRIP1 and cancers had been reported; however, most of the studies were carried on ovarian, breast, and pancreatic cancer, and most studies focused on mutation aspect(6, 11, 12). There is still a lack of multiple mechanism characteristics analysis of BRIP1 in other human tumors.

For the first time, we used multiple online databases to perform a pan-cancer analysis of BRIP1 in various human cancers in the current study. We investigated several molecular factors, including gene expression, survival, related genes enrichment, methylation, genetic alteration, and immune infiltration analysis to explore the impending mechanisms of BRIP1 in the carcinogenesis, development, and clinical outcomes of various human tumors, the design of the current study was shown in Fig. 1.

Materials And Methods

Gene expression analysis

We utilized the ONCOMINE database(13) (www.oncomine.org) to explore the mRNA expression of BRIP1 in various cancer types between normal and tumor tissues under the settings of fold change = 1.5, *P*-value = 0.05. We recoded the datasets which are statistically significant and performed a series of pooling analyses across more than two comparisons.

We also logged into the TIMER2(14) (http://timer.cistrome.org/) website to to analyze the differential BRIP1 expression between cancer and adjacent normal tissues across 33 human carcinomas of TCGA by inputting BRIP1 in the "Gene-DE" module. The expression levels of BRIP1 were performed using box plots and adjusted to log2(TPM) data. While the differences of BRIP1 expression were compared using Wilcoxon test. Since the data of normal tissues in TIMER2 database is unavailable for several cancers [e.g., SARC (Sarcoma), THYM (Thymoma), etc.], we then logged into the GEPIA2(15) (http://gepia2.cancer-pku.cn/#analysis) website to evaluate the differential expression levels of BRIP1 between these tumor tissues and their adjacent normal tissues using the "Expression DIY" module. The cutoff points were set as "Match TCGA normal and GTEx data", *P*-value = 0.05, and log2FC (fold change) = 1, and the expression levels were displayed with box plots. In addition, the pathological stage plots were

also obtained which could reflect the BRIP1 expression among different stages (stage I-IV) across all TCGA cancers.

To examine the differential proteins expression of BRIP1 among various tumors, we logged into The Human Protein Atlas(16) (https://www.proteinatlas.org) website to compare the BRIP1 protein expression between breast cancer, lung cancer, colorectal cancer, liver cancer, prostate cancer, and their adjacent normal tissues by performing immunohistochemistry image. The data of BRIP1 expression in different tumors was obtained. Besides, we also retrieved the differential expression level of BRIP1 in various tissues, blood cells, and brain tissues.

Survival analysis

Using GEPIA2 database, we also compared the survival contribution of BRIP1 in all TCGA tumors by entering “BRIP1” in the “Survival Map” module, estimated using Mantel–Cox test. The significance map of Overall Survival (OS) and Disease-Free Survival (DFS) was generated under the significance threshold *P*-value as 0.05, and the cohort with an expression over Cutoff-high (50%) values was considered as high-expression. Meanwhile, for the cancer types with statistical significance of OS and DFS, we also obtained the Kaplan-Meier curves under the “Survival Analysis” module of GEPIA2.

BRIP1 related gene enrichment analysis

At first, we logged into the STRING(17) (https://string-db.org/) website to obtain the BRIP1-binding proteins that were experimentally determined by entering “BRIP1” under the single protein name module, and “Homo sapiens” was chosen as the organism. In the “Settings” section, we set the following filters: “full network” as the network type, “evidence” as the meaning of network edges, “Experiments” as the active interaction sources, “low confidence (0.15)” as the minimum required interaction score, and “no more than 50 interactors” as the max number of interactors to show. A protein-protein interaction (PPI) analysis was conducted among the selected 50 genes, the interaction among genes were analyzed by STRING database and visualized by Cytoscape

Then, based on the datasets of all TCGA tumors, we utilized the “Similar Gene Detection” function of GEPIA2 to get the top 100 genes with highest correlation with BRIP1. For the top five BRIP1-correlated genes, we also performed a Pearson correlation analysis between BRIP1 and each selected correlated gene using GEPIA2 database under the “correlation analysis” module, and for the dot plot, the log2 TPM was implemented. Moreover, the heatmap data of the five genes were generated using the “Gene_Corr” module of TIMER2 database, the purity-adjusted P-value and partial correlation (cor) were supplied.

Thirdly, we utilized Venn online website (http://bioinformatics.psb.ugent.be/webtools/Venn/) to compare the BRIP1-interacted and binding genes by conducting an intersection analysis. Additionally, we also combined the two sets of genes to conduct Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Oncology (GO) enrichment analysis in the database of Metascape(18). There are three components contained in GO analysis, molecular functions (MF), cellular components (CC), and biological processes

(BP), which could predict the functional roles of genes closely related to BRIP1, while KEGG analysis could delineate the pathways of the genes related to BRIP1.

Mutation, Methylation and Genome-wide association of BRIP1 mRNA in cancers

By logging into the cBioPortal(19) (http://www.cbioportal.org) website, the Copy number alteration (CNA), mutation types, and alteration frequency of BRIP1 across different cancers can be observed under the "TCGA Pan Cancer Atlas Studies" module, in the "Cancer Types Summary" section. The mutation sites and three-dimensional (3D) structure of BRIP1 can be obtained in the "Mutation" section. While in the "Comparison/Survival" section, for a certain TCGA cancer type, we can get the differential data of disease-specific, progress-free, overall, and disease-free survival between altered and unaltered groups. The results were displayed as Kaplan-Meier plots, and log-rank test *P*-value was supplied.

We utilized the MEXPRESS(20) (https://mexpress.be/) database to explore the association of BRIP1 expression and methylation levels across all TCGA tumors, only cancer types with statistical significance were displayed. The methylation level of each probe was presented with a beta value, the adjusted p-value (Benjamini-Hochberg) and the Pearson correlation coefficient value (R) were also provided.

Additionally, we also logged into GSCALite(21) (http://bioinfo.life.hust.edu.cn/web/GSCALite/) website to evaluate the correlation between methylation and expression of BRIP1 and its five most correlated genes across 33 TCGA tumor, the results were displayed as bubble maps. Besides data of methylation, GSCALite also provides information of the SNV (Single Nucleotide Variation) frequency and variant types of BRIP1 and the five most related genes in all TCGA tumors, as well as the statistics of heterozygous and homozygous CNV (Copy Number Variation) of each cancer type. Besides, Pearson correlation was performed to explore the association between genes' expression and CNV of each cancer, which could help to investigate the influence of CNV on the expression of selected genes. Additionally, the differences of BRIP1 and the five most related genes' expression between pathway activation and inhibition groups across all TCGA tumors, which are defined by pathway scores, were displayed.

Moreover, we used the Cancer Regulome tools (http://explorer.cancerregulome.org/) to evaluate the association of BRIP1 expression with other genes in various tumors. The correlations were displayed as circus diagrams, based on the links between Protein level-RPPA, somatic mutation, microRNA expression, somatic copy number, DNA methylation and gene expression.

Immune infiltration analysis

To explore the correlation between the expression and immune infiltrates of BRIP1 in different cancers, the "Immune-Gene" module of TIMER2 database was implemented. We selected the cancer-associated fibroblasts and CD8 + T-cells for immune infiltration estimation, using the algorithms of EPIC, MCPCOUNTER, QUANTISEQ, CIBERSORT-ABS, CIBERSORT, XCELL, and TIMER. The associations were displayed as heatmap and scatter plots, purity-adjusted P-value and partial correlation (cor) were supplied.

Results

Expression analysis of BRIP1

In the current work, we aimed to investigate the multimolecular characteristics and role of BRIP1 (Genome location: chr7(q32.1), consensus CDS: CDS11631.1, Figure S2a). As presented in Figure S2b, conserved domain of DEAD_2 (pfam06733) was commonly consisted in BRIP1 protein structure among different species. The evolutionary relationship of the BRIP1 protein among various species was presented in phylogenetic tree (Figure S3).

We first used the ONCOMINE database to compare BRIP1 mRNA expression between tumors and the adjacent normal tissues (Fig. 2a). Higher expression of BRIP1 were noticed in Brain and CNS cancer, Breast cancer, Cervical cancer, Colorectal cancer, Gastric cancer, Head and Neck cancer, Pancreatic cancer, and Sarcoma than in the corresponding normal tissues. However, significantly downregulated expression of BRIP1 were also noticed in several tumors, the details were shown in Table 1. Collectively, 22 datasets showed a higher mRNA expression of BRIP1 in different tumors than that in normal samples, while 5 datasets showed controversy results, higher expression of BRIP1 were found in the normal tissues. We then assessed the levels of BRIP1 protein expression across different cancers in datasets from Human Protein Atlas (HPA). As shown in Figure S4a, most tumor tissues showed moderate to strong nuclear or nuclear membranous staining, especially in Colorectal cancer, Head and neck cancer, Carcinoid, Urothelial cancer, Prostate cancer, and Melanoma. The expression of BRIP1 in different tissues, blood cells, and brain tissues under the normal physiological state were also evaluated. As presented in Figure S4b, highest expression of BRIP1 was identified in Thymus, followed by Testis, and Bone marrow. Combined the three datasets (FANTOM5 (Function annotation of the mammalian genome 5), GTEx, and HPA) together, we found a BRIP1 expression in all included tissues; however, RNA tissue enhanced (lymphoid tissue) phenomenon was noticed (Figure S4b). Similarly, BRIP1 expression was detected in all blood cells, and phenomenon of low RNA blood cell type specificity was noticed, with the consensus datasets of HPA, Monaco, and Schmiedel (Figure S4c). While in brain tissues, highest expression of BRIP1 was found in Basal ganglia, followed by cerebral cortex, and olfactory region (Figure S4d).

Since the cancer types that can be used to explore the association of BRIP1 expression between tumors and normal tissues are limited on ONCOMINE, we also utilized TIMER2 database to analyze the differential expression of BRIP1 across all TCGA tumors. Figure 2b showed higher BRIP1 expressions in cancer tissues of BLCA (Bladder Urothelial Carcinoma), BRCA (Breast invasive carcinoma), CHOL (Cholangiocarcinoma), COAD (Colon adenocarcinoma), ESCA (Esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), KIRC (Kidney renal clear cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), STAD (Stomach adenocarcinoma), THCA (Thyroid carcinoma), UCEC (Uterine Corpus Endometrial Carcinoma) ($P < 0.001$), CESC (Cervical squamous cell carcinoma), and READ (Rectum adenocarcinoma) ($P < 0.01$) than in the corresponding normal tissues. As there are several cancer types in TIMER2 lacking the data of normal

tissues, we take the data of normal samples from the GTEx dataset to further explore the differential BRIP1 expression between adjacent normal tissues and cancer tissues of DLBC (Lymphoid neoplasm diffuse large B-cell lymphoma), SARC (Sarcoma), THYM (Thymoma), and UCS (Uterine Carcinosarcoma), and higher BRIP1 expressions were noticed in the cancer tissues (Fig. 2c, $P < 0.05$). However, no statistical significance was detected for other cancers (Figure S5a). Besides, the results of pooling analysis from various reports in the ONCOMINE database also verified that BRIP1 is highly expressed in breast cancer, sarcoma, colorectal cancer, and head & neck cancer (Figure S6). Additionally, the correlation between BRIP1 expression and cancers with different pathological stages was investigated using the "Pathological Stage Plot" module of HEPIA2. Significantly differences were found in ACC (Adrenocortical carcinoma), BRCA, SKCM (Skin Cutaneous Melanoma), KIRP, LIHC, LUSC, KIRC, THCA, UCS, KICH (Kidney Chromophobe), and OV (Ovarian serous cystadenocarcinoma) (Fig. 2e). Cancers without significance were shown in Figure S5b-S5d.

Moreover, we analyzed BRIP1 expression in different molecular and immune subtypes. As shown in Figure S7a, significantly different BRIP1 expression was observed in various molecular subtypes of BRCA, LGG, PCPG (Pheochromocytoma and Paraganglioma), COAD, LUSC, STAD, HNSC, KIRP, OV, UCEC ($P < 0.001$ for all), LIHC ($P < 0.01$), SKCM, and ESCA ($P < 0.05$ for all). Figure S7b showed that significant difference of BRIP1 expression exist across immune subtypes of C1 to C6 (represent would healing, IFN-γ dominant, inflammatory, lymphocyte deplete, immunologically quiet, and TGF-β dominant, respectively) in BLCA, LGG, SARC, BRCA, LUAD, SKCM, COAD, LUSC, STAD, ESCA, OV, THCA, KICH, PCPG, UCEC, KIRC, READ, and KIRP. Of interest, lowest BRIP1 expression was noticed in subtype C3 in most cancers, except for LGG and KIRC (BRIP1 expression in C5 is the lowest). For cancer types with no significant difference were presented in Figure S8. Jointly, the differential expression of BRIP1 in various molecular and immune subtypes may contribute to the differing role of BRIP1 in the prognosis of different tumors.

After exploring the differential expression patterns of BRIP1 between tumors and normal tissues, we also used the HPA dataset to examine the protein expression patterns of BRIP1 in breast cancer, lung cancer, colorectal cancer, liver cancer, and prostate cancer. As shown in Fig. 2d, high expression of BRIP1 was found in the tumor tissues, whereas low to medium expression of BRIP1 was noticed in the adjacent normal tissues.

Survival analysis of BRIP1

According to the expressional levels of BRIP1, we divided the cases into two groups (low and high expression groups) to explore the correlation of gene expression and the survival status of patients across different tumors. As performed in Fig. 3a, high expression of BRIP1 was associated with worse OS (Overall Survival) prognosis for ACC, KIRP, LGG, LUAD, MESO (Mesothelioma), and PAAD ($P < 0.05$ for all). While high BRIP1 expression was linked to better OS prognosis of COAD, READ, STAD, and THYM ($P < 0.05$ for all). Data of Disease-free Survival (DFS) analysis indicated that high expression of BRIP1 was correlated to poor DFS prognosis of ACC, LGG, LIHC, PAAD (Pancreatic adenocarcinoma), and THCA ($P < 0.05$ for all) (Fig. 3b).

Besides, evidence from Kaplan-Meier plotter tool showed that high BRIP1 expression was associated with poor OS (P = 0.026), PPS (post-progression survival) (P < 0.01), and DMFS (distant metastasis-free survival) (P < 0.001) prognosis for breast cancer (Figure S9a). For ovarian cancer, high expression of BRIP1 was correlated to worse OS (P = 0.027) and PFS (progress-free survival) (P = 0.036) of patients (Figure S9b). Similarly, high BRIP1 expression was linked to poor OS, FP (first progression), and PPS (P < 0.001 for all) prognosis for lung cancer (Figure S9c). Conversely, high expression of BRIP1 was correlated to better OS (P = 0.024), FP (P < 0.01) and PPS (P < 0.001) prognosis for gastric cancer (Figure S9d). In addition, positive correlation was found between high BRIP1 expression and worse OS (P = 0.021), PFS (P < 0.01), RFS (relapse-free survival) (P = 0.047), and FP (P < 0.001) prognosis for liver cancer (Figure S9e).

We also used the Sangerbox tool to evaluate the independent prognostic role of BRIP1 across all TCGA tumors. As shown in Figure S10, BRIP1 could serve as independent prognostic biomarker to predict the OS of patients for PCPG, ACC, KICH, LGG, READ, MESO, LIHC, KIRP, PAAD, UCEC, PRAD (Prostate adenocarcinoma), and LUAD; to predict the DSS (disease-specific survival) of patients for PCPG, ACC, KICH, LGG, KIRC, COAD, MESO, LIHC, KIRP, PAAD, PRAD, and LUAD; to predict the DFI (disease-free interval) of patients for THCA, LIHC, KIRP, and PAAD; and to predict the PFI (progress-free interval) of patients for UVM (Uveal Melanoma), PCPG, ACC, KICH, LGG, THCA, MESO, LIHC, KIRP, PAAD, PRAD, and LUAD (P < 0.05 for all). Survival analysis data of Sangerbox showed that the AUC (area under curve) for 1-year, 3-year, and 5-year was moderate to high in predicting the OS of patients for ACC, COAD, KIRP, LGG, LUAD, MESO, PAAD, READ, STAD, and THYM (Figure S11).

Moreover, we utilized the the univariate and multivariate Cox regression analysis to calculate the prognostic factors of OS for KIRP, ACC, LGG, COAD, READ, STAD, THYM, LUAD, MESO, and PAAD (Table S1). Clinical characteristics and BRIP1 expression were calculated. In addition, we combined the parameters with statistical significance from univariate analysis to construct the prognostic nomograms for predicting the 1-year, 3-year, and 5-year survival probability for the above cancers (Figure S12). Collectively, we can conclude that in most cancers, the high expression of BRIP1 is significantly correlated to the worse prognosis of patients.

Enrichment analysis of BRIP1

Based on the online tool of String and GEIP12, 50 targeting BRIP1-binding proteins and 100 BRIP1-correlated genes were selected to further explore the multimolecular characteristics and role of BRIP1 in the carcinogenesis, progress, and prognosis of various tumors. Figure 4a showed the PPI network of the 50 proteins. The top 100 most correlated gene of BRIP1 were identified using GEPIA2. As shown in Fig. 4b, BRIP1 was mostly correlated to CLSPN, FANCI, DTL, BRCA1, and TMPO, with correlation coefficient as 0.74, 0.74, 0.72, 0.71, and 0.71, respectively (P < 0.001 for all). In addition, positively correlation between the BRIP1-correlated five genes and BRIP1 was noticed in numerous of cancers (Fig. 4c). Combine the two sets of genes together, we found three commonly members, TOPB1, BRCA1, and BARD1 (Fig. 4d).

Enrichment analysis of KEGG and GO were conducted among the combination of the two sets of genes. As shown in Fig. 4e, pathways of “Basal transcription factors”, “Homologous recombination”, and “Nucleotide excision repair” might partially explain the role of BRIP1 on tumorigenesis of different cancers. Results from GO enrichment analysis further verified that most BRIP1-related genes are associated with DNA metabolism cellular biology or pathways (e.g., DNA replication, DNA repair, chromosomal region, single-stranded DNA binding, ATPase activity, etc.) (Fig. 4e).

Mutation, Methylation and Genome-wide association of BRIP1 analysis

We utilized multiple online databases to obtain the genetic alteration information of BRIP1 across different tumors. Patients with UCEC presented with the highest alteration frequency of BRIP1 (near 10%), and the major type for BRCA patients is the “amplification” of CAN, with an alteration frequency of 8% (Fig. 5a). The 3D structure of BRIP1 was performed in Fig. 5b. Figure 5c showed the case number, sites, and types of BRIP1 alteration, and the primary genetic alteration type of “missense” mutation was observed, with the number of 182. Besides, A745T/V alteration in the Helicase_C_2 domain was found in two cases of UCEC and one case of HNSC. Moreover, the correlation between BRIP1 alteration and clinical prognosis for BRCA was investigated. As shown in Fig. 5d, the alteration of BRIP1 was associated with poor prognosis in DSS ($P = 0.0252$), but not in PFS, OS, and DFS ($P > 0.05$ for all).

Using database of MEXPRESS, the potential correlation between BRIP1 expression and methylation level was explored. As shown in Table S2, significantly correlation was observed between BRIP1 expression and methylation for 24 TCGA tumors, including BLCA, BRCA, CESC, COAD, DLBC, GBM, HNSC, LAML (Acute Myeloid Leukemia), LGG, LIHC, LUAD, LUSC, OV, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS ($P < 0.05$ for all). The correlation between methylation and expression of BRIP1 among different cancers was also evaluated by GSCALite database. Generally, negatively correlations between gene expression and methylation across cancers were observed (Fig. 6a).

In addition, mutation frequency of SNV across TCGA tumors was also analyzed on GSCALite tool. As shown in Fig. 6b, we found a high SNV in many cancers, including UCEC, SKCM, COAD, BLCA, READ, CESC, LUAD, ESCA, and HNSC. The constitute of Heterozygous/Homozygous CNV of BRIP1 in 33 TCGA tumors were presented in Fig. 6c. It is worth noticing that heterozygous amplification is the major type of CNV in KIRP, while the homozygous deletion type of CNV is the primary type in KICH. Besides, the correlation between CNV and mRNA expression of BRIP1 were also explored, and the results were performed in Fig. 6d. Notably, highest correlation was observed for BRCA and LUSC, which indicated the BRIP1 expression is significantly regulated by CNV in BRCA and LUSC.

Besides, we further explored the genome-wide association of BRIP1 mRNA in cancer by analyzing the correlation between BRIP1 and other genes using the Regulome Explorer. As shown in Fig. 7, positively correlation was observed in multiple cancers, and the detailed information was presented in Table S3-S17. To further understand the multimolecular characteristics and role of BRIP1 in the tumorigenesis of cancers, we further analyzed the association between BRIP1 expression and inhibition or activation of ten

major signaling pathways, the detailed information for calculating the pathway score was described at length elsewhere(22). As shown in Figure S13, BRIP1 was highly correlated to the activation of apoptosis, cell cycle, and DNA damage response, and inhibition of hormone ER and RAS/MAPK signaling pathways.

Immune analysis of BRIP1

As a prominent part of tumor microenvironment, tumor-infiltrating immune cells are complexly interacted with the carcinogenesis, development, and prognosis of cancer(23–25). As one of the most abundant stromal cells populated in the tumor microenvironment, Cancer- associated fibroblasts (CAFs) are essentially involved in the progression of cancers(26, 27). In the current study, we utilized multiple algorithms to explore the association between immune cells' infiltration level and the expression of BRIP1 across different cancers. Generally, we observed a series of positively correlation between the expression of BRIP1 and the CAFs estimated infiltration value for CESC, ESCA, HNSC, HNSC-HPV-, KICH, KIRP, LGG, LIHC, LUAD, MESO, OV, PAAD, PRAD,THCA, and UCS (Fig. 8a). While negatively correlation for PRAD was also noticed with different algorithm. Additionally, significantly positive correlation was noticed between the BRIP1 expression and $CD8^{+}$T-cells immune infiltration for HNSC-HPV+, KIRC, LUAD, and THYM based on most algorithms (Figure S14a). Figure 8b and Figure S14b showed the scatter plots of the above cancers generated by one algorithm. As an example, BRIP1 expression in CESC is positively associated with the infiltration level of CAFs (Rho = 0.231, P = 1.02e-04) based on the EPIC algorithm (Fig. 8b). Besides, the relationship between BRIP1 expression and MSI (Microsatellite instability)/TMB (Tumor mutational burden)/neoantigen of all TCGA cancers were investigated. We observed positive correlations between BRIP1 expression and MSI for GBM, LUSC, UCEC, COAD, STAD, KIRC, READ, and KICH but noticed a negative correlation for DLBC ($P < 0.05$ for all) (Figure S15). BRIP1 expression is also positively correlated to TMB for ACC, LUAD, PRAD, UCEC, COAD, STAD, SKCM, KIRC, and KICH, but is negatively correlated to KIRP (Figure S16, $P < 0.05$ for all). Besides, only positive correlation was found between neoantigen and BRIP1 expression for PRAD, LUAD, BRCA, UCEC, and STAD (Figure S17, $P < 0.05$ for all). Additionally, statistically significant correlation of BRIP1 expression and immune checkpoints and pathways across most of TCGA tumors was observed, and the heatmaps was presented in Figure S18. The above findings are worthy of further in-depth research to explore its clinical value.

Discussion

The aberrant expression of BRIP1 have been reported associated with many human diseases, especially cancers(6, 11, 12, 28). However, the role of BRIP1 in the pathogenesis of various cancers remains to be illuminated. Pan-cancer studies have recently emerged as a novel perspective for understanding the molecular mechanisms of tumorigenesis and development of human cancers(29–35), and we failed to obtain the pan-cancer analysis of BRIP1 across human tumors from literature search. Therefore, we used the multilevel data of human databases to analyze the expression, prognostic potential, mutation, methylation, genomic characteristics, activation or inhabitation of cancer-related pathways, and immune infiltration of BRIP1 gene in 33 TCGA tumors, aimed to figure out the multimolecular characteristics and role of BRIP1 in the carcinogenesis, development, and prognosis of tumors. Our results indicated that

there was a prognosis-related differential expression of BRIP1 between various cancers and their adjacent normal tissues; genetic alteration, CNV, and SNV of BRIP1 was observed in various cancers, and BRIP1 expression could be regulated by CNV; BRIP1 methylation was negatively correlated to gene expression in many human tumors, and the expression was associated with the activation and inhabitation of several cancer relevant pathways; BRIP1 expression was also associated with the immune infiltration level of CAFs and $CD8^+$ T cell in certain cancers.

The phylogenetic tree analysis of BRIP1 in the current study suggest a conservative characteristic of BRIP1 protein structure in various species, indicating there might be similar mechanisms of BRIP1 under physiological state. Previously studies reported that BRIP1 has anti-oncogenic effect and deregulated in many cancers(6, 36, 37). Controversially, data from ONCOMINE, TIMER2, and HPA databases suggested that BRIP1 is highly expressed in most cancers, and results from GEPIA2 suggested that gene expression is associated with cancer stages, indicated that BRIP1 plays an oncogene role in human tumors. To further figure out the role of BRIP1 in tumors, we used various databases to explore the prognostic potential of BRIP1 in various cancers according to gene expression. Our analyses showed that BRIP1 expression was associated with poor prognosis of PCPG, ACC, KICH, LGG, MESO, LIHC, KIRP, PAAD, UCEC, BRCA, OV, PRAD, and LUAD, and was also correlated to better prognosis of COAD, READ, STAD, and THYM. The survival data from various databases were not completely consistent, which might because of the heterogeneity of data collection in different databases(31). We then performed a COX regression analysis to explore the BRIP1 expression and several clinical characteristics in different cancers, and the results verified that BRIP1 acted as a risk factor in KIRP, ACC, LGG, MESO, and PAAD patients, and played a protective role in COAD, READ, STAD, and THYM patients. All in all, BRIP1 have the potential to be the diagnostic/prognostic biomarker in many tumors, and may act as an effective target for treatment, which deserve further experiments to verify its clinical value.

We combined the data of BRIP1-binding proteins and BRIP1-correlated genes to perform a series of enrichment analysis and found that pathways of “Basal transcription factors”, “Homologous recombination”, “Nucleotide excision repair”, and DNA metabolism may have potential impact on the etiology and pathogenesis of tumors. In addition, association between BRIP1 mutations and breast, ovarian, and cervical cancers have been reported(11, 12, 38, 39). In the current study, we also observed a high genetic alteration frequency in BRCA, and extensive SNV of BRIP1 were found in several cancer types. Besides, CNV of BRIP1 and the influence of CNV on gene expression were evaluated across different tumors to identify the effects of BRIP1 intrinsic regulatory role on BRIP1 expression. Adhered to previous report that the increase of copy number was always followed by the increased of gene expression(40), we also found the increase of BRIP1 CNV could resulted in the upregulation of BRIP1 expression, especially in BRCA and LUSC. Besides, as one of the major forms of epigenetic modification, DNA methylation is also strongly involved in the control of gene expression(41). In the current study, statistical significance between DNA methylation and gene expression across multiple cancers were noticed. Moreover, the correlation analysis between BRIP1 expression and 10 famous cancer-related pathways among 32 cancer types suggested that BRIP1 was highly correlated to the activation of

apoptosis, cell cycle, and DNA damage response, and inhibition of hormone ER and RNS/MARK signaling pathways. Taken together, genetic changes were crucially evolved in the regulation of BRIP1 expression, and the mechanisms under the regulatory rules on BRIP1 expression across different tumors is worthy further studies and exploration.

It is well known that the tumor cells are supervised by immune cells, and the deficiency in immunity could contribute to the tumorigenesis, progression, and worse clinical outcomes(31). In the current work, we found significantly positive correlation between BRIP1 expression and immune infiltration level of CAFs and $CD8^+$ T-cells among multiple cancers, and both positive correlations between MSI, TBM and BRIP1 expression were found in COAD, STAD, KIRC, and KICH. While both positive correlations between TMB, neoantigen, and BRIP1 expression were observed in LUAD, PRAD, and STAD, suggesting the potential of BRIP1 as an immunotherapy target for these cancers. Besides, we observed positive correlations between BRIP1 expression and the immune pathways of “activated CD4 T cell”, “memory B cell”, and “type 2 T helper cell”, almost all immune checkpoints and BRIP1 expression in KIRC, THCA, and HNSC were positively correlated, which would help us to further understand the role of BRIP1 in certain tumor’s immune microenvironment.

As the first study to explore the multimolecular characteristics and role of BRIP1 in human tumors, there were several limitations needed to be recognized. First, since the current study was conducted among multiple online databases, data heterogeneity would be inevitably existing, which could result in the inconsistency of the results. Second, although our work suggested that BRIP1 has the potential to be a diagnostic/prognostic biomarker for several certain cancers and could act as an effective target for immunotherapy in some tumors; further experiments *in vitro* / *in vivo* are required to verify these hypotheses.

Conclusion

Our concentrative and systematic study of BRIP1 suggested there were significantly associations of BRIP1 expression with patients’ prognosis, genetic alterations, DNA methylation, activation or inhabitation of cancer-related pathways, immune cell infiltration, MSI/TMB/neoantigen, immune pathways and immune checkpoints across multiple human cancers, which would provide valuable resource to better understand the role of BRIP1 in cancer and maximize its clinical benefit.

Abbreviations

TME
tumor microenvironment
BRIP1
BRCA1 Interacting Protein C-terminal Helicase 1
FA
Fanconi Anemia

SARC
Sarcoma
THYM
Thymoma
OS
Overall Survival
DFS
Disease-Free Survival
PPI
protein-protein interaction
KEGG
Kyoto Encyclopedia of Genes and Genomes
GO
Gene Oncology
MF
molecular function
CC
cellular components
BP
biological processes
CAN
Copy number alteration
3D
three-dimensional
DSS
disease-specific survival
PFS
progress-free survival
SNV
Single Nucleotide Variation
CNV
Copy Number Variation
HPA
Human Protein Atlas
BLCA
Bladder Urothelial Carcinoma
KIRC
Kidney renal clear cell carcinoma
KIRP
Kidney renal papillary cell carcinoma

BRCA
Breast invasive carcinoma
HNSC
Head and Neck squamous cell carcinoma
ESCA
Esophageal carcinoma
CHOL
Cholangiocarcinoma
COAD
Colon adenocarcinoma
GBM
Glioblastoma multiforme
LUAD
Lung adenocarcinoma
LGG
Brain Lower Grade Glioma
UCEC
Uterine Corpus Endometrial Carcinoma
LUSC
Lung squamous cell carcinoma
STAD
Stomach adenocarcinoma
TGCT
Testicular Germ Cell Tumors
CESC
Cervical squamous cell carcinoma
READ
Rectum adenocarcinoma
UCS
Uterine Carcinosarcoma
SARC
Sarcoma
THYM
Thymoma
DLBC
Lymphoid neoplasm diffuse large B-cell lymphoma
ACC
Adrenocortical carcinoma
SKCM
Skin Cutaneous Melanoma

LIHC
Liver hepatocellular carcinoma
THCA
Thyroid carcinoma
KICH
Kidney Chromophobe
OV
Ovarian serous cystadenocarcinoma
PCPG
Pheochromocytoma and Paraganglioma
MESO
Mesothelioma
PAAD
Pancreatic adenocarcinoma
DMFS
distant metastasis-free survival
PPS
post-progression survival
FP
first progression
RFS
relapse-free survival
PRAD
Prostate adenocarcinoma
DSS
disease-specific survival
DFI
disease-free interval
PFI
progress-free interval
UVM
Uveal Melanoma
AUC
area under curve
LAML
Acute Myeloid Leukemia
CAFs
Cancer-associated fibroblasts
MSI
Microsatellite instability

TMB
Tumor mutational burden.

Declarations

Ethics approval and consent to participate

Ethical approval to report this case was obtained from The Committee on Medical Ethics of The Affiliated Hospital of Qingdao University (APPROVAL NUMBER/ID:QYFY WZLL 26782).

Consent for publication

Consent for publication was obtained from all participants.

Availability of data and materials

All of the data involved in this study are available in the public databases which are listed in the “Materials and methods” section.

Competing interests

The authors have declared no competing interest.

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Authors' contributions

YJ and JZ conceived the study idea. RW collected the data. XC, YN, TC, LW, JZ, and YJ contributed to the analysis of the data. RW and LW wrote the initial draft with all authors providing critical feedback and edits to subsequent revisions. All authors approved the final draft of the manuscript. All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. YJ is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Figures

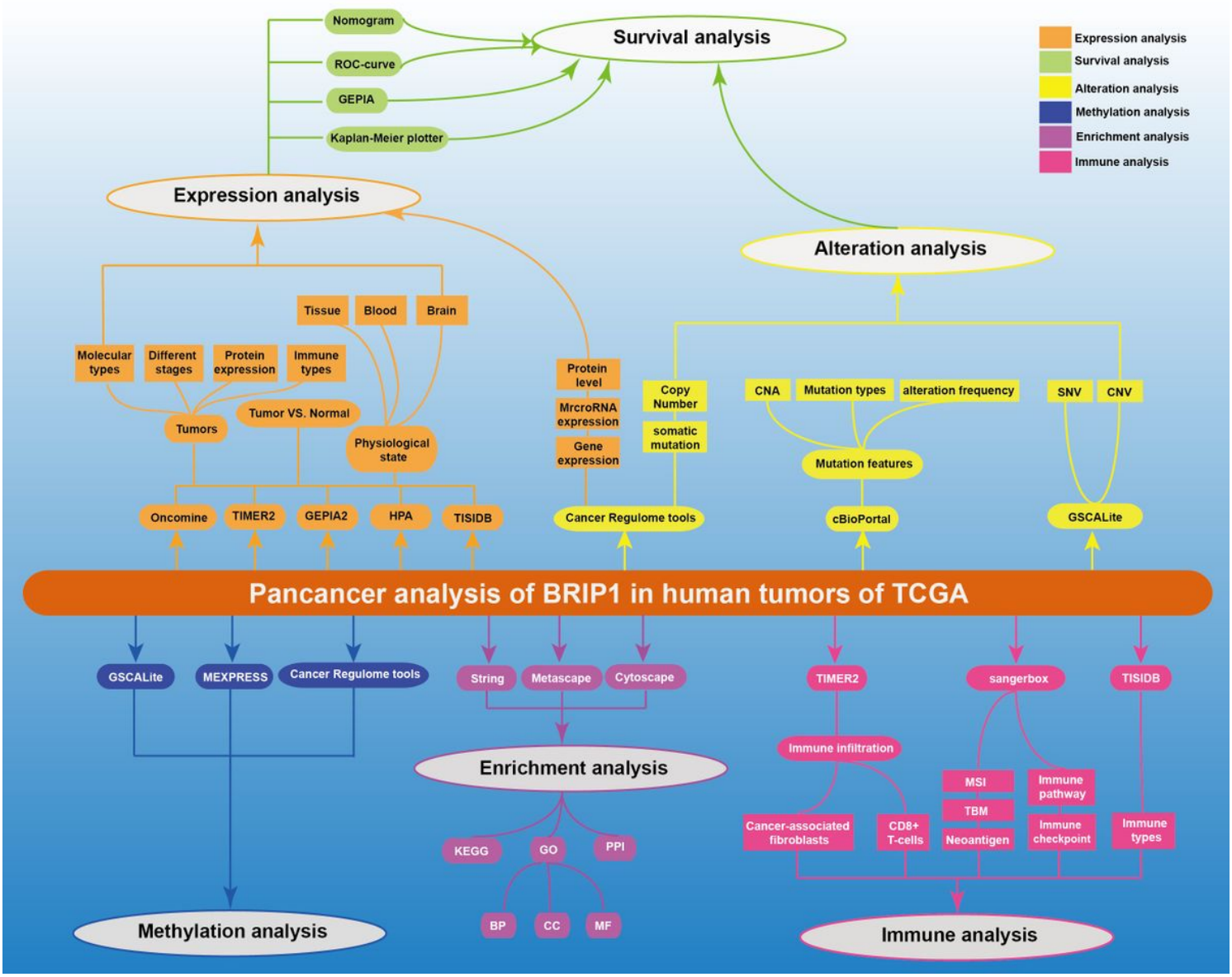

Figure 1

Study design of the current study. CNA: Copy number alteration; SNV: Single nucleotide variation; CNV: Copy number variation. PPI: Protein-protein interaction; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Oncology; MF: molecular functions; CC: cellular components; BP: biological processes; MSI: Microsatellite instability; TMB: Tumor mutational burden.

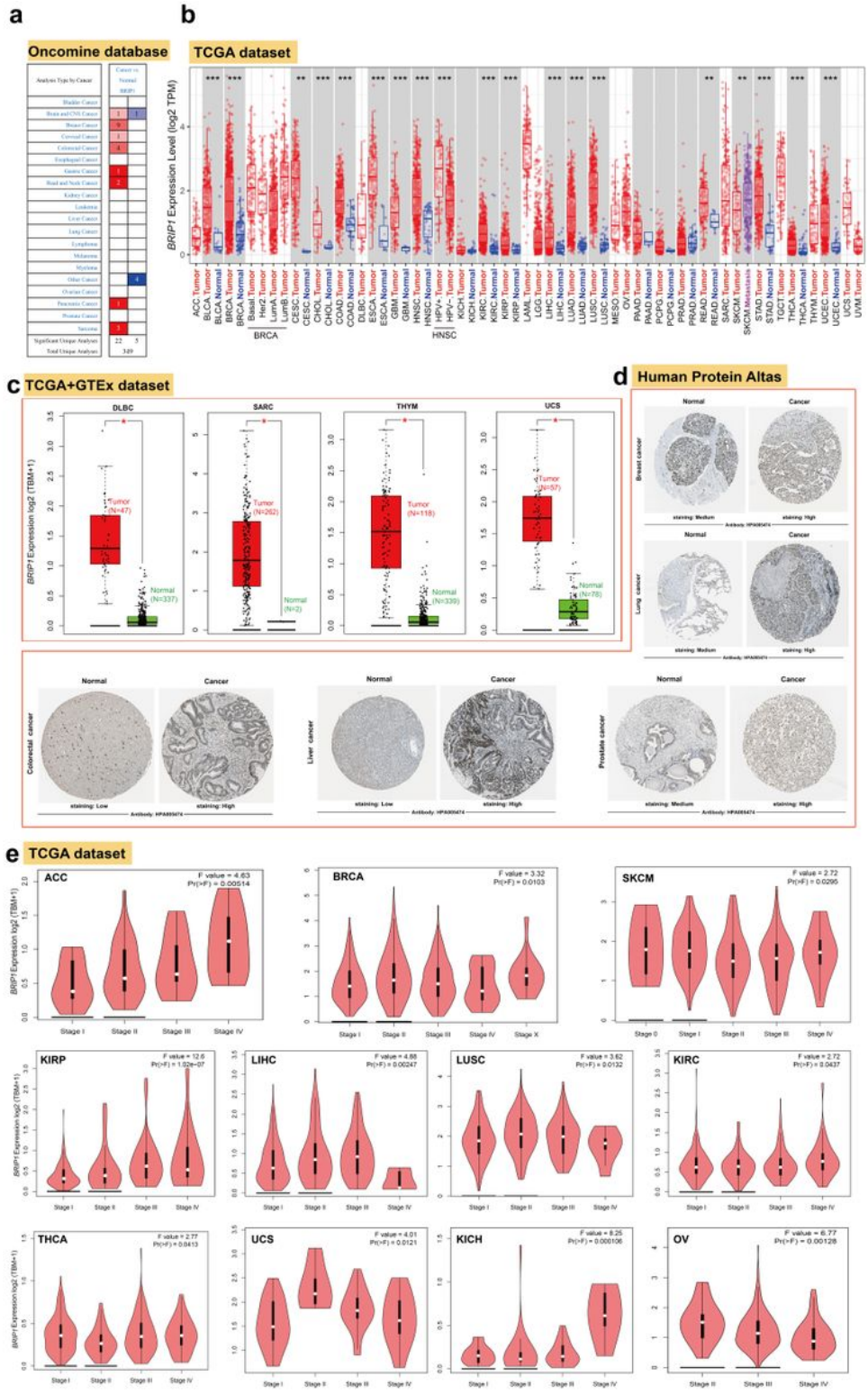

Figure 2

Expression level of BRIP1 gene in different tumors and pathological stages. (a) The transcription levels of BRIP1 in different types of human cancers (ONCOMINE). The cell number represents the dataset number that meets all the thresholds with the color blue for under-expression and color red for over-expression. Cell color is determined by the best gene rank percentile for the analyses within the cell. (b) The expression status of the BRIP1 gene in different cancers or specific cancer subtypes was analyzed

through TIMER2. * P<0.05; ** P<0.01; *** P<0.001. (c) For the type of DLBC, SARC, THYM, and UCS in the TCGA project, the corresponding normal tissues of the GTEx database were included as controls. The box plot data were supplied. * P <0.05. (d) Representative immunohistochemistry images of BRIP1 in breast cancer, lung cancer, colorectal cancer, liver cancer, prostate cancer, and their corresponding normal tissues (Human Protein Atlas). (e) Based on the TCGA data, the expression levels of the BRIP1 gene were analyzed by the main pathological stages (stage I, stage II, stage III, and stage IV) of ACC, BRCA, SKCM, KIRP, LIHC, LUSC, KIRC, THCA, UCS, KICH, and OV. Log2 (TPM+1) was applied for log-scale.

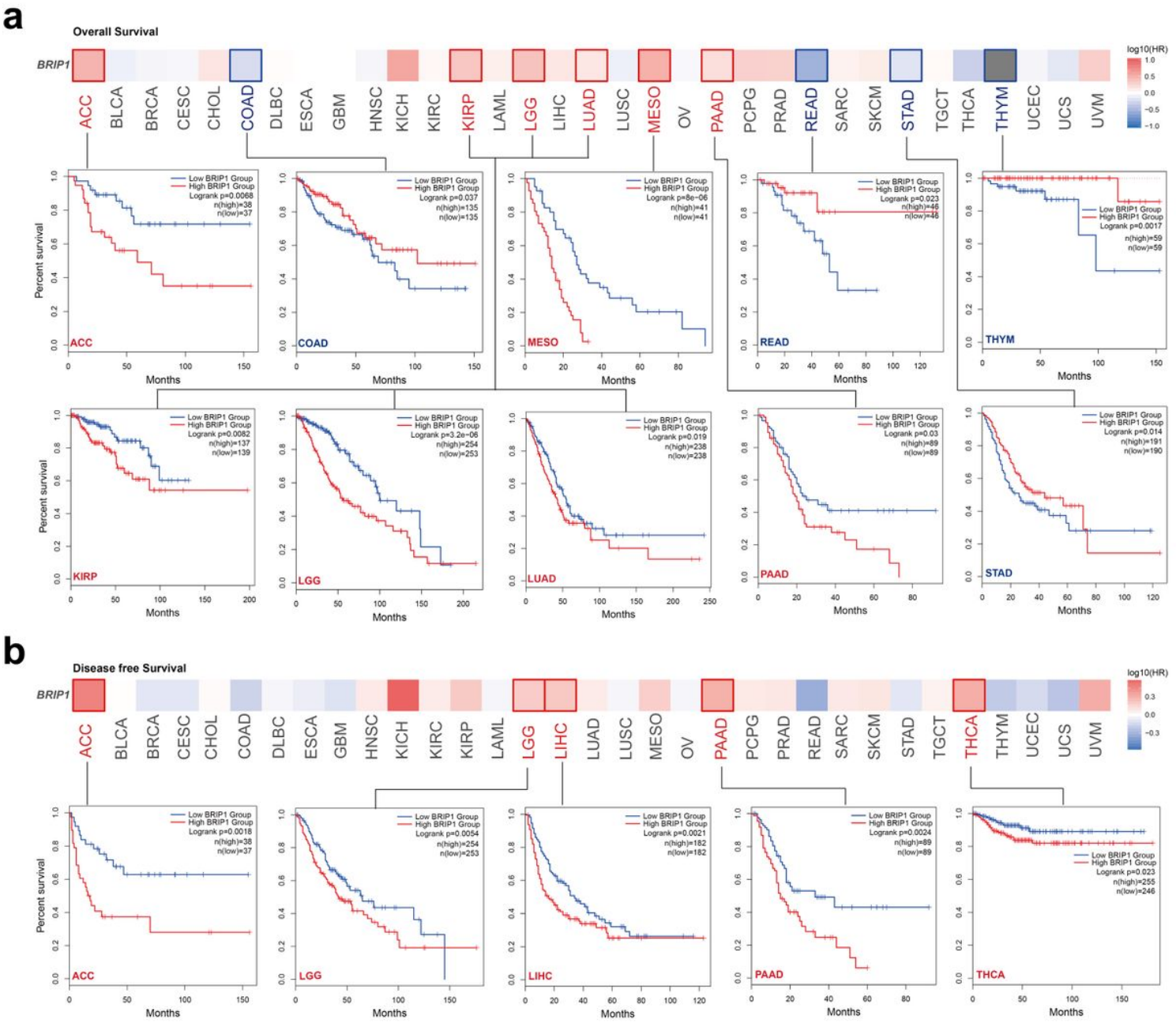

Figure 3

Correlation between BRIP1 gene expression and survival prognosis of cancers in TCGA. We used the GEPIA2 tool to perform overall survival (a) and disease-free survival (b) analyses of different tumors in

TCGA by BRIP1 gene expression. The survival map and Kaplan-Meier curves with positive results are given.

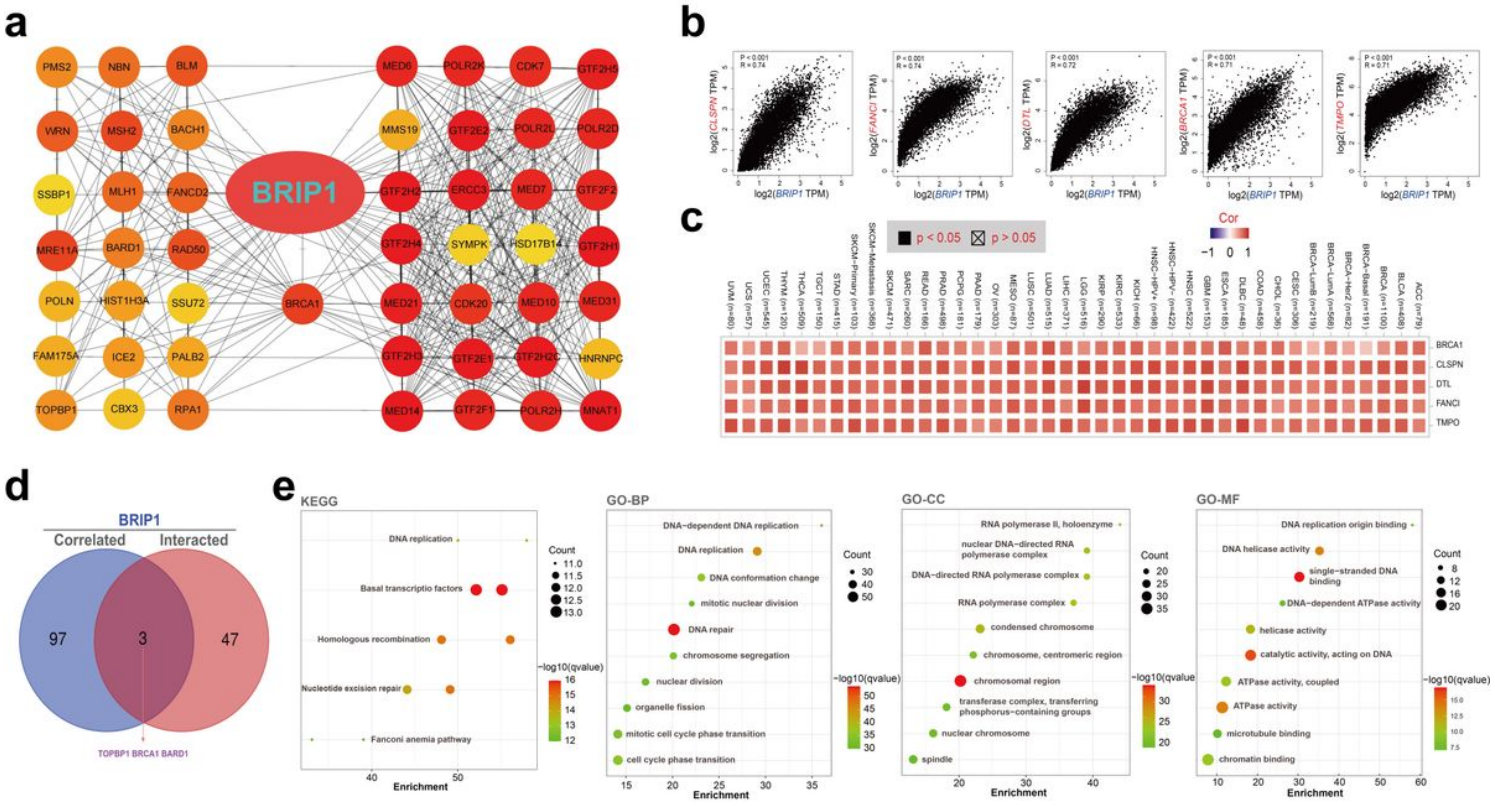

Figure 4

BRIP1-related gene enrichment analysis. (a) We first obtained the available

experimentally determined BRIP1-binding proteins using the STRING database. (b) Using the GEPIA2 tool, we also obtained the top 100 BRIP1-correlated genes in TCGA projects and analyzed the expression correlation between BRIP1 and the top five genes with highest correlation, including CLSPN, PLOD3, CALU, GCC1, and MYBBP1A. (c) The corresponding heatmap data in the detailed cancer types are displayed. (d) An intersection analysis of the BRIP1-binding and correlated genes was conducted. (e) Based on the BRIP1-binding and correlated genes, KEGG and GO enrichment analysis was performed.

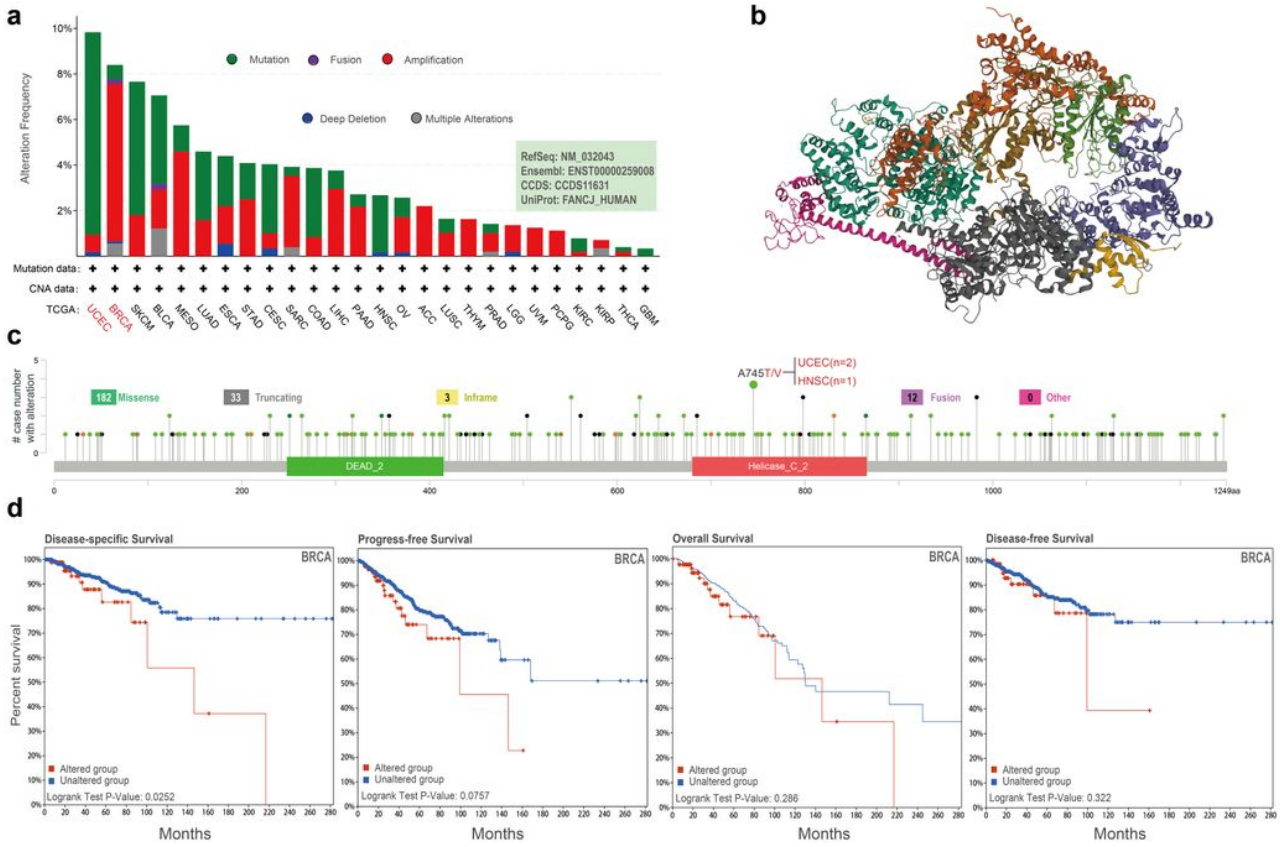

Figure 5

Mutation feature of BRIP1 in different TCGA tumors. We analyzed the mutation features of BRIP1 for the TCGA tumors using the cBioPortal tool. The alteration frequency with mutation type (a) and mutation site (c) are displayed. (b) 3D structure of BRIP1. (d) The potential correlation between mutation status and disease-specific, progression-free, overall, and disease-free survival of BRCA.

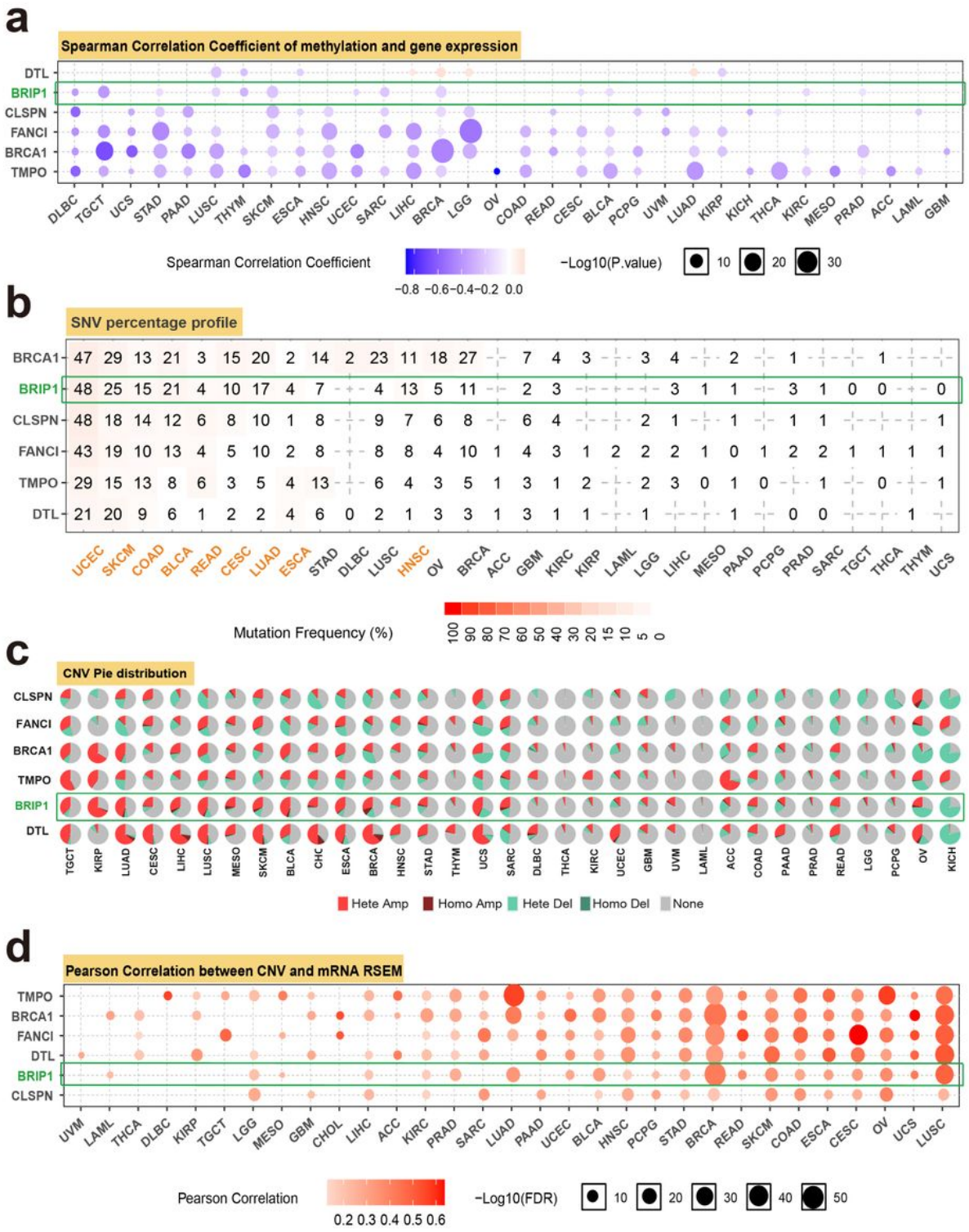

Figure 6

Regulatory mechanism of BRIP1 and the correlated genes expression and its role in Cancer. (a) The correlation between DNA methylation and expression level of BRIP1 and the correlated genes in different cancers. (b) SNV frequency of BRIP1 and the correlated genes in different cancers. The deeper of color, the higher of mutate frequency. (c) Global profile that shows the constitute of Heterozygous/Homozygous CNV of BRIP1 and the correlated genes in each cancer. Hete Amp:

heterozygous amplification; Hete Del: heterozygous deletion; Homo Amp: homozygous amplification; Homo Del: homozygous deletion; None: no CNV. (d) The association between paired mRNA expression and CNV percent samples, based on Person's product moment correlation coefficient.

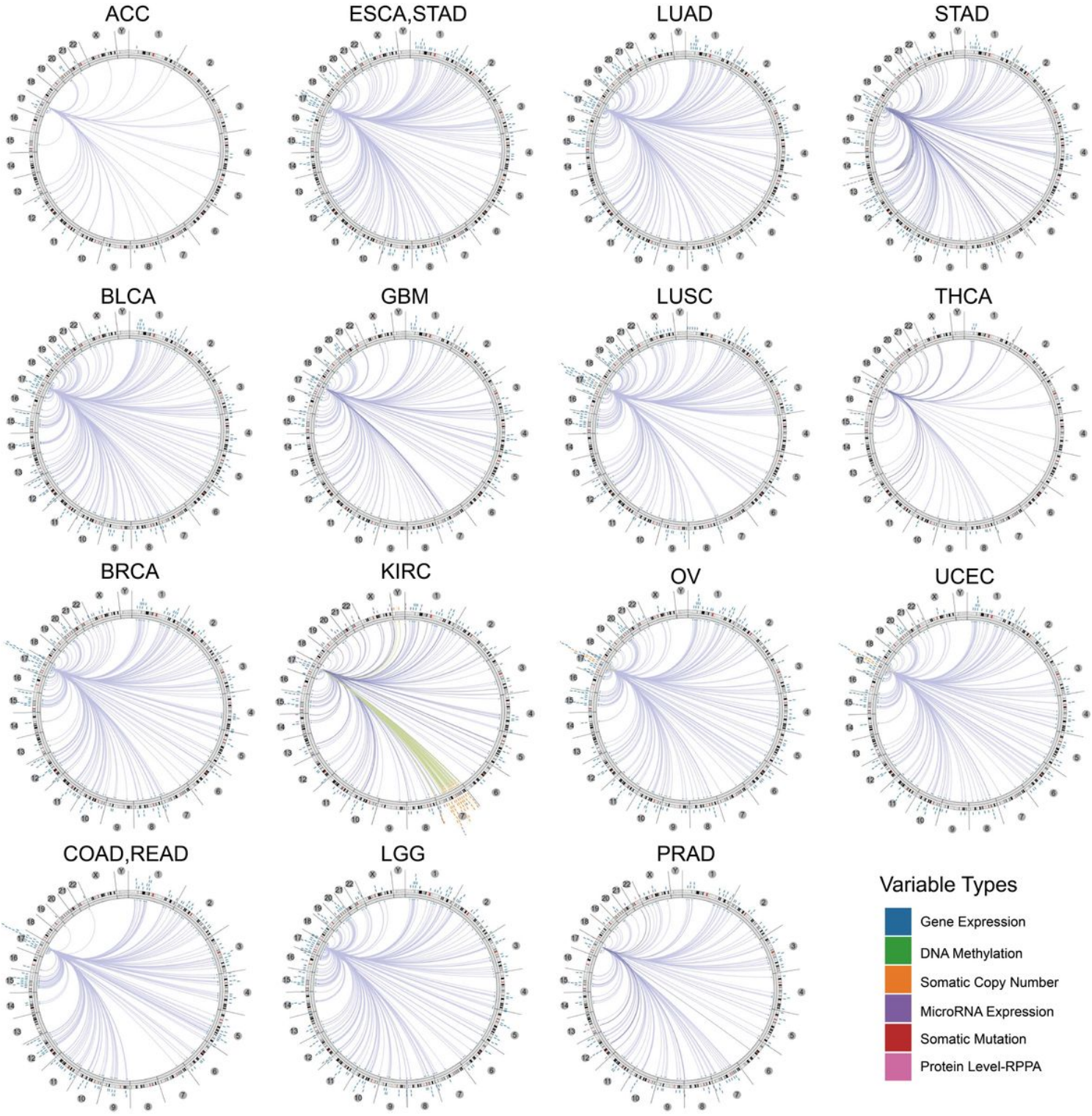

Figure 7

The correlation between BRIP1 and other genes from the TCGA database (Regulome program). Note: The circular layout displays the associations as edges in the Center connecting the features (with genomic

coordinates) displayed around the perimeter. The outer ring displays cytogenetic bands. The inner ring displays associations that contain features lacking genomic coordinates.

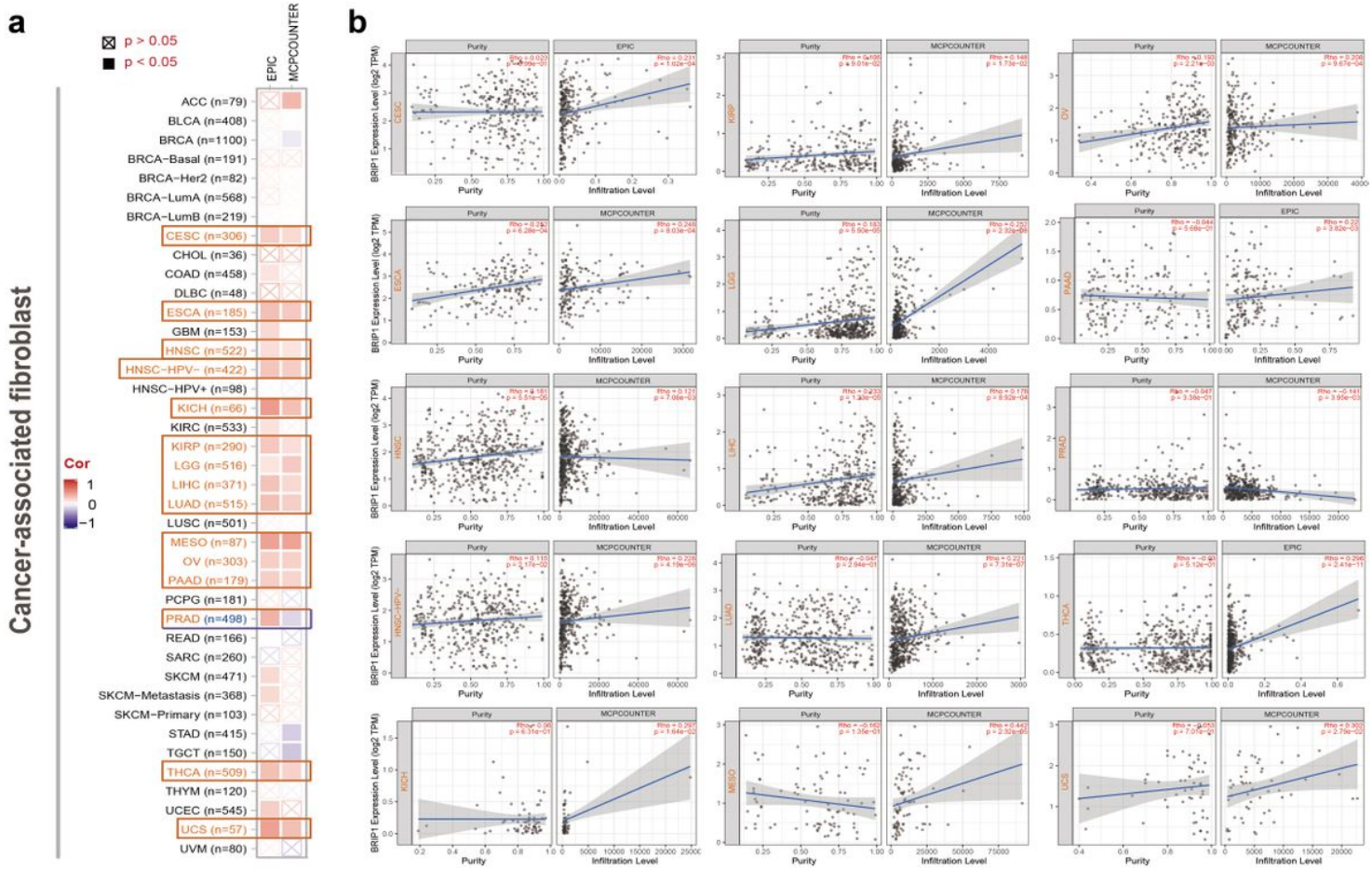

Figure 8

Correlation analysis between BRIP1 expression and immune infiltration of

cancer-associated fibroblasts. Different algorithms were used to explore the potential correlation between BRIP1 expression level and the infiltration level of cancer-associated fibroblasts across all TCGA tumors.

Supplementary Files

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