Hindawi
Advanced Gut & Microbiome Research
Volume 2022, Article ID 1591377, 28 pages
https://doi.org/10.1155/2022/1591377

Research Article

A Pan-Cancer Analysis of the Oncogenic Roles of RAD51 in Human Tumors

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Received 27 May 2022; Revised 1 August 2022; Accepted 18 August 2022; Published 17 September 2022

Academic Editor: Jiong Yu

Objective. RAD51 homolog 1 (RAD51) plays significant roles in DNA damage repair and apoptosis. These pathways are tightly associated with tumor initiation and progression. To unravel the roles of RAD51 in oncogenesis and progression of different cancers, herein, a comprehensive analysis of the RAD51 was carried out using multiomics datasets of 33 cancers. *Methods*. Raw data were obtained from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. We analyzed the correlation between RAD51 expression and drug response using datasets from CellMiner. Next, clinical characteristics and prognostic values of RAD51 were conducted based on TCGA data. The correlation between RAD51 expression and tumor immune infiltration was explored. This was followed by gene set enrichment analysis by Rsoftware. In addition, pan-cancer analysis was conducted to investigate genetic and epigenetic alterations, respectively. *Results*. RAD51 was upregulated in most tumors, and this was associated with poor overall survival (OS), progression-free survival (PFS), and disease-specific survival (DSS). The expression level of RAD51 is significantly associated with the IC50 of multiple antitumor drugs and the proportion of stromal and immune components in tumor microenvironment (TME). Moreover, RAD51 expression showed a positive relationship with multiple key immune checkpoint and immunosuppressive genes, including death-ligand 1 (PD-L1), cytotoxic T-lymphocyte associated protein 4 (CTLA-4), CD28, and several TNF-related immune genes. Gene set enrichment analysis uncovered that RAD51 correlated with cell cycle, cell division, and immune system pathways in diverse cancers. Our results revealed a strong link between RAD51 expression and microsatellite instability (MSI) or tumor mutation burden (TMB). *Conclusions*. Our pan-cancer analysis provides a comprehensive overview of the roles of RAD51 in multiple human cancers and infers that RAD51 has the potential as a biomarker for progression and immune infiltration of different tumor types.

1. Introduction

Genomic instability is a critical feature of tumorigenesis. The absence of DNA damage repair (DDR) pathway may lead to many catastrophic consequences, such as mutations or chromosomal rearrangements, promoting genomic instability and tumor progression [1]. RAD51, a DNA repair protein, functions in mediating the pairing of homologous DNA sequences and strand invasion [2]. RAD51 is central to homologous recombination repair (HRR), which is one of the major pathways for DNA double-strand break repair, directly taking charge of genomic stability [3]. There was a host of animal and cell-based data connecting RAD51 to several types of malignancies. RAD51 was shown to be elevated in brain cancer [4], head and neck cancer [5], thyroid cancer [6], nonsmall-cell lung cancer [7], and breast cancer

[8]. Therefore, RAD51 has the potential to be a biomarker and a key mediator in a variety of human malignancies.

DNA damage response of tumor cells has a profound impact on the organization of tumor microenvironment (TME) that plays a critical role in the beginning and progression of human malignancies [9, 10]. TME can function in malignant transformation through inhibiting DDR pathways [11]. Hypoxia, one of the TME characteristics, was demonstrated to downregulate c-MYC, a proliferation promoting transcription factor that combines with promoters of Mlh1 and Mlh2, which are known to be involved in the mismatch repair (MMR) pathway [12]. And, it is worth noting that RAD51 and BRCA1 in the HRR pathway are also associated with hypoxia, so the TME can regulate genomic instability via key repair factors [13, 14]. And, some of the most common single nucleotide polymorphisms (SNPs) found in RAD51 may increase drug resistance of cancers [15]. SNPs in RAD51 were also manifested to increase the risk of glioblastoma and non-small cell lung cancer and influence overall survival (OS) [16, 17]. Despite these findings imply that TME and mutation are closely associated with tumorigenesis, additional research is required. As a result, it is paramount to elucidate the interactions between the biomarker gene and TME, mutation, or epigenetic modification.

Herein, a comprehensive analysis of the RAD51 was carried out using multiomics datasets of 33 cancers, including the response of antitumor drugs, expression profile comparison, survival status, immune cell infiltration, TMB, and microsatellite instability (MSI). In addition, genetic alteration, methylation, and relevant cellular pathways were investigated. In conclusion, the obtained results indicate the potential molecular mechanism of RAD51 in the clinical prognosis of multiple human cancers.

2. Materials and Methods

2.1. Data Collection. RNA sequence and related clinical data (10201 samples for 33 types of cancer) were retrieved from The Cancer Genome Atlas (TCGA) database [18]. Gene profile data from normal human tissues were retrieved from Genotype-Tissue Expression (GTEx) [19]. The R package "metafor" was used for data processing (filtering, deleting missing, and duplicated results) and transformed by $\log2\left[\mathrm{TPM}\left(\text{Transcripts per million}\right)+1\right]$ (R version: 4.1.2).

2.2. Gene Expression Analysis and Relationship with Pathological Stages. We used Tumor Immune Estimation Resource (TIMER) to investigate the expression profiling of RAD51 between tumors and adjacent normal tissues [20]. However, some samples lacked normal tissue. We combined the data from TCGA and GTEx to perform further analysis of the expression of RAD51 in tumors and adjacent normal tissues. The $\log 2(\mathrm{TPM}+1)$ transformed expression data were applied for the box and radar plots. Gene Expression Profiling Interactive Analysis (GEPIA2) [21] is a web-based platform, and it was used for expression profiling based on pathological stages.

2.3. Survival Analysis and Cox Regression Analysis. We used UCSCXenaShiny [22] to perform survival analysis of RAD51 for pan-cancer based on TCGA. Cutoff-low (50%) and cutoff-high (50%) values were used as the thresholds of RAD51 expression to split the low-expression and high-expression cohorts. The log-rank test was utilized for differential analysis (P value < 0.05 as significant). With the aid of R package "survival," univariate Cox regression analysis was used to explore the correlation of RAD51 expression with patients' OS, progression-free survival (PFS), disease-specific survival (DSS), and disease-free survival (DFS) in each cancer type based on TCGA databases [23].

2.4. RAD51 Expression and Drug Response. CellMiner (https://discover.nci.nih.gov/cellminer/home.do) was utilized to explore the correlation between RAD51 expression and drug response. CellMiner database was designed to investigate transcript and drug patterns data in the NCI-60 cancerous cell line set, which is a dataset of 60 diverse human cancer cell lines used by the Developmental Therapeutics Program of the U.S. National Cancer Institute to screen over 100,000 chemical compounds [24]. RNA expression data (RNA: RNA-SEQ) and drug data (compound activity: DTPNCI-60) were downloaded. To ensure the reliability of the analysis results, clinical trials and FDA-approved drug results were selected for the analysis of drug sensitivity. The half-maximal inhibitory concentration (IC50) that represented drug response was estimated employing the pRRophetic package [25].

2.5. Immune Infiltration Analysis. TIMER was used to calculate immune cell infiltration scores for six main immune cell types ($CD4^+$ T cells, CD 8^+ T cells, B cells, dendritic cells, neutrophils, and macrophages) [26]. To increase the accuracy of the results, we also used xCell to calculate the correlation of RAD51 with various immune cells. ESTIMATE [27] (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) is a tool for predicting tumor purity and the presence of infiltrating stromal/immune cells in tumor tissues using gene expression data [27]. We downloaded disease-centric results containing stromal, immune, and ESTIMATE scores of pan-cancer from the ESTIMATE database and combined them with the results of RAD51 expression in diverse tumors in TCGA, and the correlation of RAD51 expression with immune infiltration scores was performed (P value < 0.05 as significant).

The correlation of RAD51 expression with immune-related genes, including immunosuppressive genes, histocompatibility complex (MHC) genes, chemokine, and chemokine receptors downloaded from TISIDB [28], was calculated by Spearman correlation coefficients.

2.6. Gene Set Enrichment Analysis. Gene Set Enrichment Analysis (GSEA) was employed to explore the potential biological process of RAD51 in diverse tumors using clusterProfiler package [29]. Adjusted P value < 0.05 was considered statistically significant. RAD51-related genes were selected by calculating the Spearman correlation coefficient

between RAD51 and other genes in all samples of 33 tumors downloaded from TCGA (P value < 0.05 as significant).

2.7. Analysis of RAD51 Expression with TMB and MSI. We obtained the scores of tumor mutation burden (TMB) and microsatellite instability (MSI) from TCGA. Correlation analysis of the RAD51 expression with TMB and MSI was determined employing the Spearman correlation coefficient (P value < 0.05 as significant).

2.8. CNV and Methylation Profile of RAD51. We investigated the copy number variation (CNV) and methylation profile of RAD51 by using Gene Set Cancer Analysis (GSCA) [30], which is a web platform providing methylation, immune infiltration, and CNV based on the TCGA database. Also, the correlation of RAD51 expression with the level of CNV and methylation in pan-cancer was performed by the GSCA website (P value < 0.05 as significant).

2.9. Statistical Analysis. Correlation analysis between RAD51 expression and targets of research, such as drug sensitivity, immune cell infiltration scores, immune checkpoint, CNV, methylation, TMB, or MSI, was determined by using the Spearman Correlation coefficient. Paired t-test or t-test was performed for comparison of RAD51 expression levels in cancer tissues and normal tissues. For survival analysis, the hazard ratio (HR) and P value were calculated using univariate Cox regression analysis. Kaplan-Meier analysis was utilized to examine the survival time of patients stratified according to the degree of RAD51 expression. P value < 0.05 was considered the significant threshold for all statistical analyses. The R tools ggplot2, ggunchained, ggpubr, and forestplot were used for visualization.

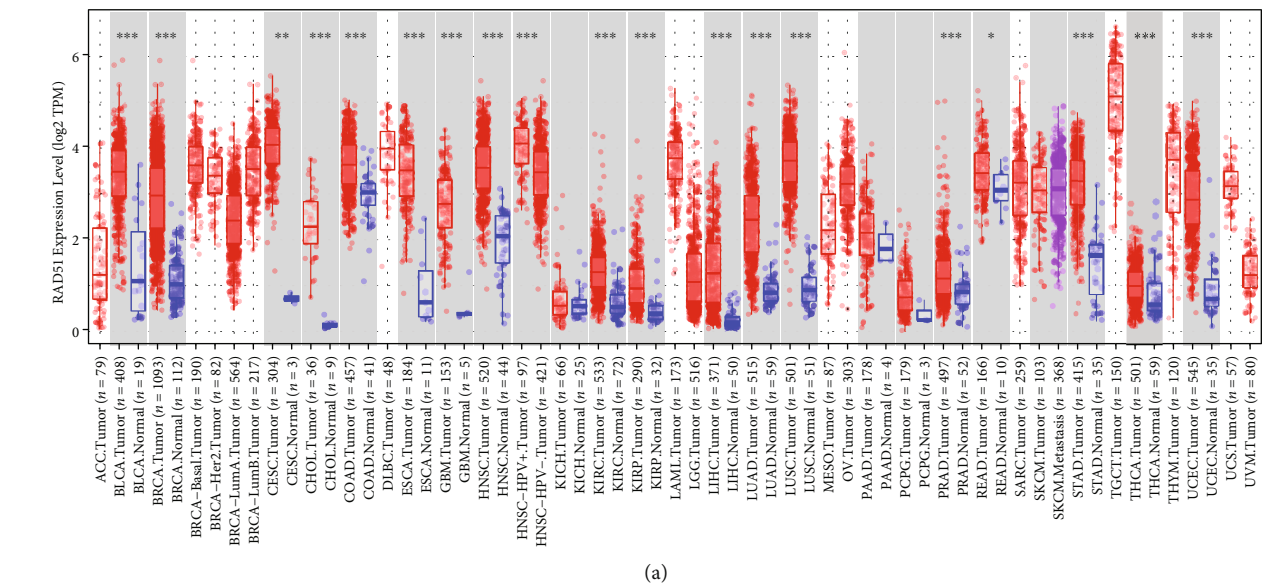
3. Results

3.1. Pan-Cancer Expression Landscape of RAD51. TIMER database was utilized to study the differential expression of RAD51 between tumors and adjacent normal tissues presented in TCGA. As shown in Figure 1(a), the RAD51 expression was significantly higher in cancers versus adjacent normal tissues in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma, endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal papillary cell carcinoma (KIRP), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine carcinosarcoma (UCEC). Some normal tissue data were not available in TCGA, and we further evaluated the expression differences of RAD51 between the tumor and normal tissues employing the GTEx dataset. According to the results from the GTEx database, the differences were highly significant for 27 of the 33 cancers (Figure 1(b)). Profiles of RAD51 expression across various types of cancers and corresponding tissues were presented and ranked from low to high (Figures 1(c) and 1(d)).

3.2. Correlation of RAD51 Expression with Pathological Stages of Cancer. GEPIA2 was utilized to examine the relationship between RAD51 expression and the pathological stages of cancers. We found that the expression profiles of RAD51 in kidney chromophobe (KICH), KIRP, LIHC, ACC, KIRC, LUAD, BRCA, COAD, THCA, testicular germ cell tumor (TGCT), and uterine carcinosarcoma (UCS) were all significantly associated with their pathological stages (Figure 2).

3.3. RAD51 and Drug Sensitivity. Targeted therapies have an important role in cancer management. Genetic alterations that contribute to cancer heterogeneity are associated with the action of targeted therapies. Reliable prediction of RAD51 for targeted therapies remains lacking in pan-cancer. Thus, we performed the correlation analysis of RAD51 expression and drug sensitivity in the NCI-60. RAD51 expression was positively connected with drug sensitivity in NCI-60 treated with methylprednisolone, nelarabine, vorinostat, ribavirin, 6-thioguanine, chelerythrine, cyclophosphamide, parthenolide, ZM-336372, 8-chloroadenosine, and 6-thioguanine. Differently, there was a negative correlation between RAD51 expression and the anticancer drug BPTES, INK-128, LY-3023414, mithramycin, and GDC-0349 (Figure 3(a)). Further analysis was conducted to uncover whether RAD51 expression was predictive for drug responses in cancer patients. We noted that the high-expression group of RAD51 had significantly lower estimated IC50 of INK-128, LY-3023414, and GDC−0349, indicating that the subpopulations with high-expression RAD51 presented higher sensitivity to INK-128, LY-3023414, and GDC−0349. In the meantime, the low-expression group showed significantly lower estimated IC50 of vorinostat, indicating that low-expression subpopulations preferred to respond to vorinostat (Figure 3(b)). Therefore, RAD51 might be a favorable predictive biomarker for targeted therapies with the aforementioned drugs.

3.4. Effects of RAD51 on Pan-Cancer Prognosis. Cox proportional hazard model was utilized to examine the relationship between RAD51 expression and OS in various cancer types through TCGA. As shown in Figure 4(a), high RAD51 level was significantly linked to worse OS in patients with adrenocortical carcinoma (ACC), BRCA, KICH, KIRP, brain lower grade glioma (LGG), LIHC, LUAD, mesothelioma (MESO), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), PRAD, rectum adenocarcinoma (READ), and THYM. With the aid of survival analyses, Kaplan–Meier curves revealed that high expression of RAD51 was associated with shorter survival times in patients with ACC, BRCA, KICH, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PCPG, and PRAD (Figures 4(b)–4(l)). Conversely, the high expressions of RAD51 in READ and THYM were associated with longer OS times (Figures 4(m) and 4(n)), and this was consistent with the result through Cox proportional hazards model.



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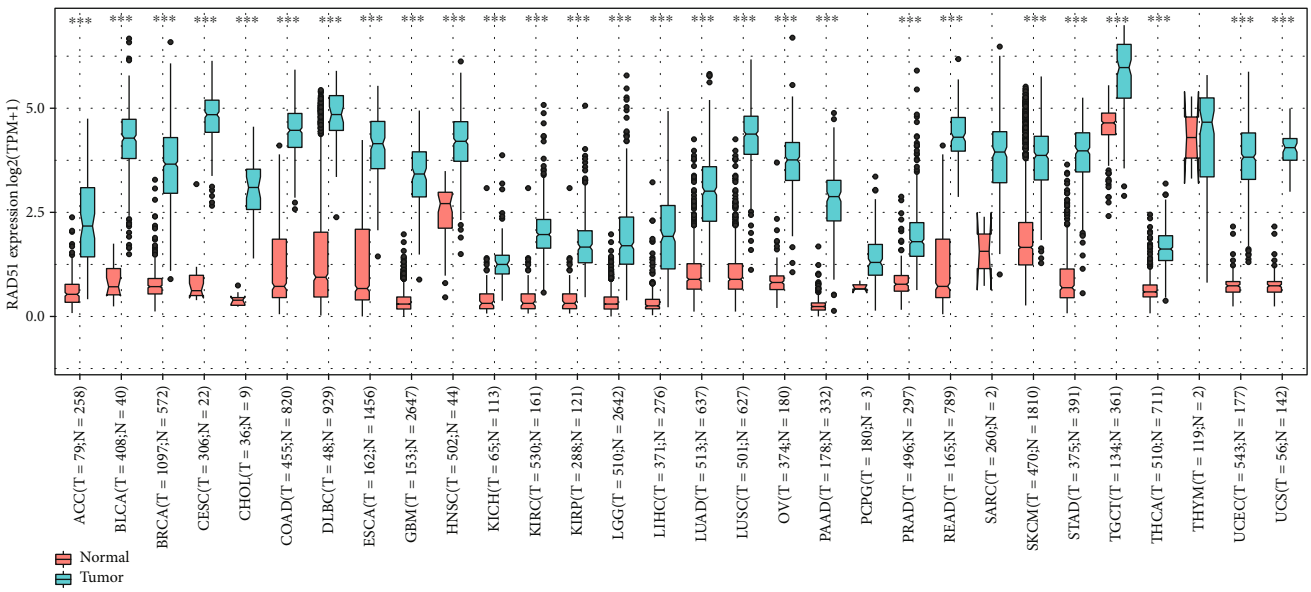

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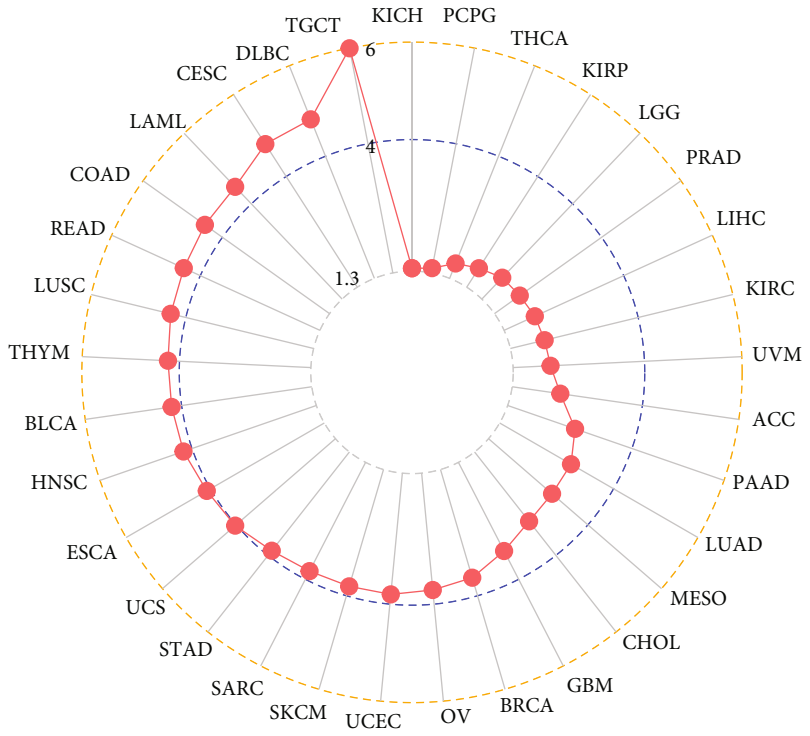

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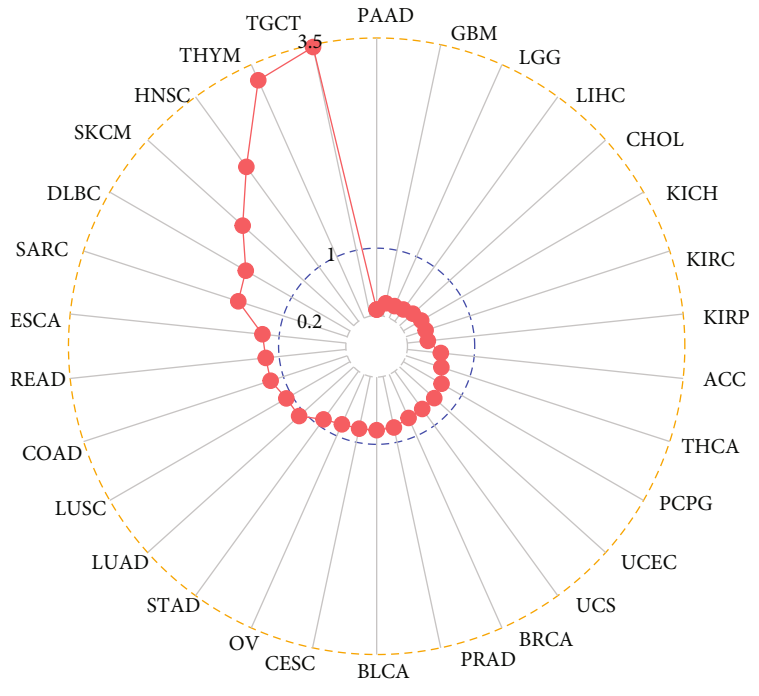
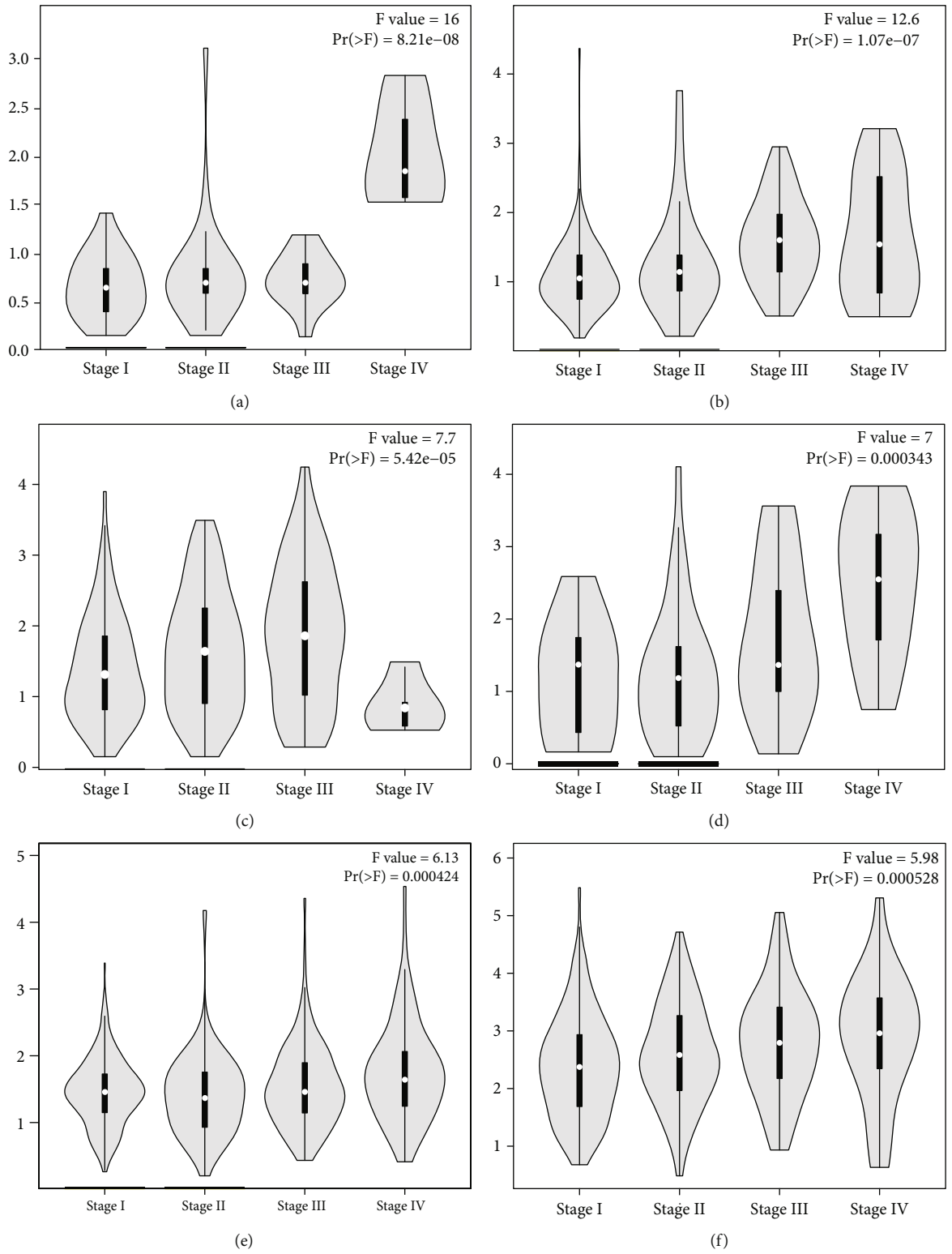


(d)

Figure 1: RAD51 expression level varies between tumors. (a) The expression level of RAD51 in TCGA tumors and adjacent tissues was visualized by TIMER. (b) The comparison of RAD51 expression between tumors from TCGA and the corresponding normal tissues from GTEx. (c) Profiles of RAD51 expression in 33 cancer types. (d) RAD51 expression in homologous normal tissues. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Next, we performed Cox regression analysis for PFS, DSS, and DFS to elucidate the relationship between RAD51 expression and various survival indicators. For PFS, the high expression of RAD51 was significantly associated with poor prognosis in cancer patients with ACC, BLCA, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PCPG, PRAD, SKCM, THCA, and UVM (Figure 5(a)). On the other side, it was demonstrated that high RAD51 level was correlated with



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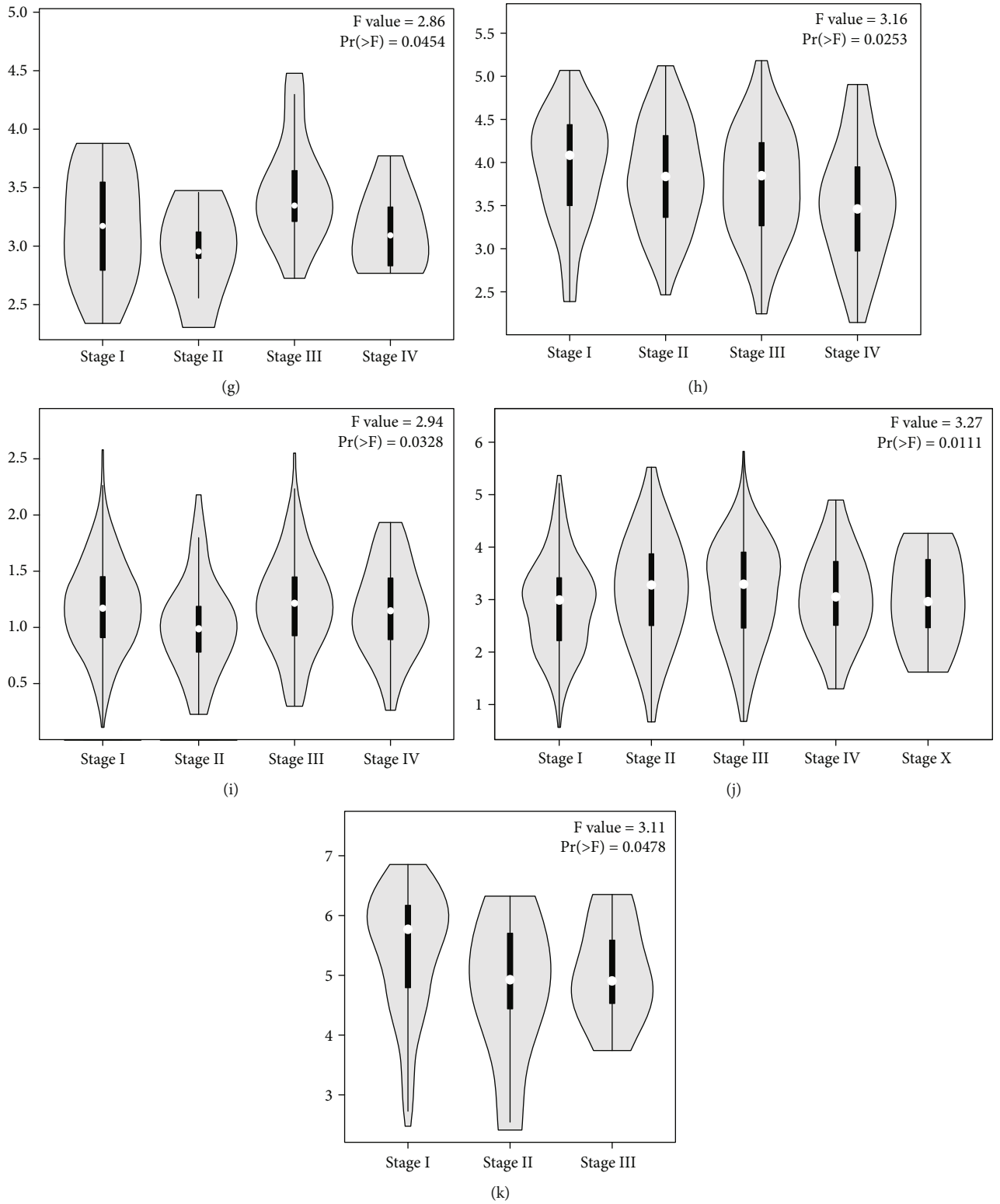


Figure 2: The correlation between RAD51 expression and the pathological stages of cancers, including (a) KICH, (b) KIRP, (c) LIHC, (d) ACC, (e) KIRC, (f) LUAD, (g) TGCT, (h) COAD, (i) THCA, (j) BRCA, and (k) UCS using GEPIA2. P value < 0.05 as significant.

significantly worse DSS in patients with ACC, BLCA, BRCA, KIRC, KIRP, LGG, LIHC, LUAD, MESO, SKCM, and STAD (Figure 5(b)). Similarly, a high level of RAD51 expression was also concerned to have a correlation with an unfavorable DFS in the TCGA cancer types, which are exhibited in Figure 5(c).

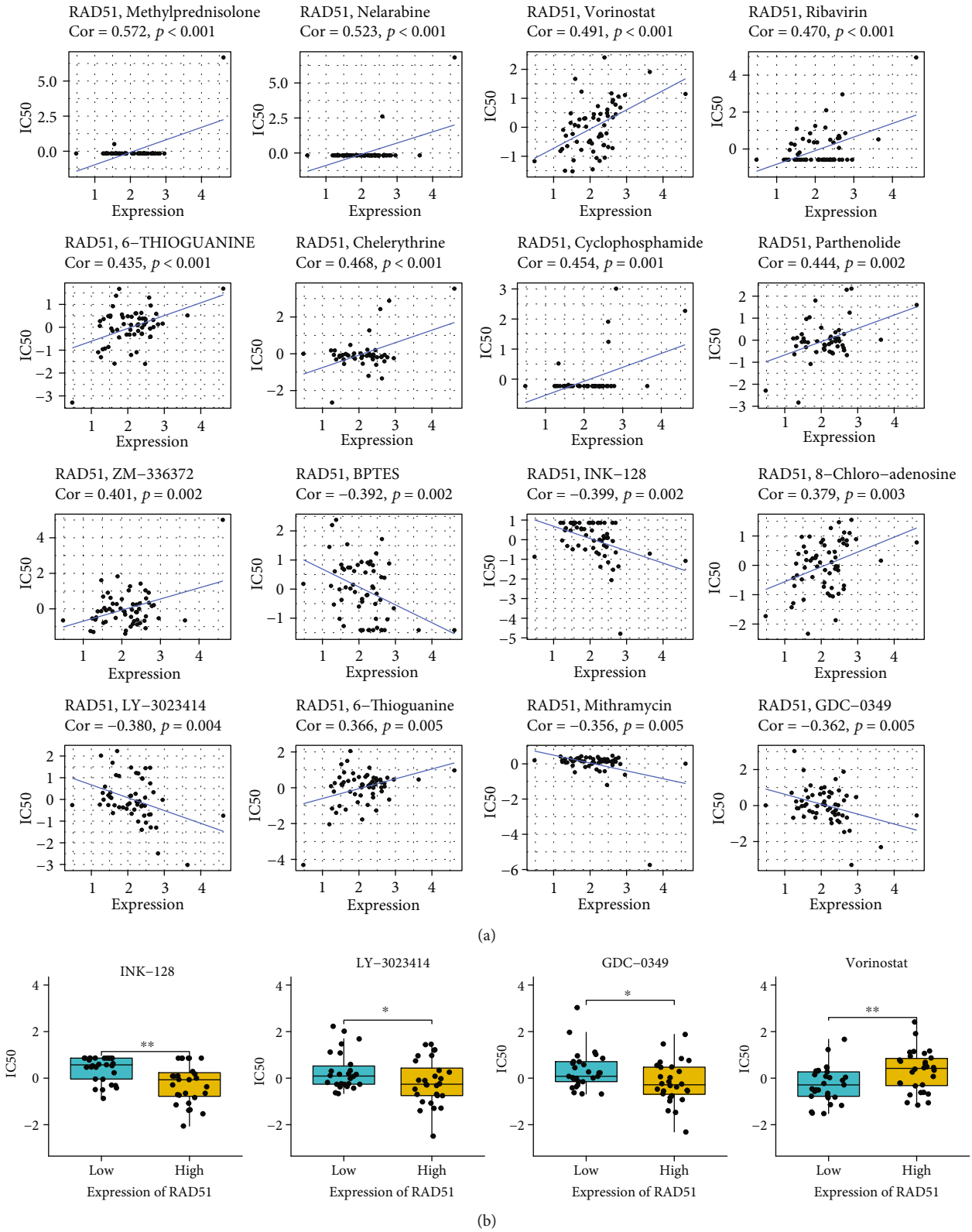
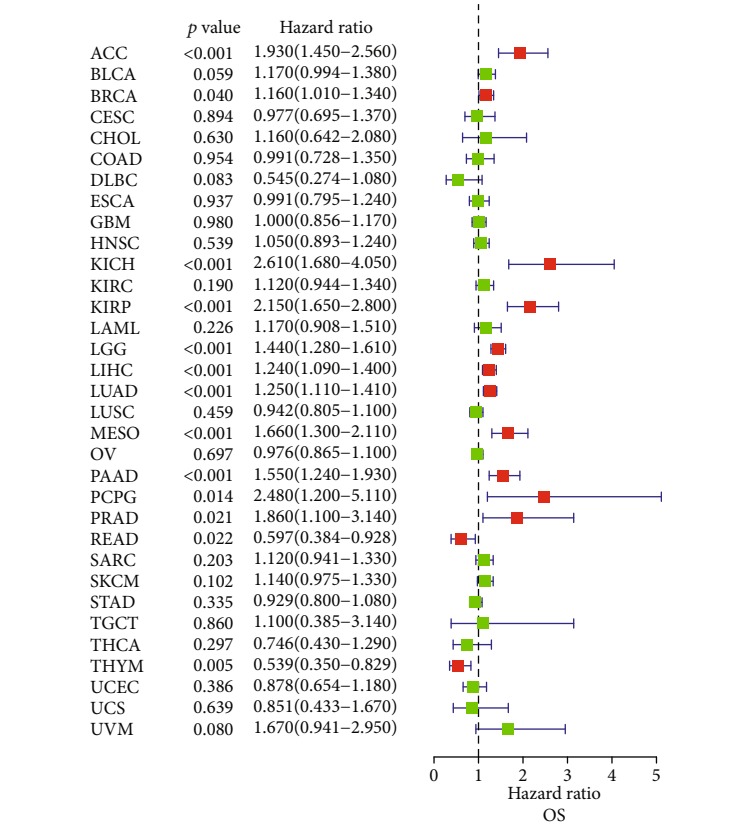


Figure 3: RAD51 expression and drug responses. (a) The relationship between RAD51 expression and the estimated IC50 values of anticancer drugs. (b) Comparison of the estimated IC50 values of INK-128, LY-3023414, and vorinostat between high- and low-expression RAD51 subpopulations. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.



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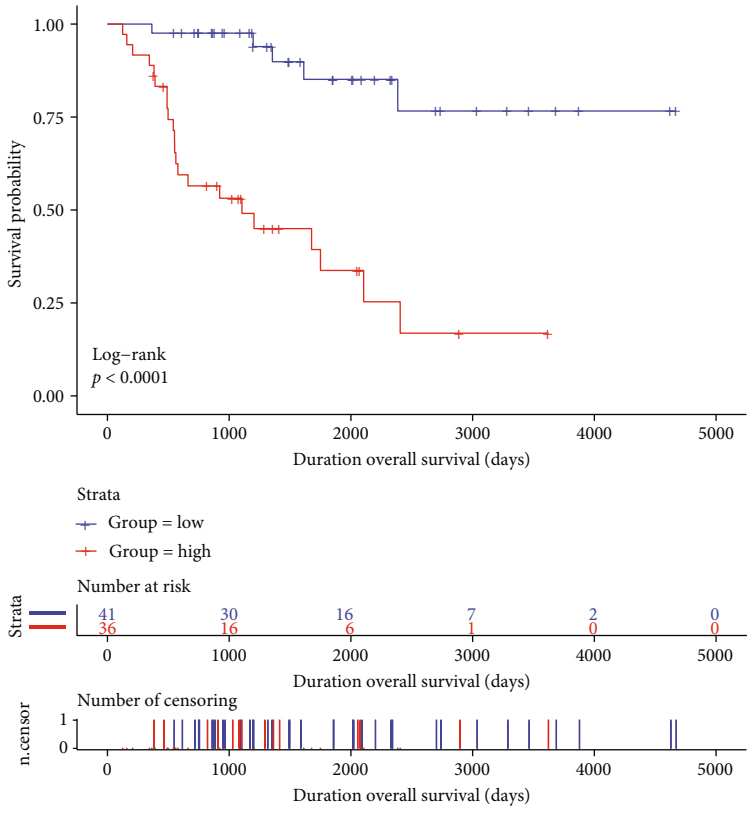

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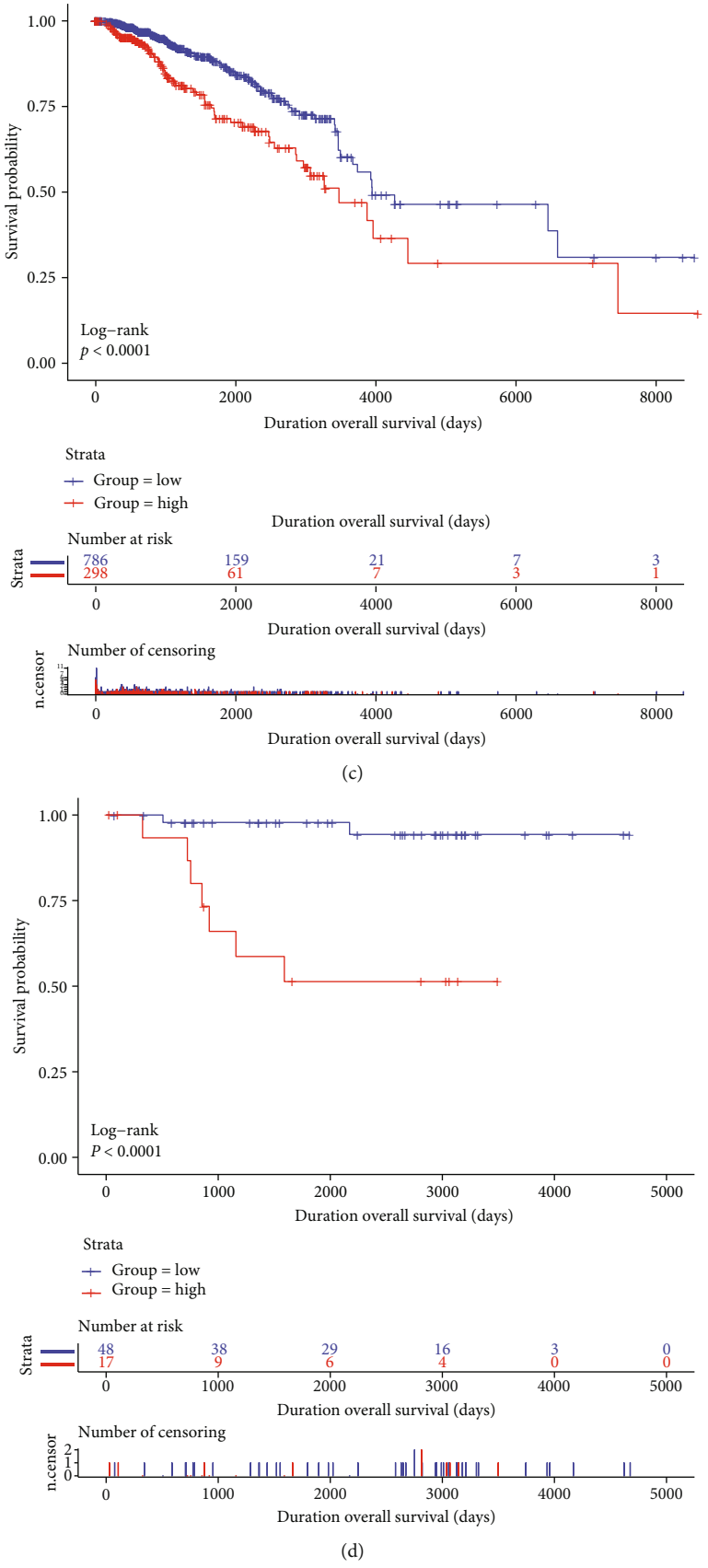

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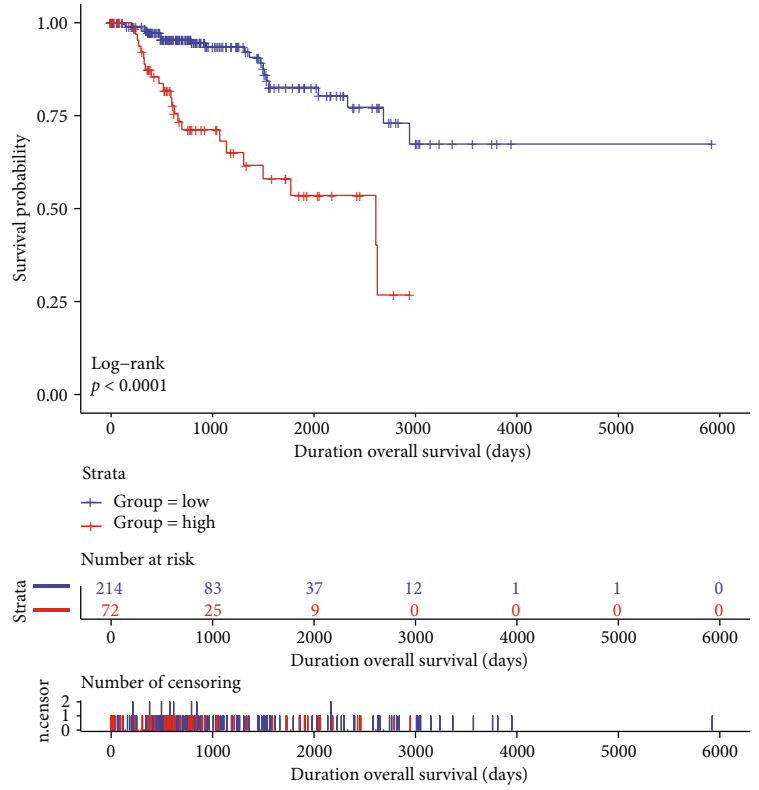

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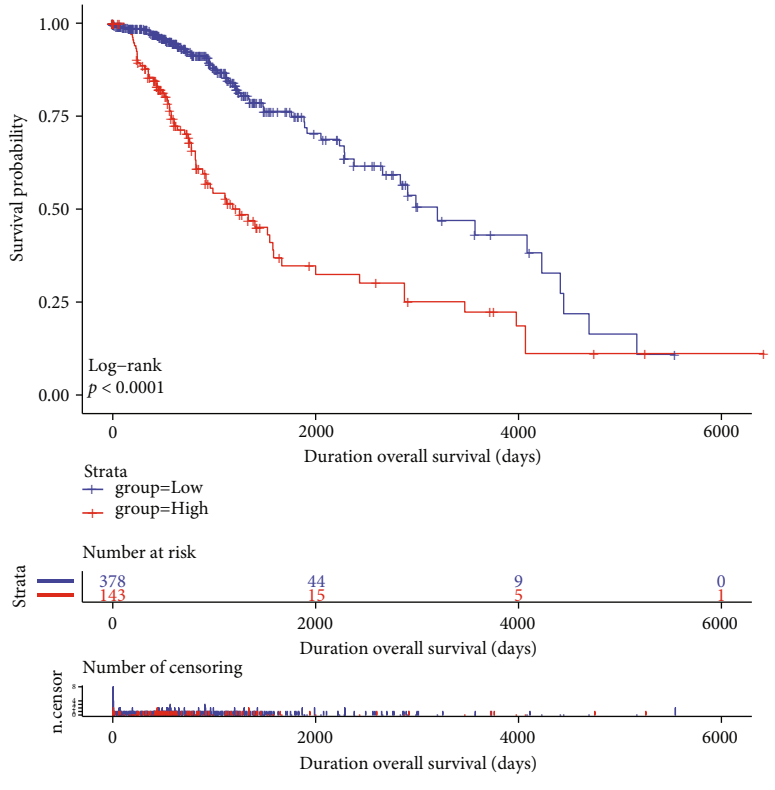

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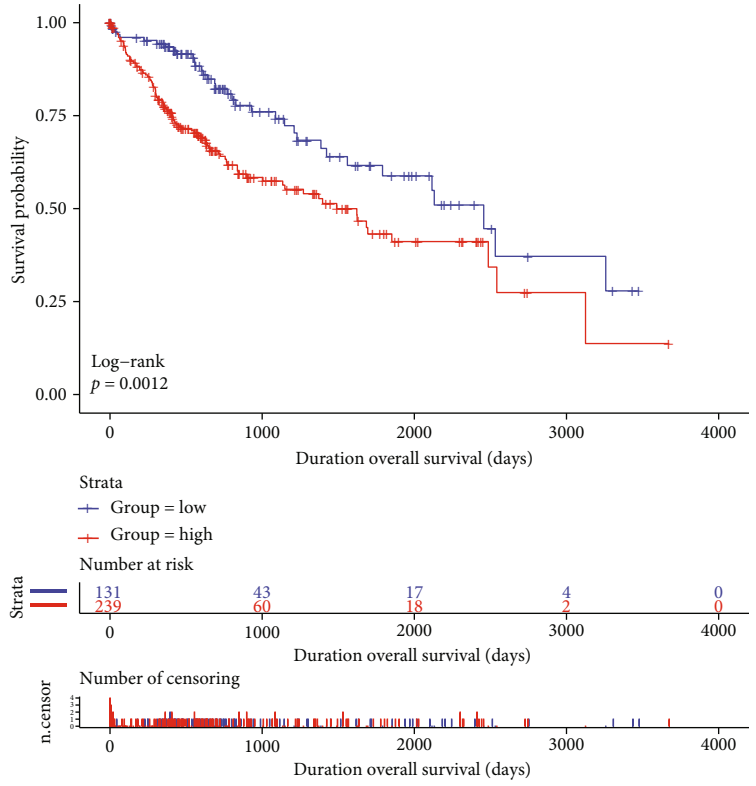

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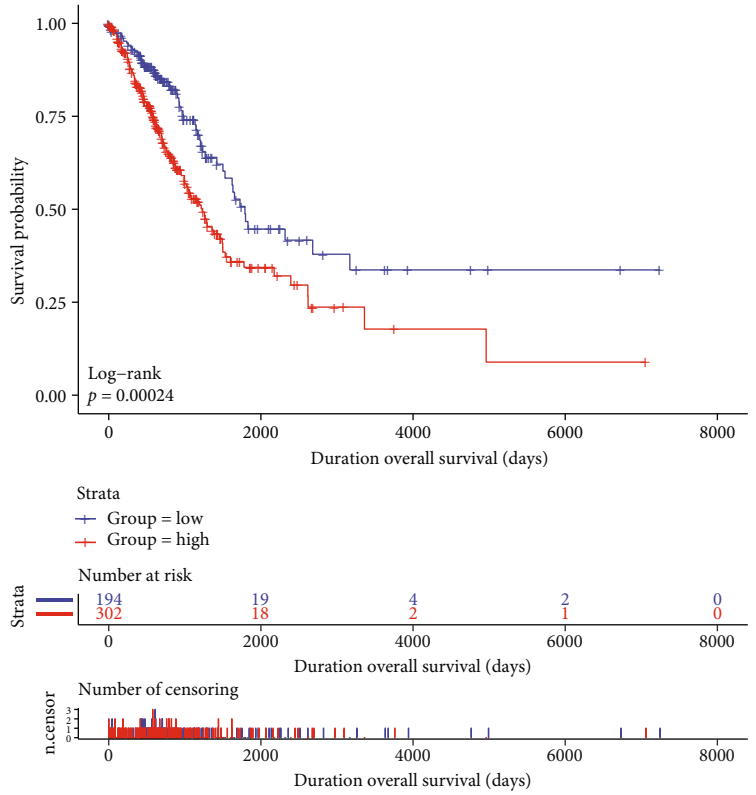

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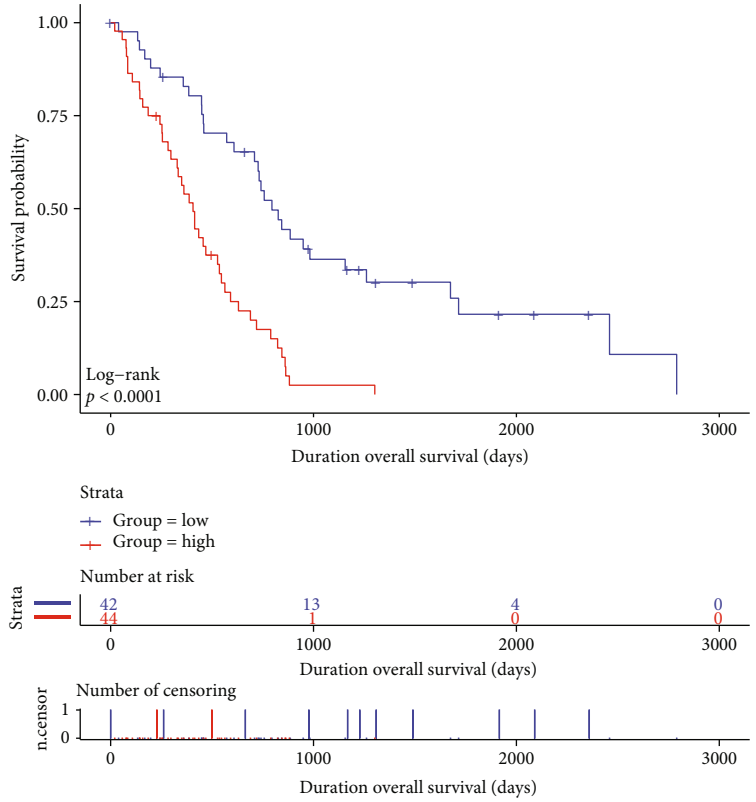

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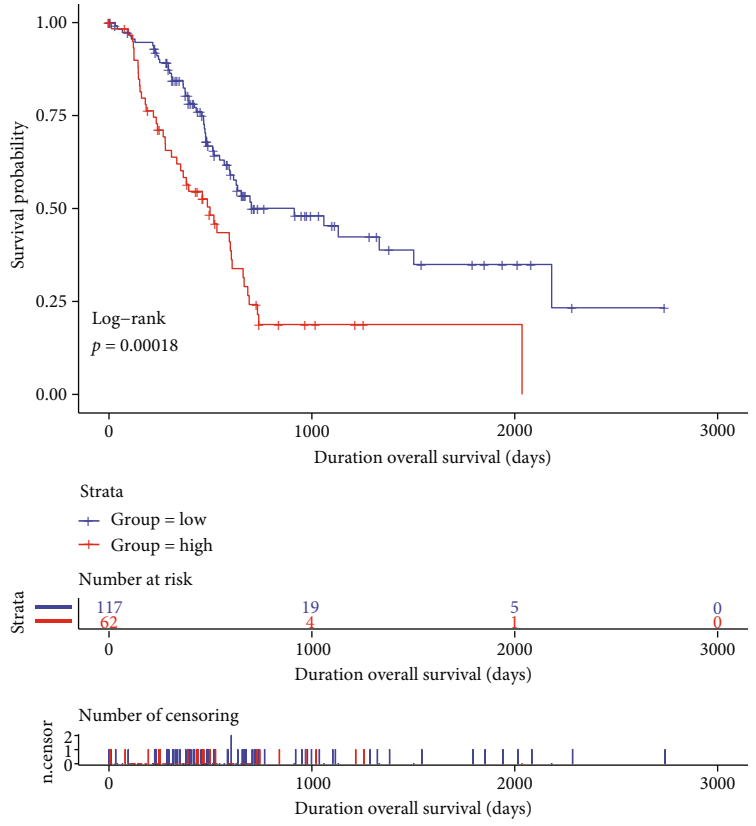

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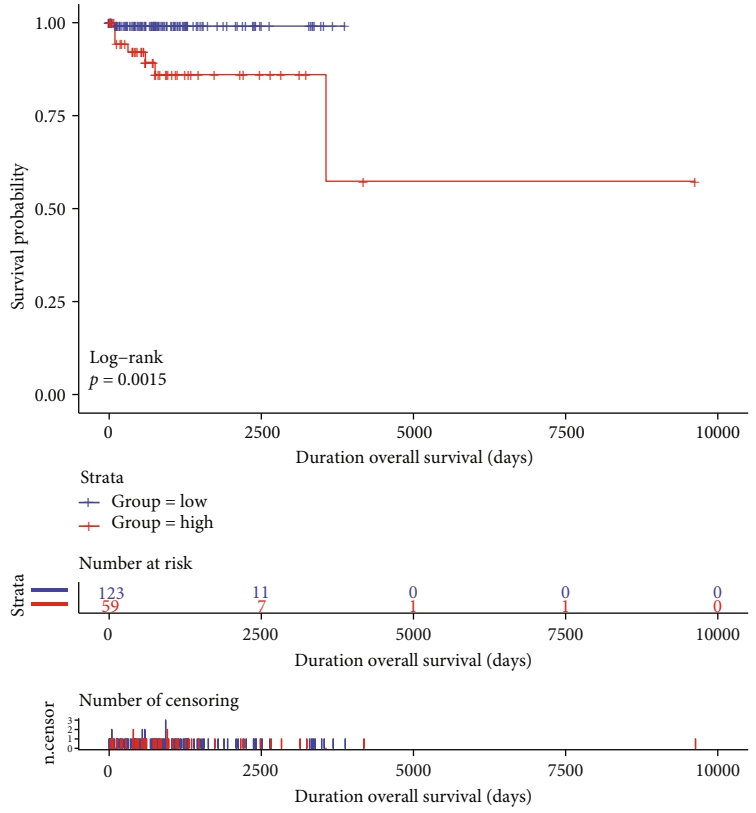

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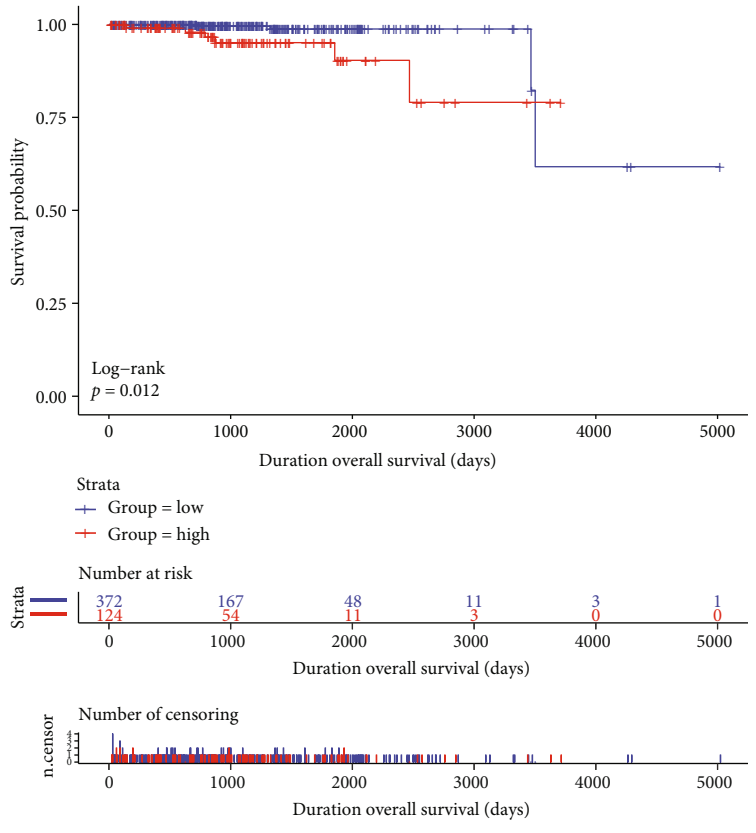

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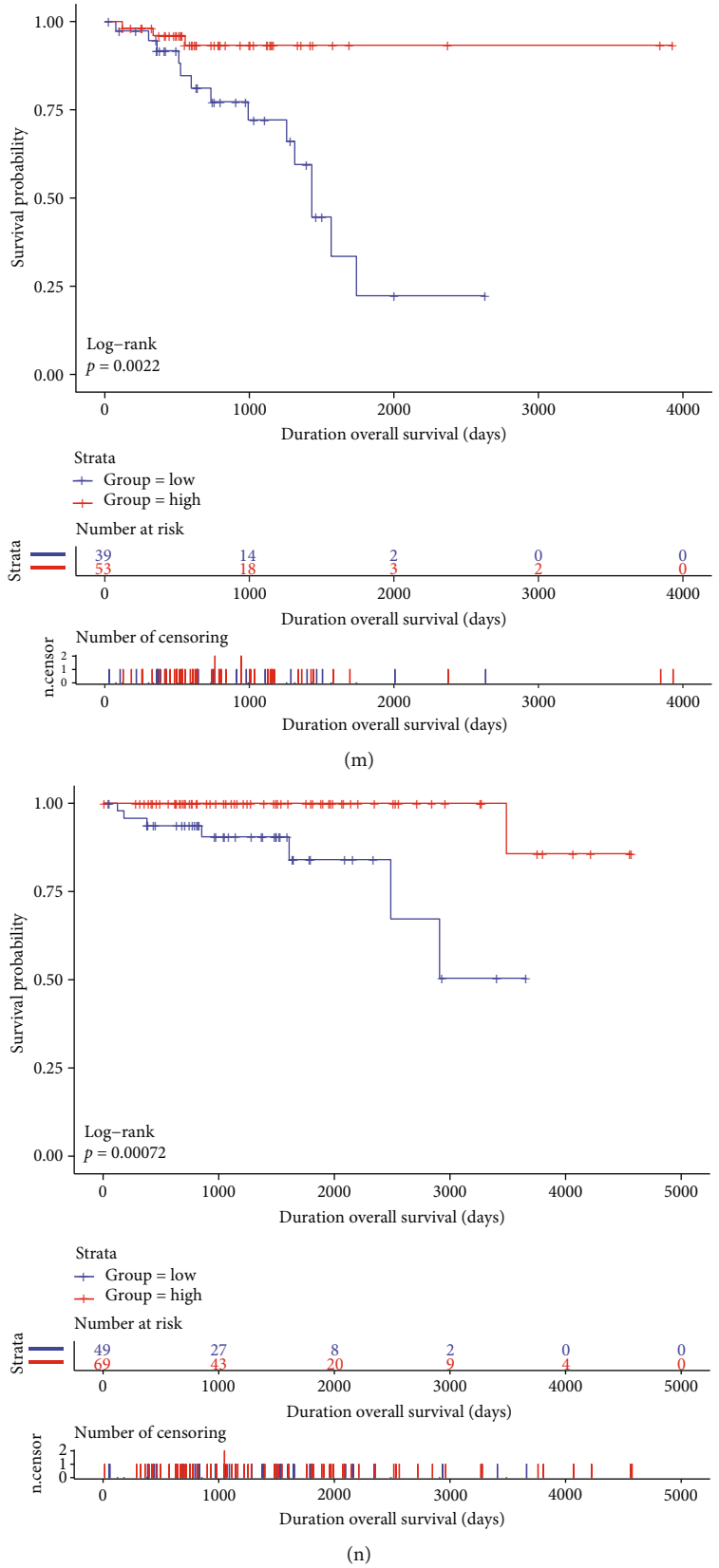


Figure 4: Relationship between RAD51 expression level and OS. Red box represents significant results (P value < 0.05). (a) Forest plot showing OS through Cox regression analysis in various cancer types. (b–n) The survival analyses for median expression value in diverse tumors using data from the TCGA database. Only significant results were shown.

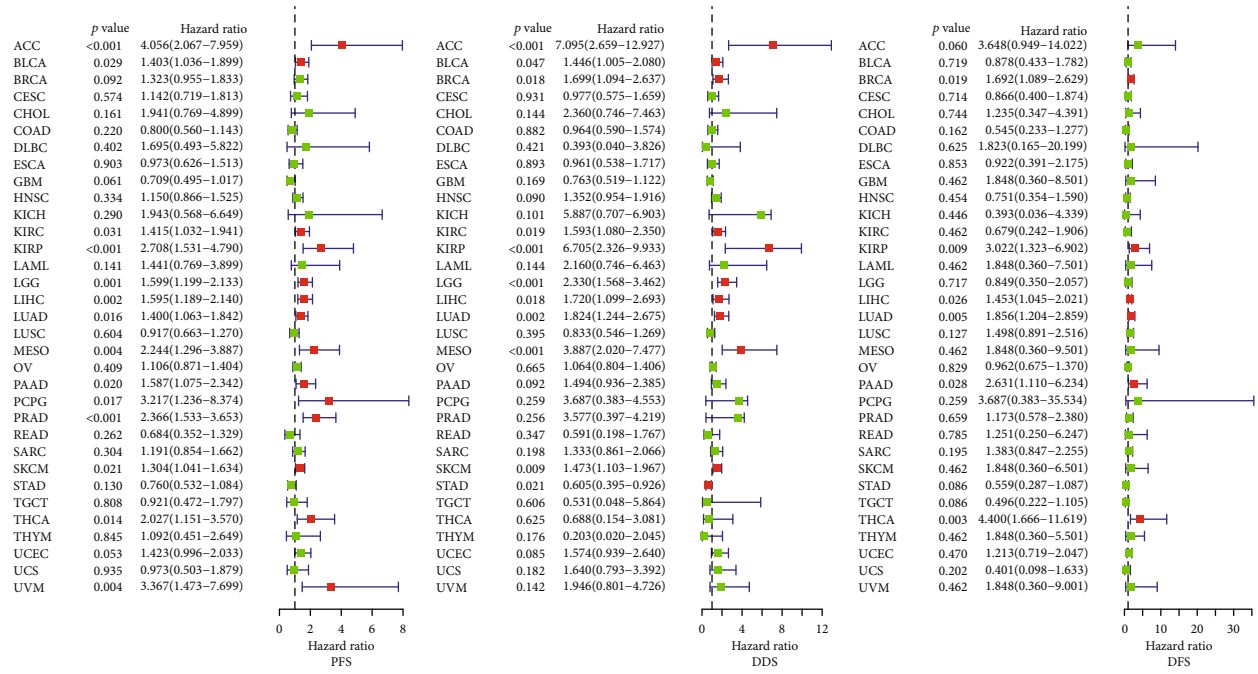


FIGURE 5: Prognostic analyses of RAD51 based on TCGA in various cancer types. Forest plots showing results of univariate Cox regression analysis for (a) PFS, (b) DSS, and (c) DFS. Red box represents significant results (P value < 0.05).

3.5. Correlation between RAD51 Expression and Immune Infiltrating Level in Cancers. As the central of HRR, RAD51 participates in the regulation of DDR and plays a key role in genomic stability. It is known that TME regulates genomic stability via DDR pathways [1], suggesting the close connection between RAD51 and TME in tumorigenesis. To determine whether RAD51 affects TME, we used TIMER to examine the correlation between RAD51 expression and the infiltrating degree of six immune cell types ($CD8^+$ T cell, $CD4^+$ T cell, neutrophil, dendritic cell, macrophage, and B cell). A clustering heat map revealed a significant correlation between RAD51 expression and six major immune cells: $CD8^+$ T cells in 13 types of cancer, $CD4^+$ T cells in 18 types of cancer, neutrophils in 13 types of cancer, dendritic cells in 18 types of cancer, macrophages in 19 types of cancer, and B cells in 10 types of cancer (Figure 6(a)). KIRC, LIHC, THCA, and THYM were the top four tumor groups that had a strong association with these six immune cells accessible in TIMER (Figure 6(a) and Figure S1). We further performed a pan-cancer analysis of the relationship between the RAD51 expression and the immune infiltration level based on xCell. As shown in Figure 6(b), RAD51 expression was significantly negatively associated with 38 subtypes of immune cells in most tumor types. Remarkably, Th2 $CD4^+$ T cells exhibited a highly significant positive correlation with RAD51 expression in these cancers (Figure 6(b)).

We used the ESTIMATE algorithm to estimate the relationship between the level of RAD51 expression and the proportion of immune matrix components (immune cells, stromal cells, and the sum of the previous two) in the TME presented in the form of ImmuneScore, StromalScore, and ESTIMATEScore in cancers. The degree of RAD51 expression significantly correlated with the proportion of stromal and immune components of TME in UCEC, LUSC, THCA, LUAD, SKCM, CESC, GBM, STAD, KIRC, HNSC, ESCA, and PAAD (Figure 7).

3.6. Correlation of RAD51 Expression with Immune-Related Genes. Immunosurveillance has crucial influences on the therapy and prognosis of cancer patients. Immune checkpoints provide opportunities for tumors to evade immune responses. We determined gene coexpression analyses to illustrate the relationship between RAD51 expression and checkpoint-related genes that are tightly linked to and recognized as immune checkpoint components. Our results revealed that RAD51 expression was positively correlated with multiple checkpoint genes (Figure 8). The coexpression analysis of RAD51 and immunostimulatory gene indicated a high correlation with TNF-related immune genes, including TNFSF9, TNFSF4, TNFRSF18, TNFRSF9, TNFRSF8, TNFRSF4, TNFRSF25, TNFRSF18, TNFRSF14, and CD276 (Figure 8). PDCD1 (PD-1)/CD274 (PD-L1) and CD28/CTLA-4 axis are two fundamental pathways of immune checkpoint, which makes a huge effect on cancer therapy [31, 32]. Our results manifested that PDCD1 (PD-1), CD274 (PD-L1), CD28, and CTLA-4 significantly correlated with RAD51 expression in multiple tumor types (Figure 8). We also found a strong connection between RAD51 expression and immune-related genes, including immunosuppressive genes, MHC genes, and chemokine (Figure S2B-S2D). These results may indicate the potential mechanism of RAD51 influencing infiltration of TAMs.

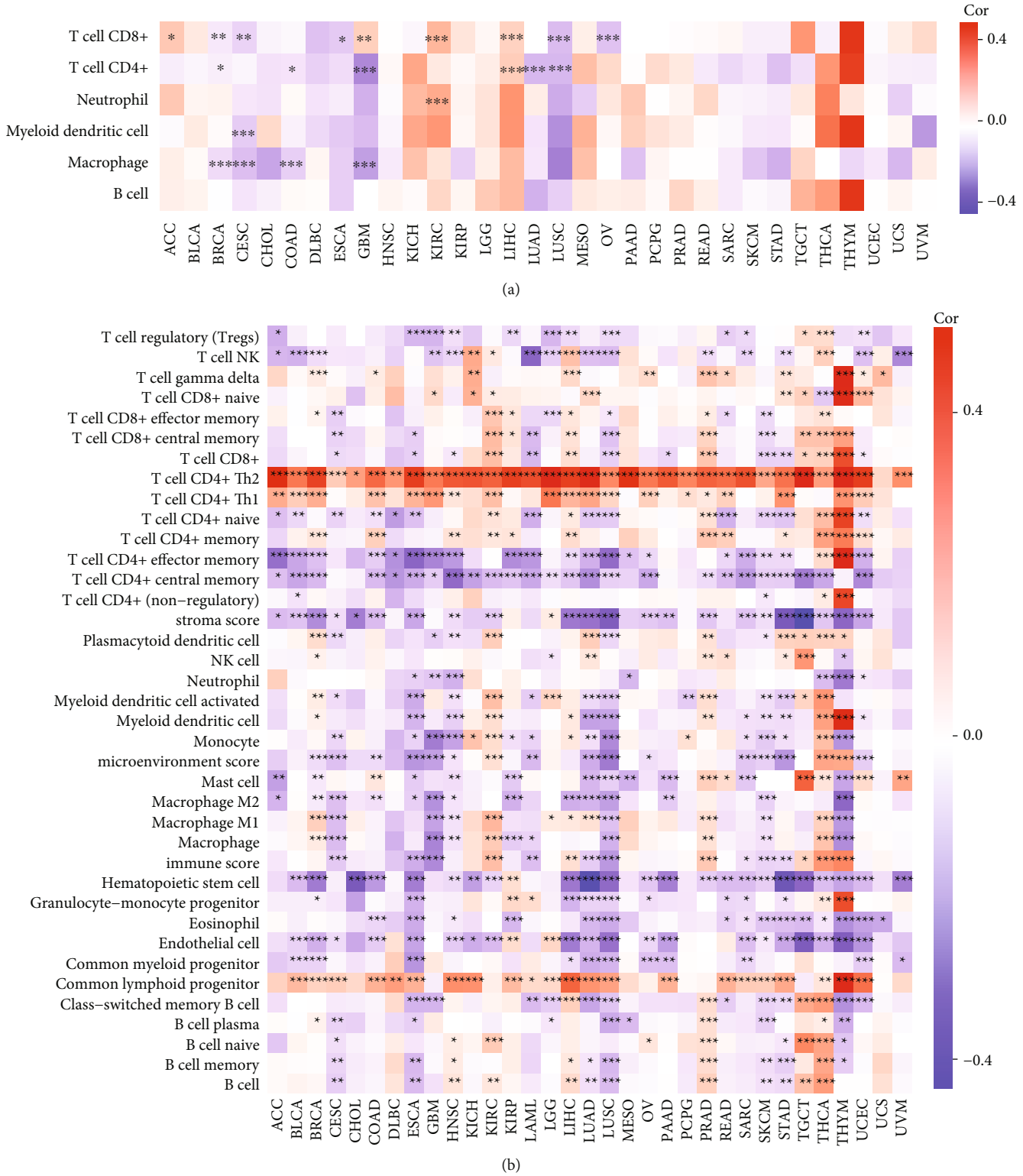


Figure 6: Correlation between RAD51 expression and the degree of immune cell infiltration. (a) The RAD51 expression significantly correlated with the infiltration levels of various immune cells based on the TIMER database. (b) The RAD51 expression significantly correlated with the infiltration levels of various immune cells based on xCell. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

3.7. Correlation between RAD51 Expression and TMB or MSI in Cancers. TMB and MSI are two emerging biomarkers associated with the immunotherapy response. We determined a pan-cancer analysis of the correlation of the RAD51 expression with TMB and MSI. In STAD, ACC, LUAD, LGG, PAAD, BRCA, and PRAD, RAD51 expression positively correlated with TMB (Figures 9(a) and 9(c)–9(h)). It is worth noting that THYM exhibited a remarkably

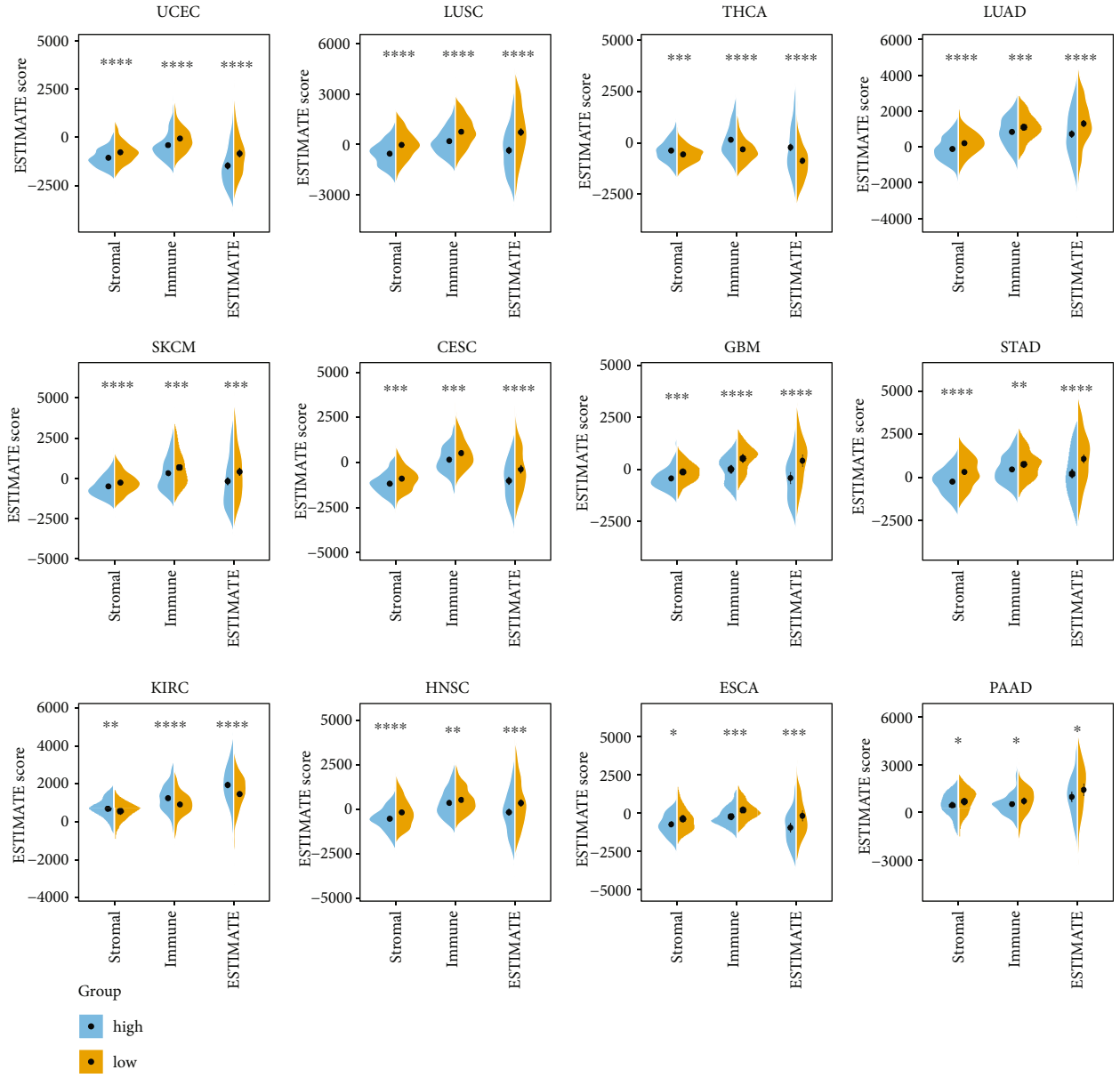


Figure 7: The relationship between the degree of RAD51 expression and the proportion of immune matrix components (immune cells, stromal cells, and the sum of the previous two) in the TME presented in the form of ImmuneScore, StromalScore, and ESTIMATEScore. Blue represents the high-expression group of RAD51, and yellow represents the low-expression group of RAD51. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

negative correlation with TMB (Figure 9(a)). In STAD, CHOL, UCEC, MESO, COAD, ACC, UVM, LIHC, BLCA, and UCS, RAD51 expression positively correlated with MSI, whereas in TGCT and CESC, it was adversely connected with MSI (Figures 9(b) and 9(i)–9(n)).

3.8. GSEA Analysis of RAD51 in Pan-Cancer. To deeply examine potential biological pathways of RAD51 regulation in diverse tumors, we performed GSEA analysis using "clusterprofiler" in pan-cancer subjects. Then, we selected the 12 types of tumor with similar results. These results indicated that these pathways enriched by GESA focused on cell cycle, cell division, immune system, cell communication, DNA repair, DNA metabolic process, and DNA replication (Figure 10 and Figure S3). The etiologic fundamental of cancer is deregulation of cell cycle arrest, activation of genes, and gene products involved in DNA repair when DNA damage occurs [33]. Therefore, it can lead to the loss of heterozygosity and genetic instability, which is the kernel of carcinogenesis, if not regulated properly [33]. Besides, GSEA results also indicated that RAD51-related genes were enriched in immune-related pathways, which implied the correlation between RAD51 and tumor immunity.

3.9. CNV and DNA Methylation of RAD51 in Pan-Cancer. We investigated the genetic alterations of RAD51 using the cBioPortal. The results demonstrated that the highest frequency of alteration in RAD51 (>5%) occurred in patients

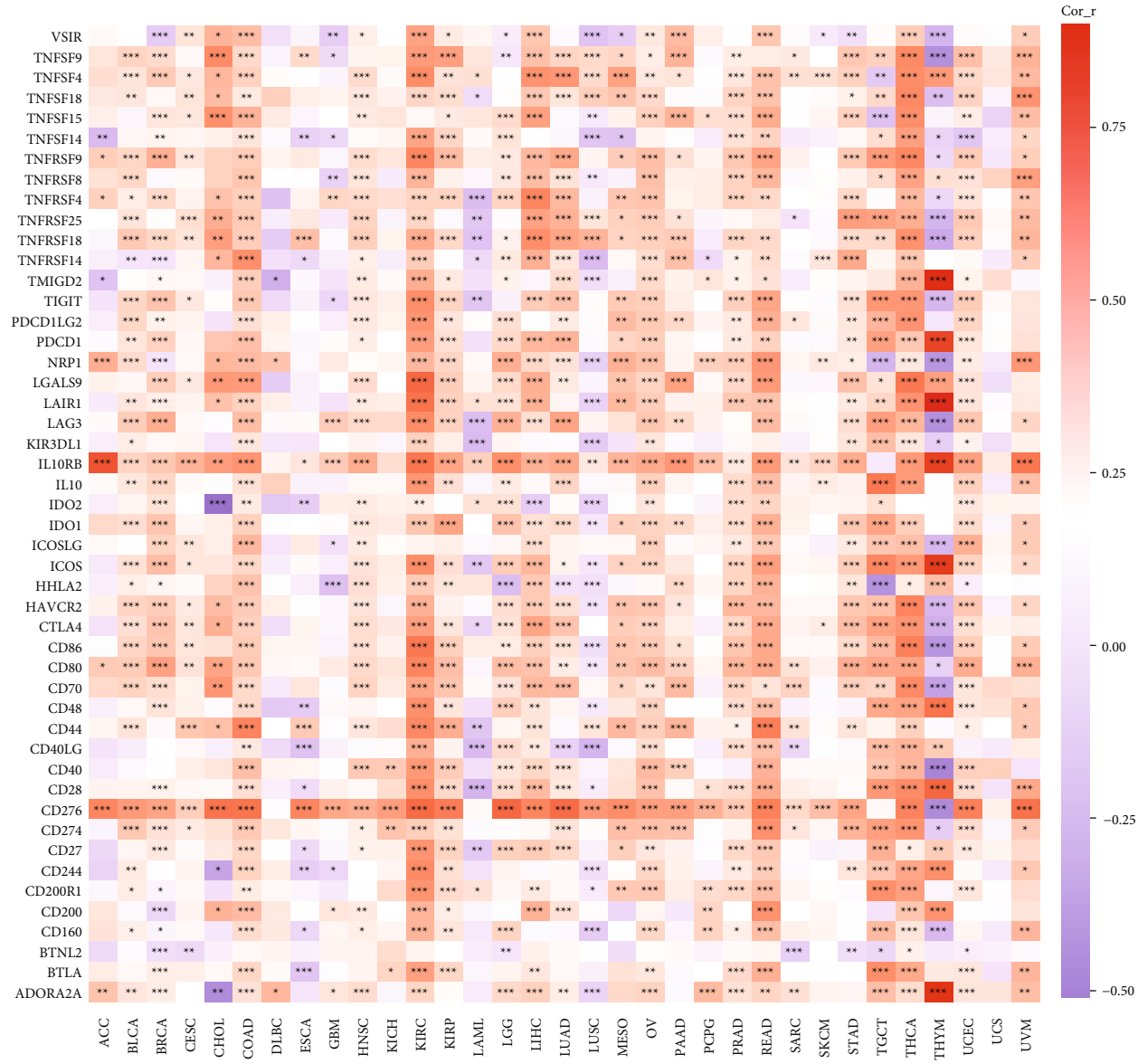


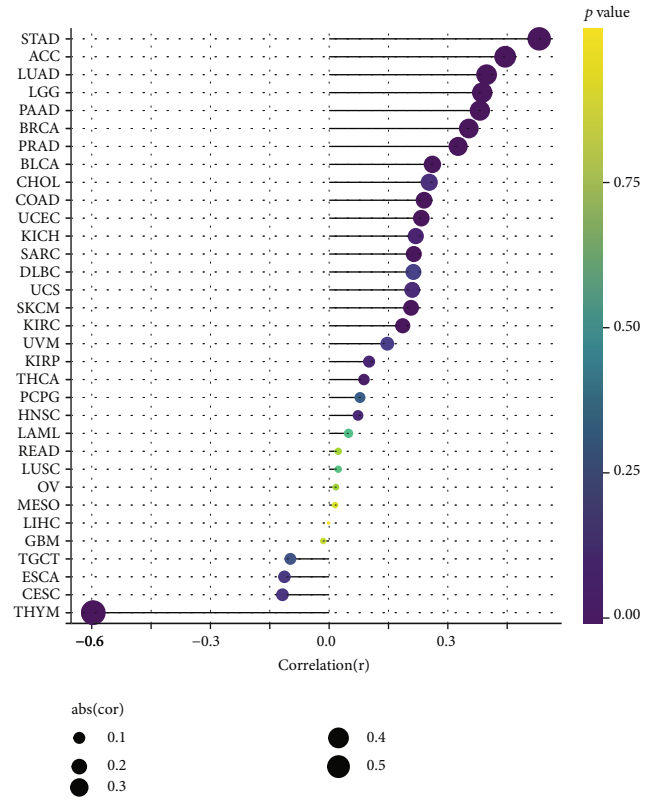
Figure 8: Relationship between RAD51 expression and immune checkpoint genes. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

with lung cancer, where "deep deletion" and "amplification" were the primary types. The "amplification" type of CNV was also the primary type in the soft tissue sarcoma and pancreatic cancer and occupied an important part in breast cancer (Figure 11(a)). Thereafter, we explored the relationship between CNV and mRNA expression. The results revealed a significantly positive correlation of RAD51 expression with CNV in LUSC, COAD, CESC, HNSC, TGCT, ESCA, SKCM, OV, STAD, READ, UCEC, UCS, SARC, THYM, and BLCA (Figure 11(b)).

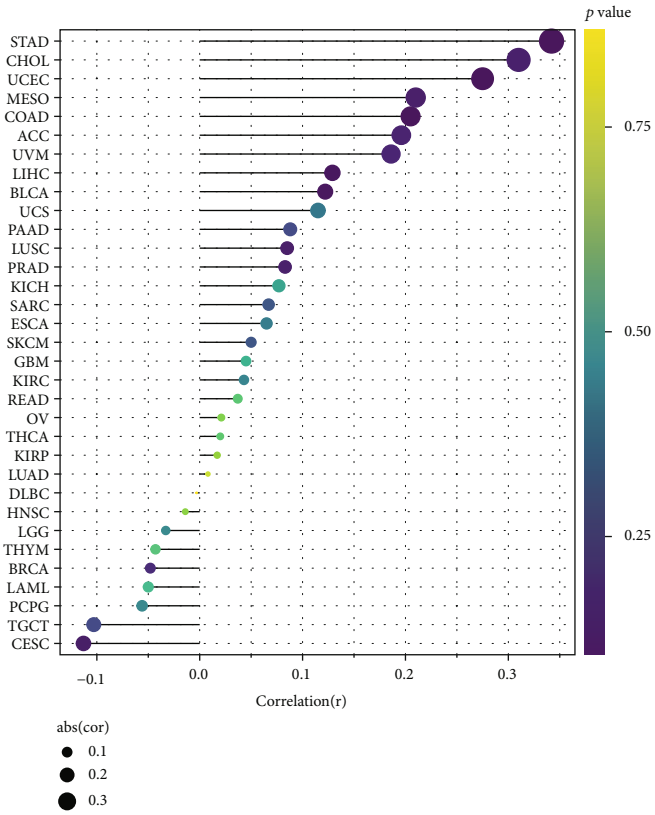
DNA methylation directly affects tumor occurrence and progression [34]. We utilized Spearman's correlation to explore the relationship between DNA methylation and the expression of RAD51. A significantly negative correlation of DNA methylation with RAD51 expression was observed in LIHC, OV, LGG, PRAD, SKCM, ACC, THYM, HNSC, SARC, and BRCA (Figure 11(c)).

4. Discussion

The maintenance of genome integrity is indispensable for any organism survival and the inheritance of traits. Deficiency in DDR results in prolonged DNA damage and can give rise to gene mutations, chromosome rearrangements, carcinogenesis, and cancer progression. These defects in DDR may have the potential to provide therapeutic implications for the clinical practice of malignancies. Previous studies have shown that the impact of a range of defects in the HRR is relevant to cancer inception, progression, and therapeutics [15]. RAD51, the vital molecular actor of HRR, directly takes charge of genomic stability. With the help of accessory proteins, RAD51 performs the tricky task of revolving around a single broken strand of DNA, then captures the backup DNA copy, matching the sequence of the broken stand to the homologous sequence. In the complex



(a)



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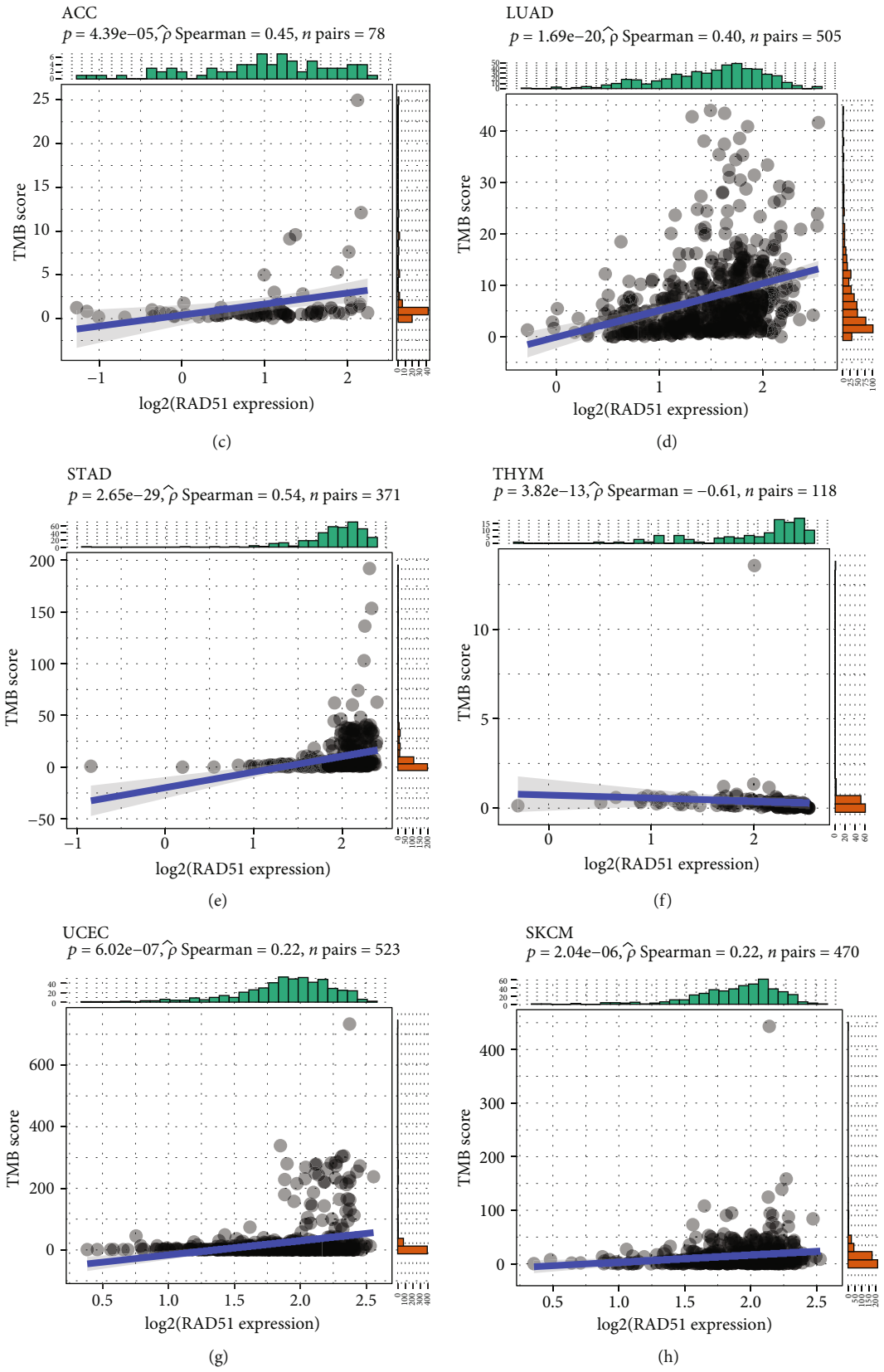

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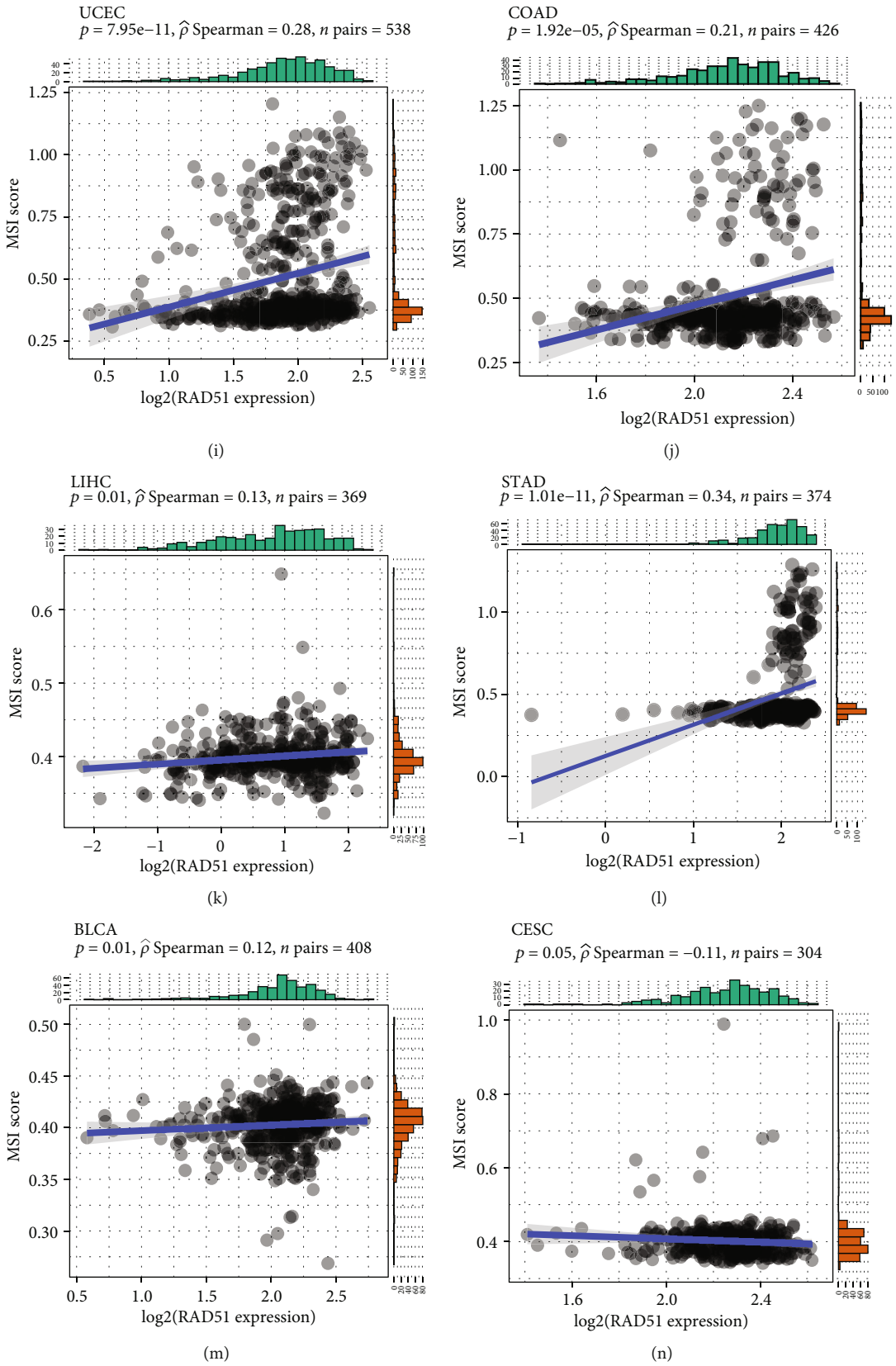


Figure 9: (a) Correlation between RAD51 expression and TMB in pan-cancer. The size of the dots represents the degree of the correlation coefficient, and different colors represent the significance of *P* value. (b) Correlation between RAD51 expression and MSI in pan-cancer. Relationship between the RAD51 expression and TMB (c–h) and MSI (i–n) in diverse tumors. Correlation analysis was performed using Spearman's method.

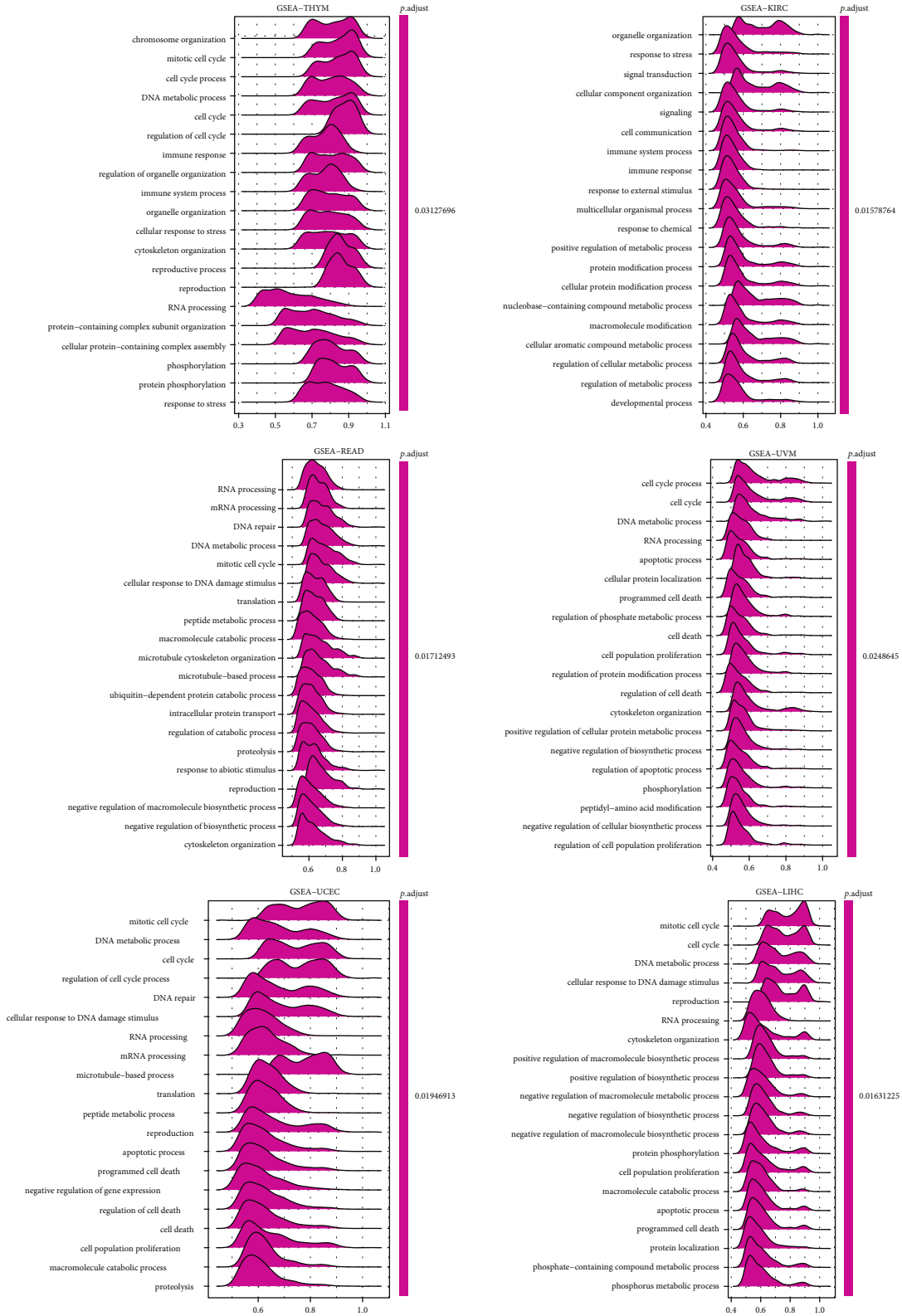
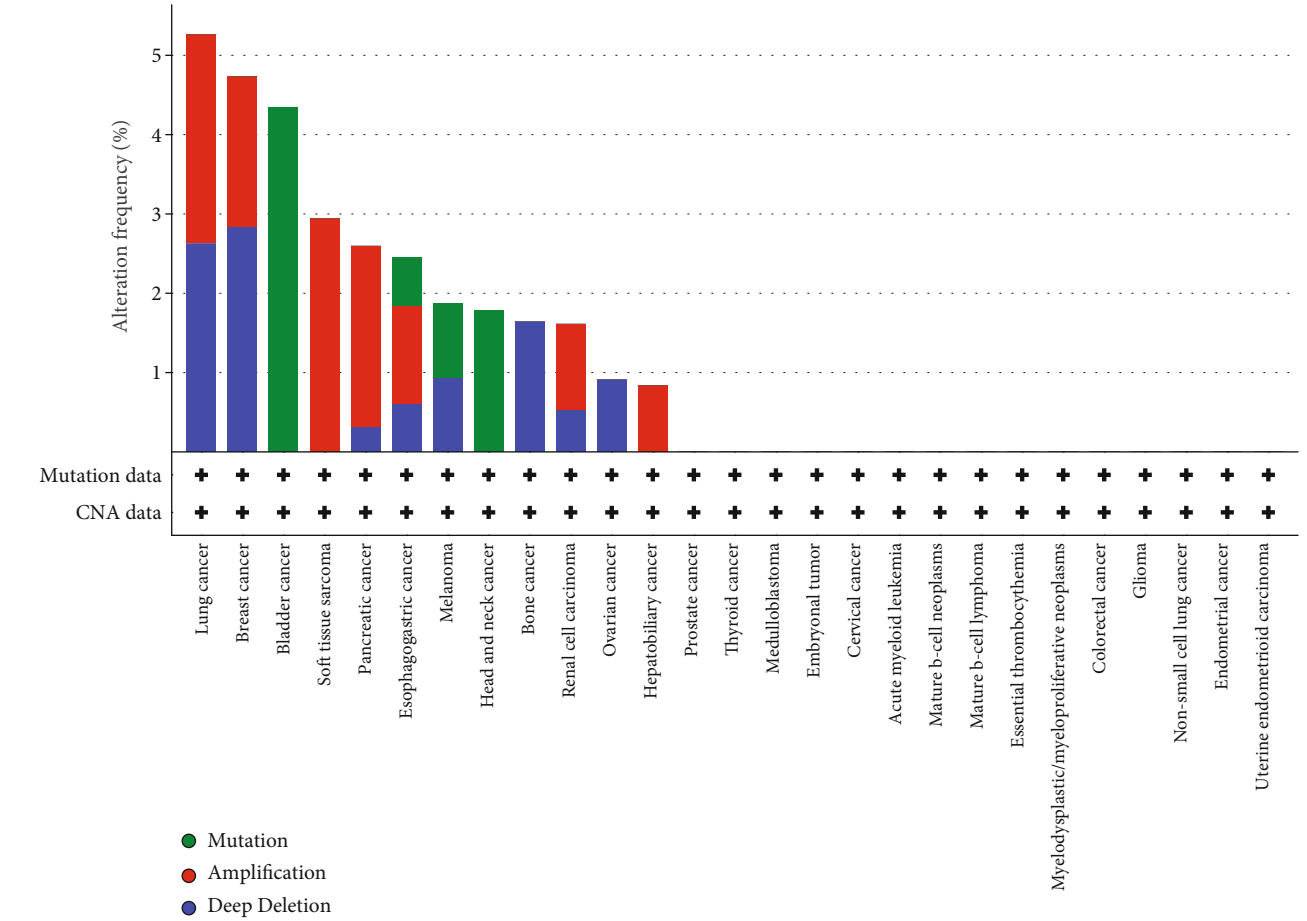
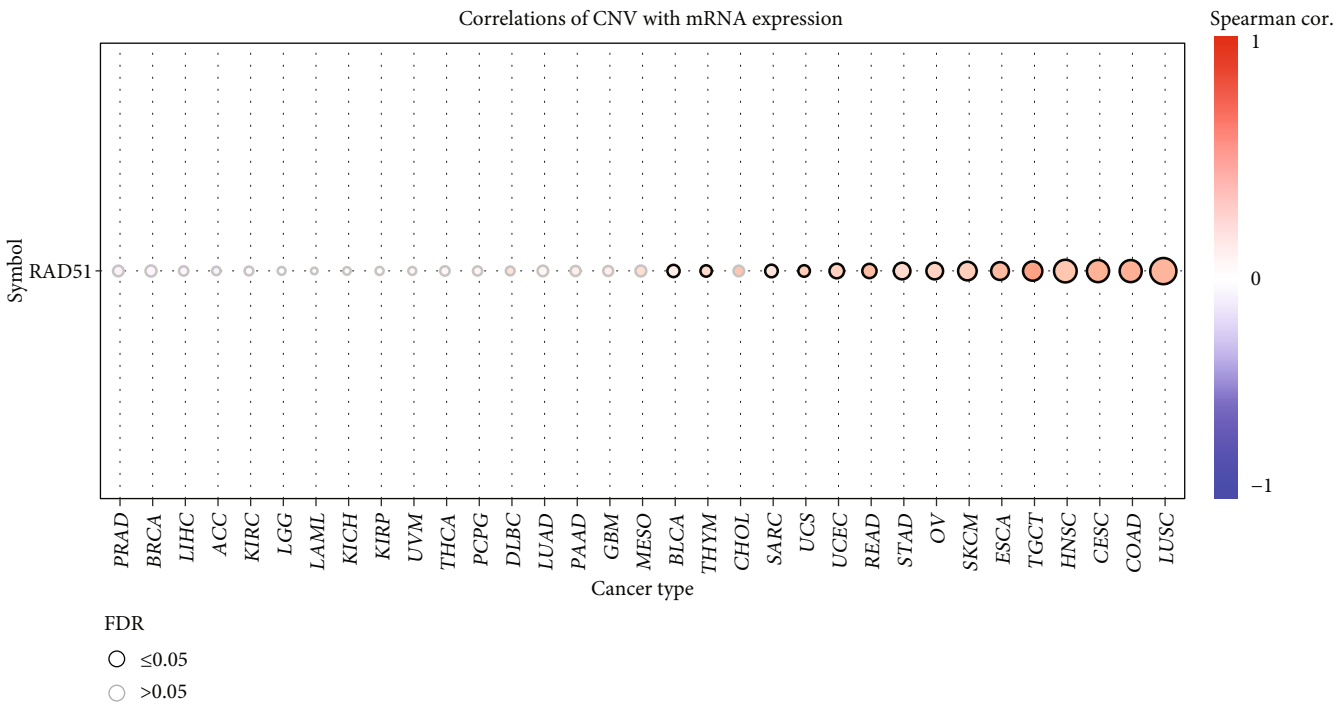


Figure 10: GSEA of RAD51 in TCGA. The top 20 significant pathways of GSEA results across the indicated tumor types.

of RAD51, the single strand is swapped for another strand in the double-stranded DNA. Finally, a plethora of other proteins fill in the missing sections of DNA and restore two matching copies [3]. RAD51 was expressed at high levels in multiple types of cancers and shown to be elevated in several types of malignancies compared with corresponding normal tissues [35]. The reports abovementioned provide the evidence that RAD51 has the potential to be a biomarker



(a)



(b)

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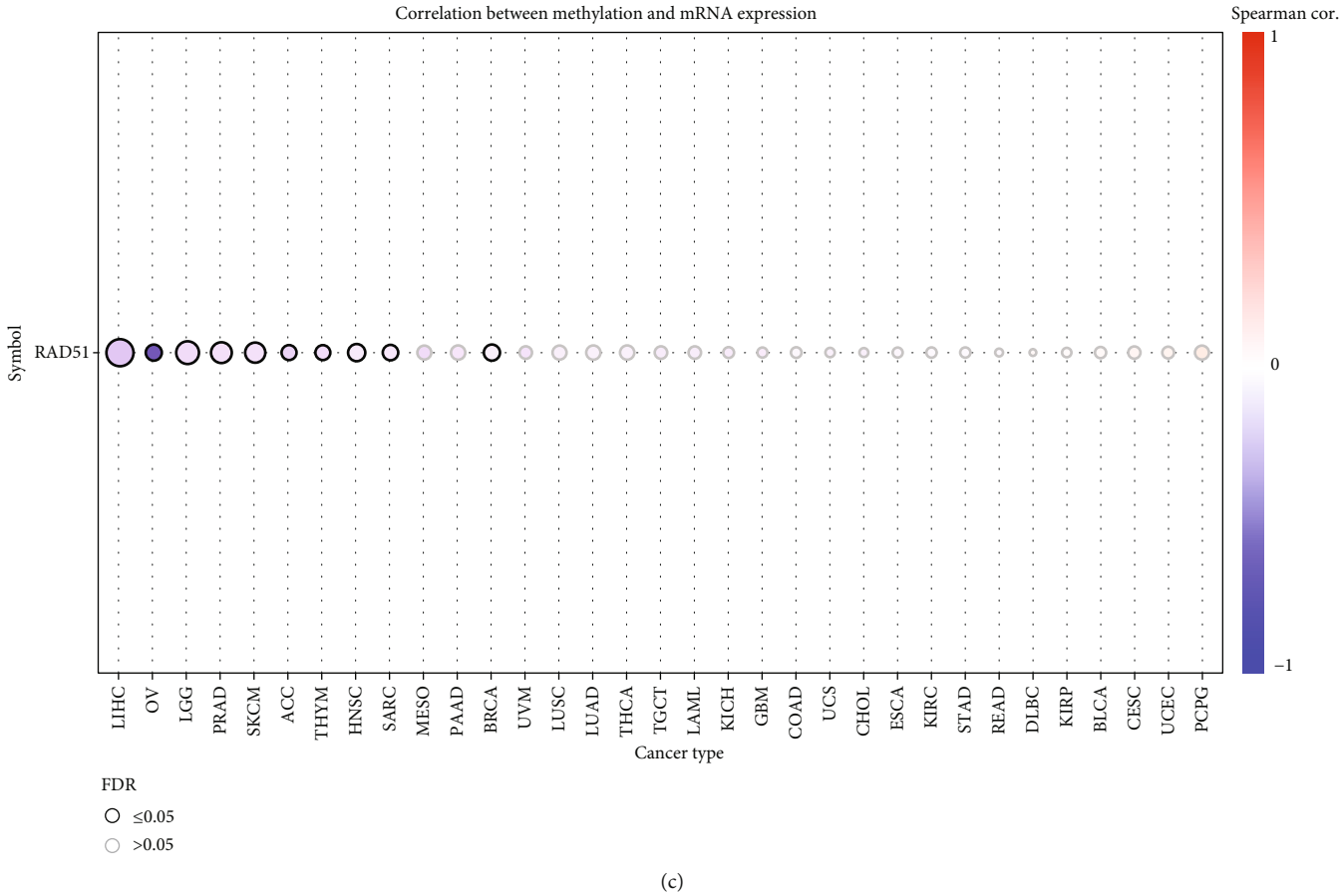

(c)

Figure 11: Correlation of CNV and methylation with RAD51 expression in pan-cancer. (a) The alteration frequency with different types of mutations was examined using the cBioPortal database. (b) Correlation between the expression levels of RAD51 and DNA copy number. Solid circle represents significant results (FDR < 0.05). (c) Correlation between the expression levels of RAD51 and methylation.

in a variety of human malignancies. Thus, here, we set out to perform a pan-cancer analysis of RAD51.

In our result, the analyses of 33 cancer datasets from the TCGA were consistent with previous studies and indicated that the expression of RAD51 was significantly higher compared to adjacent normal tissues in BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRP, KIRC, LIHC, LUAD, LUSC, PRAD, STAD, THCA, and UCEC (Figures 1(a) and 1(b)). Cox proportional hazard model analysis and Kaplan-Meier survival curves in various cancer types revealed that the high expression of RAD51 was associated with worse OS in patients with ACC, BRCA, KICH, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PCPG, and PRAD (Figures 4(b)–4(l)). In contrast, the high expressions of RAD51 in READ and THYM were associated with longer OS times (Figures 4(m) and 4(n)). The upregulated expression of RAD51 correlated with worse DSS, PFS, and DFS in several cancers. It is worth noting that KIRP LIHC and LUAD simultaneously exhibited worse DSS, PFS, and DFS (Figures 5(a)–5(c)). Next, we will emphatically discuss the situation of RAD51 in LIHC and KIRC, which exhibited worse DSS and DFS. RAD51 expression closely correlated with its CNV and DNA methylation in diverse tumors, and a high RAD51 methylation level or CNV could serve as a biomarker of prognosis in patients with cancer. In particular, LIHC displayed the highest correlation with CNV. These results implied that RAD51 can be used as a significant prognostic marker in indicated tumor types.

It was reported that elevated levels of RAD51 contributed to drug resistance in BCR-ABL transformed cells, which is the etiological cause of chronic myeloid leukemia (CML) [36]. Thus, RAD51 could be assigned as a potential alternative approach for overcoming drug resistance in cancer therapeutics [37]. We performed the correlation analysis of RAD51 expression and drug sensitivity in the NCI-60. In our results, the drug sensitivity of methylprednisolone, nelarabine, vorinostat, ribavirin, 6-thioguanine, chelerythrine, cyclophosphamide, parthenolide, ZM-336372, 8-chloro-adenosine, and 6-thioguanin were positively associated with RAD51 expression (Figure 3(a)). Inversely, a negative correlation was exhibited between RAD51 expression and BPTES, INK-128, LY-3023414, mithramycin, and GDC-0349 (Figure 3(a)). Further, high-expression RAD51 presented higher sensitivity to INK-128, LY-3023414 and GDC−0349, and low-expression subpopulations preferred to respond to vorinostat (Figure 3(b)). These results not only implied the potential role of RAD51 in the mechanisms of tumor drug resistance but also provided new therapeutic regimens when RAD51 might be used for targeted therapies with the aforementioned drugs.

TME plays a critical role in tumorigenesis [38]. Alterations driven by oncogene may influence TME and inhibit immune responses. Tumor cells might escape immune surveillance by generating TAMs to restrict the cytotoxicity from immune cells [39]. In our results, TAM infiltration significantly correlated with RAD51 expression in multiple tumor types, especially M2-like macrophages, indicating that RAD51 may affect macrophage polarization (Figure S2A). $CD8^+$ cytotoxic T lymphocytes (CTLs) are preferred immune cells for tumor immunotherapy [40]. We indeed found a significant association between CTLs and RAD51 expression in multiple malignancies (Figure 6). $CD4^+$ and $CD8^+$ T cell responses are part of the cancer immune cycle, and both populations significantly influence the clinical outcomes [41]. The balance between $CD4^+$ and $CD8^+$ T cells play important roles in tumor-suppressive mechanisms. In our results, Th2 and Th1 cells exhibited a highly significant positive correlation with RAD51 expression in these cancers (Figure 6). KIRC, LIHC, THCA, and THYM were the top four tumor groups that had a strong association with these six immune cells. (Figure 6(a)). The function of RAD51 in tumor-suppression may mainly depend on $CD4^+$ T cells, which can regulate and cooperate with CTLs. Immune effector cells in the TME can suppress tumor growth [42].

Our results further demonstrated a strong link between RAD51 level and immune checkpoint genes, such as CD274 (PD-L1), CTLA-4, CD28, and TNF-related immune genes. PDCD1 (PD-1)/CD274 (PD-L1) axis is responsible for cancer immune escape and plays a crucial part in cancer therapy [31]. CTLA-4 and CD28 are members of immunoglobulin-related receptors that are responsible for T-cell immune regulation [32]. The correlation between RAD51 expression and immune checkpoint genes is very high in LIHC and KIRC, especially KIRC. These results suggested that RAD51 may influence the regulation of the tumor immune response via immune checkpoint activities. And, we estimated the proportion of immune matrix components in the TME for pan-cancer, suggesting that the degree of RAD51 expression significantly correlated with the proportion of stromal and immune components of TME in UCEC, LUSC, THCA, LUAD, SKCM, CESC, GBM, STAD, KIRC, HNSC, ESCA, and PAAD (Figure 8). Moreover, GSEA results indicated that RAD51-related genes were involved in the immune system pathway, which implies the relationship between RAD51 and the immune process (Figure 10). RAD51 is an essential protein related to HRR and directly functions in DNA damage repair. The above results prove that RAD51 also participates in immune infiltrating and has high correlation with immune checkpoint, implying RAD51 has crucial roles in DNA damage repair and immune infiltrating. These results provide evidence for RAD51 as a potential drug target for tumor immunotherapy. TMB and MSI are two emerging biomarkers associated with the immunotherapy response [43, 44]. Our results also revealed a strong link between RAD51 expression and MSI or TMB (Figure 9).

However, the limitations of our research should be pointed out. Our study was mainly based on bioinformatics. The patient data obtained from open databases have not been verified by experiments. Therefore, more studies to validate the expression and function of RAD51 *in vivo* and *in vitro* are needed in the future.

5. Conclusions

Overall, our study determined an integrative analysis to uncover the statistical association between the level of RAD51 expression and drug response, survival status, immune cell infiltration, tumor mutation burden, microsatellite instability, and genetic alteration in diverse tumors, contributing to elucidate the roles of RAD51 in tumorigenesis from multiple perspectives. Particularly, in LIHC and KIRC, RAD51 exhibited high correlation with prognosis, immune cell infiltration, and immune checkpoint.

Data Availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare no competing interests.

Authors' Contributions

H.L., L.L., H.W., and X.Z. initiated the study. H.L. and Z.L. performed the analysis. Z.L., Y.T., A.Z., Y.Z., and X.M. collected the literature. H.L., Z.L., and H.W. wrote the manuscript. L.L., H.W., and X.Z. supervised sample collection and data processing.

Acknowledgments

We acknowledge the contributions from TCGA, GTEx, xCell, TIMER, ESTIMATE, CellMiner, and cBioPortal databases.

Supplementary Materials

Figure S1: relationship between RAD51 expression and the infiltration scores of six immune cell types (B cell, $CD4^+$ T cell, $CD8^+$ T cell, neutrophil, macrophage, and dendritic cell) in several tumors (KIRC, LGG, LIHC, THCA, and THYM) accessible in TIMER. Figure S2: correlation of RAD51 expression with (a) macrophages, (b) chemokine, (c) immunosuppressive genes, and (d) MHC genes. Red represents positive correlation, blue represents negative correlation, and the darker the color, the stronger the correlation. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. Figure S3: GSEA of RAD51 in TCGA. The top 20 significant pathways of GSEA results across the indicated tumor types. Adjusted P value < 0.05 was considered statistically significant. *(Supplementary Materials)*

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