Pan-Cancer Analysis of the Prognostic and Immunological Role of Thymocyte-Expressed Positive Selection-Associated Protein 1 (TESPA1) in Human Tumors

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Research Article

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Abstract

Background: Thymocyte-expressed positive selection-associated protein 1 (TESPA1) was identified playing a critical role responsible for T cell development in the thymus. Evidence has built the relationship between TESPA1 and cancers, but no pan-cancer analysis is available.

Methods: We explored the expression patterns, prognostic values and immunological roles of TESPA1 across thirty-three cancers based on the datasets of TCGA and GEO via multiple databases and analysis tools, including Oncomine, TIMER 2, GEPIA 2, Kaplan–Meier Plotter, Prognoscan, UALCAN, cBioPortal web, STRING website, DNMIVD and GO/KEGG analysis.

Results: We have demonstrated the different expression level of TESPA1 gene across pan-cancers and pathological stages. Furthermore, different expression level of TESPA1 gene correlated with prognosis of different cancer. A group of factors, such as DNA methylation, genetic alteration, protein phosphorylation and relevant cellular pathways are included differently compared with normal tissues, further related to prognosis. In addition, TESPA1 expression correlate positively with immune infiltrates, especially CD8 + T cells. Moreover, blood coagulation disorders were involved in the functional mechanisms of TESPA1.

Conclusions: Our findings supported TESPA1 could serve as a pan-cancer prognostic and immune-related biomarker and play a vital role in tumorigenesis.

Background

Given the complex nature of tumorigenesis, pan-cancer expression analysis of meaningful gene to explore its relation with clinical prognosis and potential underlying molecular mechanisms should be valued highly[1–4]. Gene data bank The Cancer Genome Atlas (TCGA) and The Gene Expression Omnibus (GEO) database are two comprehensive databases applied to catalogue and discover major cancer-causing genomic alterations in large sample populations around the world in public, thus allow us to develop pan-cancer analysis[5–7].

Thymocyte-expressed positive selection-associated protein 1 (TESPA1) protein, also known as KIAA0748 or HSPC257, is a T cell-expressed protein which play a dominant and necessary role in development and maturation of T-cells and involved in the late stages of thymocyte development through the regulation of T-cell antigen receptor-mediated signaling[8, 9]. TESPA1 affects the regulation of inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} release and mitochondrial Ca^{2+} uptake via the mitochondria-associated endoplasmic reticulum membrane compartment[8, 10–12]. Given the important role of these signaling pathways and immune in cancer, increasing focus is being placed on understanding the role of TESPA1 in cancer. TESPA1 has been shown credible prognostic value in lung adenocarcinoma(LUAD) patients in TCGA through GEPIA database online analysis and verification in the Kaplan-Meier plotter database[13].

In this study, we used the TCGA project and GEO databases to conduct a pan-cancer analysis of TESPA1 for the first time. Based on analysis of the expression of TESPA1 in various tumors using Oncomine and the Tumor immune estimation resource, version 2 (TIMER 2). Then, the expression of TESPA1 and its correlation with cancer prognosis were analyzed via Gene Expression Profiling Interactive Analysis, version 2 (GEPIA 2), Kaplan–Meier Plotter approach and Prognoscan. Moreover, we explored the relationship between TESPA1 expression and immune cells using the TIMER. A group of another factors, such as DNA methylation, genetic alteration, protein phosphorylation and relevant cellular pathways are included to investigate the mechanisms of TESPA1 in the pathogenesis or clinical prognosis of different cancers.

Materials And Methods

2.1 Gene expression analysis

We input “KIAA0748” in the “Gene DE” module of TIMER2 web (http://timer.cistrome.org/) to analyze the different expression of TESPA1 between tumor or specific tumor subtypes and adjacent normal tissues of the TCGA project[14]. Given certain tumors without normal tissues, the expression difference between the these tumor tissues and the corresponding normal tissues of the Genotype-Tissue Expression (GTEx) database was obtained by the “Expression analysis-Box Plots” module of the GEPIA2 web server (http://gepia2.cancer-pku.cn/#analysis) in box plots form, under the settings of *P*-value cutoff = 0.01, log2FC (fold change) cutoff =1, and “Match TCGA normal and GTEx data”[15]. The expression difference between skin cutaneous melanoma (SKCM) tissues and the corresponding normal tissues of the GTEx database obtained by xiantao tools (https://www.xiantao.love/)[16].
Oncomine(https://www.oncomine.or) as a comprehensive database that collects all published cancer microarray data, was concurrently utilized to analyze the expression levels of the TESPA1 gene in various tumors[17]. “P-value 0.001 and fold change 1.5” as threshold values were used. Additionally, we obtained the different expression of TESPA1 expression in different pathological stages (stage 0, stage I, stage II, stage III, and stage IV) across all TCGA tumors through the “Pathological Stage Plot” module of GEPIA2 and shown in violin plots form[15]. Expression data after log2 [TPM (Transcripts per million) +1] transformed were applied for the violin plots and box.

2.2 TESPA1-related phosphorylation analysis

Clinical Proteomic Tumor Analysis Consortium (CPTAC) is a convenient but powerful dataset in UALCAN(http://ualcan.path.uab.edu/index.html)[18], provided proteomic characterization of more than 500 human cancers[19]. We also compared the differences in TESPA1 phosphorylation levels between normal tissues and primary tumor tissues in breast cancer, clear cell RCC and LUAD, were analyzed (No data was available for the other tumors).

2.3 Survival prognosis analysis

GEPIA 2 was a user-friendly tool used to analyze the effects of TESPA1 expression on survival including overall survival(OS) and disease-free survival(DFS)[15]. We analyzed OS and DFS significance map data of TESPA1 across all TCGA tumors by the “Survival Map” module of GEPIA2. We choose Cutoff-high (50%) and cutoff-low (50%) values as expression thresholds for separating the high-expression and low-expression cohorts. The log-rank test was used in the hypothesis test. Furthermore, the survival plots were also obtained by the “Survival Analysis” module of GEPIA2. For more rigorous results, we analyzed the relationships between TESPA1 expression and survival in various cancer types using PrognoScan, Kaplan–Meier Plotter as well. PrognoScan (http://gibk21.bse.kyutech.ac.jp/PrognoScan/index.html) provides a powerful platform accessible for evaluating potential tumor markers and therapeutic targets[20]. Adjusted Cox P-value < 0.05 was as threshold value. Kaplan–Meier Plotter(https://kmplot.com/analysis/) is a gene expression data downloaded from GEO, EGA and TCGA, can be used for analyzed the correlations between relapse free or overall survival information and expression of genes[21]. Kaplan–Meier Plotter analyzed the relationship of TESPA1 expression with OS and RFS across different cancers, hazard ratio (HR) values with 95% confidence intervals and log-rank P-values were calculated.

2.4 Genetic alteration analysis

After logging into the cBioPortal web (https://www.cbioportal.org/) [22, 23], we click the “TCGA Pan Cancer Atlas Studies” and next choose the “Query by gene” section and entered “TESPA1” for queries of the genetic alterations of TESPA1. Alteration frequency, mutation type and copy number alteration of TESPA1 across all TCGA tumors were shown in the “Cancer Types Summary” module. We also searched the “Comparison” module to explore the correlations between TESPA1 genetic alterations and overall, disease-free, progression-free, and disease-free survival differences for different TCGA cancer cases. Kaplan-Meier plots with log-rank *P*-value were calculated as well.

2.5 Methylation analysis

DNA methylation patterns vary greatly between tumor and adjacent normal tissues. Identification signatures may provide potential cancer-specific prognostic biomarkers for pan-cancer[24]. DNMIVD web (http://119.3.41.228/dnmivd/index/) serve as a user-preferred database to visualize the DNA methylation interactive action[25]. We chose the “gene symbol” in the “Quick Search” section and input “TESPA1” for searches of the genetic methylation characteristics of TESPA1. The results of differential methylation level of this gene in the promoter region of across all tumors were observed in the “DMG” module. We also used the “Survival” module to obtain the data on the overall, disease-free, progression-free, and disease-free survival differences for the TCGA cancer cases with or without TESPA1 genetic methylation using two methods to make prognostic grouping. One method was to group the patients according to the median value, the other method was to group based on pre-methylation data < 0.3 and >0.7. Kaplan-Meier plots with log-rank *P*-value were generated as well.

2.6 Immune infiltration analysis

TIMER2.0 also served as a web server for comprehensive analysis of tumor-infiltrating immune cells[26]. We input the “TIMER2” in “Immune-Gene” module, and selected different types of infiltrating immune cells to explore the association between TESPA1 expression and immune infiltrates across all TCGA tumors. The immune cells of CD8+ T-cells, monocyte, Treg, T cell CD4+, CAF, NK cell, macrophage, DC cell, mast cell, B cell were selected. The TIMER, EPIC, MCPCOUNTER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ and XCELL algorithms were applied for immune infiltration assessments. Purity-adjusted Spearman’s rank correlation test as the statistical methods to calculate *P*-values and partial correlation (cor) values, and visualized as a heatmap and a scatter plot.

2.7 TESPA1-related gene enrichment analysis

Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) website (https://string-db.org/) analyzed protein-protein networks[27]. We explored TESPA1 internetworks by using the query of a single protein name (“TESPA1”) and organism (“Homo sapiens”). We set the following main parameters in “setting” module: minimum required interaction score [“Low confidence (0.150)”], meaning of network edges (“evidence”), max number of interactors to show (“no more than 50 interactors” in 1st shell) and active interaction sources (“experiments”). Finally, the available visualized experimentally comfirmed TESPA1-binding proteins were obtained. Subsequently, based on the datasets of all TCGA tumor and normal tissues, we used the “Similar Gene Detection” module of GEPIA2 to obtain the top 100 TESPA1-correlated targeting genes. Then, we performed a pairwise gene Pearson to analyze the correlation analysis of TESPA1 and selected genes by the “correlation analysis” module of GEPIA2. Log2 TPM-based *P*-value and correlation coefficient (R) were indicated in dot plot. Furthermore, we utilized the “Gene_Corr” module of TIMER2 to visualize the heatmap data of the correlations between TESPA1 and selected genes. Partial correlation (cor) and *P*-value in the purity-adjusted Spearman’s rank correlation test were calculated. Moreover, we combined the two sets of TESPA1-correlated targeting genes and TESPA1-interrelated genes. Enriched pathways and Gene ontology (GO) enrichment analysis were finally conducted and visualized with the cnetplot function by Xiantao tools(https://www.xiantao.love/products) [28]. The data for biological process, cellular component, molecular function and Kyoto encyclopedia of genes and genomes (KEGG) pathway were visualized as cnetplots. Two-tailed $P < 0.05$ was considered statistically significant.

Results

3.1 Gene expression analysis data

The expression status of the mRNA expression levels of TESPA1 across various cancer types in TCGA through TIMER 2 is shown in Fig. 1a. TESPA1 is enriched in the tumor tissues of head and Neck squamous cell carcinoma (HNSC) ($P<0.01$), kidney chromophobe (KICH) ($p<0.001$), kidney renal clear cell carcinoma (KIRC) ($p<0.001$), kidney renal papillary cell carcinoma (KIRP) ($p<0.001$), pheochromocytoma and paraganglioma (PCPG) ($p<0.05$). Oppositely, TESPA1 expression level in bladder urothelial carcinoma (BLCA) ($p<0.001$), colon adenocarcinoma (COAD) ($p<0.001$), glioblastoma multiforme (GBM)

(p<0.001), lung adenocarcinoma (LUAD) (p<0.001), lung squamous cell carcinoma (LUSC) (p<0.001), rectum adenocarcinoma (READ) (p<0.001), thyroid carcinoma (THCA) (p<0.001), uterine corpus endometrial carcinoma (UCEC) (p<0.001) is lower than normal tissues. Furthermore, the analyzed results in Oncomine shown that TESPA1 is higher in leukemia, but lower in brain and CNS cancer and colorectal cancers than the corresponding control tissues (Fig. 1b). Given the tumors without controls, we further evaluated the expression difference of TESPA1 between the normal tissues of the GTEx dataset as controls and tumor tissues of DLBC (*P*<0.05), LAML (*P*<0.05), brain lower grade glioma (LGG) (*P*<0.05), ovarian serous cystadenocarcinoma (OV) (*P*<0.05), testicular germ cell tumors (TGCT), thymoma (THYM) (*P*<0.05), uterine carcinosarcoma (UCS) (*P*<0.05) as well as skin cutaneous melanoma (SKCM) (p<0.001) (Fig. 1c). TESPA1 expression associated with the pathological stages of cancers presented in "Pathological Stage Plot" module of GEPIA2 including LUAD(p<0.001), SKCM (p<0.001), stomach and esophageal carcinoma (STAD) (p<0.01), TGCT(p<0.05), Thyroid carcinoma (THCA) (p<0.05) (Fig. 1d) but no shows significant differences in other cancers.

3.2 Protein phosphorylation analysis data

Phosphorylation sites and the significant differences of phosphorylation level TESPA1 in various cancers are summarized in Fig. 2. The S311 locus of TESPA1 exhibits a higher phosphorylation level in breast cancer, clear cell RCC compared with normal tissues (Fig. 2a, d, both *P* <0.001). S454 is another increased phosphorylated locus in breast cancer (Fig. 2b, P=7.025442e-04). Additionally, the S311 locus of TESPA1 exhibits a lower hypo-phosphorylated level in clear cell RCC (Fig. 2c, P=1.06088519024596e-15).

3.3 Survival analysis data

Cancer cases are divided the into high-expression and low-expression groups according to the expression levels of TESPA1 for investigating the correlation of TESPA1 expression with the prognosis of patients with different tumors. Up-regulation of TESPA1 was linked to good prognosis of OS for cancers of breast cancer (BRCA) (*P*=0.00049), HNSC(*P*=0.0077), KIRC(*P*=0.038), LIHC(*P*=0.0032), LUAD(*P*=0.00043), SARC (sarcoma)(*P*=0.0027), SKCM(*P*=6.3e−06), THYM(*P*=0.013) and poor prognosis of OS for uveal melanoma (UVM) (*P*=0.0038) within the TCGA project (Fig. 3a). Higher TESPA1 expression could forecast good disease-free survival rate in TCGA cases of adrenocortical carcinoma (ACC) (*P*=0.019), CHOL(*P*=0.034), KICH(*P*=0.022), LIHC(*P*=0.0088), SKCM(*P*=0.005) (Fig. 3b).

Next, we analyzed the prognostic value of TESPA1 using different databases. In PrognoScan, data mainly comes from GEO database, increased TESPA1 expression had a protective effect in four tumor types: brain (OS: total number = 77, HR = 0.69, Cox P = 0.028742), colorectal (OS: total number = 177, HR = 0.16, Cox P = 0.002736), colorectal (DSS: total number = 177, HR = 0.14, Cox P = 0.006587), eye (distant metastasis-free survival: total number = 63, HR = 0.00, Cox P = 0.043517), skin (OS: total number = 38, HR = 0.51, Cox P = 0.030600) (Fig. 4b-f). TESPA1 only displayed a detrimental role in blood cancer (OS: total number = 163, HR = 1.39, Cox P = 0.028519) (Fig. 4a). Next, we explored the prognostic survival including OS and RFS of TESPA1-related cancers in Kaplan–Meier Plotter, which data mainly within TCGA data. Higher TESPA1 expression is related to good prognosis including OS and RFS in BRCA(OS: HR = 0.52, log-

rank P = 4.6e-05; RFS: HR = 0.61, log-rank P = 0.022)(Fig. 5a,b), cervical squamous cell carcinoma (CESC) (OS: HR = 0.32, log-rank P = 0.00037; RFS: HR = 0.39, log-rank P = 0.015) (Fig. 5e, f), LIHC(OS: HR = 0.56, log-rank P = 0.0011; RFS: HR = 0.52, log-rank P = 0.0021) (Fig. 5g, h), OV (OS: HR = 0.72, log-rank P = 0.017; RFS: HR = 0.62, log-rank P = 0.0084) (Fig. 5i, j), TGCT (OS: HR = 1769571832.57, log-rank P =0.011; RFS: HR = 0.29, log-rank P = 0.017) (Fig. 5k, l), uterine corpus endometrial carcinoma (UCEC)(OS: HR = 0.5, log-rank P = 0.00066; RFS: HR = 0.43, log-rank P = 0.0011) (Fig. 5n, o). In contrast, higher TESPA1 is related to poor both OS and RFS probability as detrimental role in esophageal carcinoma (ESCA) (OS: HR = 2.78, log-rank P = 0.017; RFS: HR = 3.32, log-rank P = 0.01) (Fig. 5c, d), KIRP (OS: HR = 2.35, log-rank P = 0.0089; RFS: HR = 2.22, log-rank P = 0.04) (Fig. 5r, s). Individually, TESPA1 had a positive impact on OS (HR = 0.08, log-rank P = 9.9e-05) but sample number too low to do meaningful analysis of PFS in THYM (Fig. 5m); In LUAD, TESPA1 was a protective prognostic factor for OS (HR = 0.48, log-rank P = 3.1e-05) but not RFS (HR = 0.71, log-rank P = 0.22) (Fig. 5p). In PAAD (Pancreatic Adenocarcinoma), TESPA1 was beneficial to OS (OS: HR = 0.65, log-rank P = 0.045) but had no influence on RFS (RFS: HR = 0.62, log-rank P = 0.39) (Fig. 5q), whereas the opposite was true in KIRC (OS: HR = 0.86, log-rank P = 0.33; RFS: HR = 0.15, log-rank P = 0.031) (Fig. 5u). Higher TESPA1 positively improved overall survival in HNSC (OS: HR = 0.62, log-rank P = 0.00045; RFS: HR = 1.89, log-rank P = 0.093) (Fig. 5t), SARC (OS: HR = 0.41, log-rank P = 9.3e-06; RFS: HR = 0.65, log-rank P = 0.11) (Fig. 5w), READ (Rectum Adenocarcinoma) (OS: HR = 0.3, log-rank P = 0.0014; RFS: HR = 2.61, log-rank P = 0.36) (Fig. 5y), and RFS in BLCA (OS: HR = 0.82, log-rank P = 0.17; RFS: HR = 0.43, log-rank P = 0.024) (Fig. 5x), but negatively inhibited with RFS probability of STAD (OS: HR = 0.88, log-rank P = 0.47; RFS: HR = 3.91, log rank P = 0.0055) (Fig. 5v).

3.4 Genetic alteration analysis data

Genetic alteration status of TESPA1 in different tumor samples of the TCGA cohorts were shown in Fig. 5a. Patients with skin cutaneous melanoma with “mutation” as the primary type accounts for the highest alteration frequency of TESPA1 (>6%). Patients with adrenocortical carcinoma with “amplification” type of copy number alteration as the primary and only type shown an alteration frequency above 4% (Fig. 6a). The types and sites of the TESPA1 genetic alteration were further displayed in Fig. 6b. Among these mutations, missense mutation of TESPA1 was the main type of genetic alteration, and R184Q alteration in the KRAP_ inositol 1,4,5-trisphosphate receptor (IP3R) _bind domain, which was detected in 5 cases of 1 case of GBM, 1 case of UCEC, 1 case of COAD and 2 cases of SKCM (Fig. 6b), is able to induce mutation of the TESPA1 gene, translation from R (Arginine) to Q (Glutamine) at the 184 sites of TESPA1 protein (Fig. 6b). Additionally, we analyzed the possible correlations between genetic alteration of TESPA1 and survival rate of cases with different types of cancer. The data of Fig. 6c indicate that esophageal adenocarcinoma patients with altered TESPA1 mutation were victims with worse prognosis in OS(*P*=0.0192), but not DSS (disease-specific survival) (*P*=0.136), DFS (disease-free survival) (*P*=0.603), and progression-free survival (PFS) (*P*=0.652). However, adrenocortical carcinoma patients with altered TESPA1 mutation were victims with worse prognosis in PFS (*P*=0.0181) and DSS (*P*=0.0491), but not OS(*P*=0.0831) and DFS (*P*=0.359) (Fig. 6c).

3.5 DNA methylation analysis data

We used the DNMIVD approach to investigate the potential association between TESPA1 DNA methylation and the tumorigenesis in different tumors based on the TCGA project. Compared with normal tissues, we observed a reduced methylation level of TESPA1 in tumor tissues of THCA(p=1.81e-07), COAD(p=1.58e-19), READ(p=6.01e-08), LUSC(p=6.64e-21), BLCA(p=1.02e-04), GBM(p=3.38e-12), LUAD(p=2.47e-05), and an increased methylation level of TESPA1 in tumor tissues of KIRC(p=1.18e-48), KIRP(p=8.07e-05) and PCPG(p=0.026) (Fig. 7a). Furthermore, we observed that highly methylated level of TESPA1 positively correlated with OS(p=0.044), PFI(p=7.08e-03) other than DFI(p=0.48) in GBM. Up-methylated level of TESPA1 positively correlated with OS(p=4.95e-05), progression free interval (PFI) (9.00e-03) other than disease free interval(DFI) (p=0.394) in KIRC. Higher methylation level of TESPA1 positively correlated with OS(p=4.81e-03), PFI(p=7.75e-03), and DFI(p=0.233) in KIRP. For THCA, hypermethylation level of TESPA1 negatively correlated with PFI(p=0.004) other than OS(p=0.984), DFI(p=0.346) (Fig. 7b).

3.6 Immune infiltration analysis data

Tumor-infiltrating immune cells, as prominent components of the tumor microenvironment, were closely linked to the almost all process of cancer. After a series of analysis, we observed a statistical positive correlation between the immune infiltration of CD8+ T cells and TESPA1 expression in the tumors of 12 tumors including BRCA, CESC, COAD, KIRC, KIRP, LUAD, MESO(Mesothelioma), SARC, SKCM, SKCM-Metastasis, STAD and TGCT (Fig. 8a). The detail scatterplot data of these tumors generated using one algorithm are presented in Fig. 8b. For example, TESPA1 expression level in KIRC is positively correlated with the infiltration level of CD8+ T cells using XCELL algorithm (Fig. 8b, cor=0.721, *P*=4.00e-75). The correlations between monocyte, Treg, CD4+ T cells, CAF, NK cell, macrophages, DC cells mast cell, B cell and TESPA1 expression shown in Fig. 8c.

3.7 Enrichment analysis of TESPA1-related partners

To further investigate the molecular mechanism of the TESPA1 gene in pathogenesis of cancer, we screened out TESPA1-binding proteins and TESPA1-correlated genes for a series of pathway enrichment analysis. We obtained a total of 22 experimented TESPA1-binding proteins and the interaction networks of them were shown via STRING tools in Fig. 9a. Concurrently, we obtained the top 100 TESPA1-correlated genes used the GEPIA2 tools. Nucleotide-oligomerization domain-like receptor subfamily C3 (NLRC3) (R=0.72), solid-pseudopapillary neoplasm (SPN) (R=0.72), integrin alpha4 (ITGA4) (R=0.68), Protein tyrosine phosphatase nonreceptor type 7 (PTPN7) (R=0.68), CBFA2/RUNX1 partner transcriptional co-repressor 3(CBFA2T3) (R=0.67) genes (all $P<0.001$) were selected to analyzed the correlation with TESPA1 expression level in dot plot, which proved a positive relationship (Fig. 9b). The heatmap data also showed a significant positive correlation between TESPA1 and the above five genes across almost all cancer types consistently (Fig. 9c). We combined the two datasets to perform KEGG and GO enrichment analyses. The KEGG data of Fig. 9d suggest that “coagulation”, “hemostasis” and “blood coagulation” might be involved in the effect of TESPA1 on tumor pathogenesis. The GO enrichment analysis further indicated that most of these genes are linked to the pathways or cellular biology of

coagulation, hemostasis, gap junction, growth hormone synthesis, secretion and action, oocyte meiosis and others. (Fig. 9e).

Discussion

TESPA1, a key gene in thymocyte development, is a rare and potentially pathogenic variant in 14 systemic diseases, such as rheumatoid arthritis, and plays an important role in the pathogenesis of these diseases, such as autoimmune arthritis[8, 29, 30]. In the process of double-positive (DP) thymocytes become mature single-positive CD4+ and CD8+ T cells, TESPA1 have been reported as a necessary gatekeeper of thymic-specific TCR signaling regulator that are able to improve the sensitivity of the TCR signal to facilitate positive selection[8, 9, 31–33],The mechanism of Tespa1 in T cell development and the regulation of TCR is that Tespa1 interacts with a transmembrane Ca^{2+} channel protein in endoplasmic reticulum inositol 1,4,5-trisphosphate receptor (IP3R), then induced subsequent calcium signaling and MAPK activation[11, 34]. Interestingly, Tespa1 protein is phosphorylated in response to store-operated calcium entry[10]. For B cells, Tespa1 is essential for T cell-dependent (TD) B cell responses. However, Tespa1 does not influence the development of B cells, but Tespa1-deficient has a significant reduced impact on antibody concentrations in serum due to inhibit the activation and proliferation of B cells induced by TD antigens[9]. Mast cell is also activated by Tespa1, which orchestrates by tuning the balance of LAT1 and LAT2 signalosome assembly[35]. Tespa1 also participate in mitochondrial Ca^{2+} uptake in the MAM compartment[12]. Owing to the close relationship between TESPA1 and immune system, more and more attention focused on the role of TESPA1 in cancer. Novelty, TESPA1 has been discovered credible prognostic value in evaluating the survival/prognosis of patients, invasion and progression of tumors in LUAD patients[13]. Whether TESPA1 plays a role in the pathogenesis of different tumors through some common molecular mechanisms remains unclear. Therefore, based on TCGA, CPTAC and GEO database data, as well as molecular characteristics of gene expression, genetic changes, DNA methylation or protein phosphorylation, we comprehensively examined TESPA1 genes in a total of 33 different tumors.

TESPA1 expression level is aberrantly high or aberrantly low in different tumors. However, the survival prognostic analysis data of TESPA1 gene showed that there were significant differences among different tumors. Our study first used four independent datasets in PrognoScan, and TCGA data in GEPIA and Kaplan-Meier plotter approach to explore the expression of TESPA1 and its prognostic value across pan-cancers for a more rigorous conclusion. For breast cancer and liver hepatocellular carcinoma, the expression of TESPA1 shown no difference in cancers compared with normal tissues. But in TCGA data in GEPIA and Kaplan-Meier plotter displayed consistently TESPA1 was beneficial to OS, DFS and RFS. Overexpressed TESPA1 gene may be a new target for breast cancer and liver hepatocellular carcinoma treatment. For skin cutaneous melanoma, high expressed TESPA1 was correlated with OS, DFS but not RFS. Kaplan-Meier plotter approach shown that in skin cancers, high expressed TESPA1 was correlated with OS. The expression of TESPA1 in different stage of skin cutaneous melanoma is also shown obvious difference. TESPA1 may play an important role in the development and treatment of. For the

three cancers in kidney, TESPA1was all highly expressed. High expressed TESPA1 was related to good OS and RFS in kidney renal clear cell carcinoma and DFS of kidney chromophobe, but had a detrimental effect on prognosis kidney renal papillary cell carcinoma. This contradictory conclusion needs to be unified in a large number of samples. For tumor of reproductive system, including cervical squamous cell carcinoma, Ovarian serous cystadenocarcinoma, uterine carcinosarcoma and uterine corpus endometrial carcinoma. TESPA1 expressed lowly compared with controls. OS and RFS of Ovarian serous cystadenocarcinoma, cervical squamous cell carcinoma and uterine corpus endometrial carcinoma benefit from high expressed TESPA1. The contradiction also exists in testicular germ cell tumors, TESPA1 was beneficial to RFS of TGCT, but was detrimental to OS of TGCT. For eye cancers, uveal melanoma, contradictory conclusion that higher TESPA1 was beneficial DMFS of eye cancer via Prognoscan, but higher TESPA1 was related to poor survival of uveal melanoma via GEPIA 2. For lung cancer, we analyzed the datasets of the TCGA-LUSC and TCGA-LUAD projects and found a correlation between TESPA1 high expression and good overall survival prognosis of lung adenocarcinoma but not lung squamous cell carcinoma. The expression of TESPA1 in every stage of lung adenocarcinoma is also shown obvious difference. Previous study has obtained the consistent results and proposed prognostic value of TESPA1[13]. Our results demonstrated the prognostic value of TESPA1 in lung adenocarcinoma, and the possible of its value in lung adenocarcinoma treatment. Larger sample sizes and experiments are required to confirm these results. In blood cancers, results from Oncomine and TIMER2 consistently shown that TESPA1 is high expressed in leukemia, but the survival analysis by Prognoscan has demonstrated that higher TESPA1 expression could led to poor survival in blood cancers. The prognostic value of TESPA1 in blood cancer is prospective. For tumor of digestive system, TESPA1 is has no different expression in cholangiocarcinoma esophageal carcinoma, stomach adenocarcinoma compared with controls. But the expression level of TESPA1 had an influence on the survival of tumor of digestive system. Moreover, TESPA1 has been demonstrated to be associated with the development of gastric carcinoma[36]. For brain cancers, TESPA1 expression is reduced in glioblastoma and low-grade glioma, The Prognoscan demonstrated high-expressed TESPA1 is related the good OS of brain cancer. Up-regulated TESPA1 maybe a new treatment of brain cancers. In summary, considering the above contradiction between TESPA1 expression level and the prognosis of some cancers. There are three possible reasons. First, a larger sample size is needed to further verify the above conclusions. Second, other clinical features should also be fully considered. Finally, further in vitro and in vivo molecular experimental evidence is needed to determine whether the high expression of TESPA1 plays an important role cancer mentioned above or is merely an accompanying result of the immune response[1].

We found a previously uncharacterized and closed correlation between TESPA1 expression and immune cell infiltration across pan-cancers and reveal its critical mechanisms and immunological role in tumor microenvironment. After the analysis by TIMER2, TESPA1 expression levels in cancers were significant positively correlated with infiltration of immune cells, including CD8 + T cells, CD4 + T cells, B cells, macrophages, mast cells, CAF, Treg, monocytes and dendritic cells. Particularly, TESPA1 correlation with B cells, T cells, dendritic cells are almost comparable high, which confirmed that TESPA1 possibly involved in tumor antigen presentation and tumor killing critically. Not only B cells, T cells, dendritic cells,

but macrophage and Treg is also correlated with expression level of TESPA1. Due to the complex of tumor microenvironment, the mechanism of TESPA1 affects cancers through the regulation of the immune microenvironment is unclear, which is need further research to explore.

Furthermore, we integrated the information on TESPA1-binding components and TESPA1 expression-related genes across all tumors for a series of enrichment analyses and identified the potential impact of “coagulation”, “hemostasis” and “blood coagulation” in the etiology or pathogenesis of cancers. Interestingly, previous studies have demonstrated that cancer and the hemostatic system interact with each other and trigger coagulation abnormalities[37]. Hemostatic factors have been reported play a critical role tumor progression, growth and metastasis through effecting on the key event of neovascularization[37–39]. But the mechanism is not clear, our study may provide a new sight to solve this problem. Also, the treatments of cancer aimed at hemostatic system are bifunctional therapeutic approaches that are both able to attack the malignant process and resolve the coagulation impairment[37]. Last but not least, the coagulation disorders of tumor patients are different from those of other diseases. Finding cancer specific biomarkers can better guide the treatment of tumor patients[40].

We also found significant differences in TESPA1 DNA methylation compared with normal tissues, and different levels of TESPA1 DNA methylation were also associated with different survival outcomes, including OS, DFI and PFI. It’s a pity that the data of total protein of TESPA1 is not clear. We only used the CPTAC dataset to analyzed of the TESPA1 phosphorylated protein in breast cancer, clear cell renal cell carcinoma, lung adenocarcinoma. We observed high expression level of phosphorylated TESPA1 protein level at the S311 locus in the primary tumors compared with normal controls in breast cancer and clear cell renal cell carcinoma, but low expression level in lung adenocarcinoma. We also found that S454 phosphorylation of TESPA1 is increased in primary tumor. Additional experiments are required to further evaluate the total protein level of TESPA1 and more phosphorylated locus in other types of cancer, and their role in tumorigenesis.

There are some limitations still exist in our study[3]. Firstly, systematic bias may be introduced into our analysis given that the data were collected by analysis of a large number of tumor tissues from different types of gene chip and methods of sequencing. Higher-resolution methods such as single-cell RNA sequencing is very necessary[3, 41–43]. Secondly, due to the complex disease-related clinicopathological characteristics of cancers, more clinical data need to be involved into the correlation analysis between TESPA1 expression and clinical outcome[44]. Finally, in vivo or in vitro experiments were essential to validate the results of the prognostic value and immunological role in cancer obtained by bioinformatics analysis. Finally, owing to the complexity of tumor microenvironment and hallmarks of cancer[45], we just observed a phenomenon of the close correlation between TESPA1 expression and immune cell infiltration and patient survival in cancer, we could not directly conclude whether TESPA1 affects patient survival via immune cell infiltration. Our study laid the foundation for further exploration of the mechanism of interactions between the expression of TESPA1 and the infiltration of tumor immune cells. Future studies on up or down TESPA1 expression and immune cell infiltration in cancer populations will help to provide a clear answer to this question[3].

Conclusions

In conclusion, our pan-cancer analysis confirmed the prognostic value of level of TESPA1 expression, DNA mutation and methylation and protein phosphorylation in various of cancers. We also observed the close correlation between TESPA1 with immune cell infiltration, which uncovered its immunological role in tumor. Novelty, we also further founded that TESPA1 could also involve tumor progression by influencing blood coagulation. This work builds a foundation for us to explore TESPA1 in tumorigenesis.

Abberations

TESPA1 : Thymocyte-expressed positive selection-associated protein 1;

TCGA: The cancer genome atlas;

GEO : Gene expression omnibus;

LUAD : lung adenocarcinoma;

TIMER 2 : Tumor immune estimation resource, version 2;

GEPIA 2 : Gene Expression Profiling Interactive Analysis, version 2;

DLBC : Lymphoid Neoplasm Diffuse Large B-cell Lymphoma;

LAML : Acutemyeloid leukemia;

GTEx : Genotype-Tissue Expression;

TPM : Transcripts per million;

CPTAC : Clinical Proteomic Tumor Analysis Consortium;

OS : overall survival;

DFS : disease-free survival;

HR : hazard ratio;

KEGG : Kyoto encyclopedia of genes and genomes;

HNSC: Head and Neck squamous cell carcinoma;

KICH : Kidney Chromophobe;

KIRC : Kidney renal clear cell carcinoma;

KIRP : Kidney renal papillary cell carcinoma;

PCPG : Pheochromocytoma and Paraganglioma;

BLCA : Bladder Urothelial Carcinoma;

COAD : Colon adenocarcinoma;

GBM : Glioblastoma multiforme;

LUSC : Lung squamous cell carcinoma;

READ : Rectum adenocarcinoma;

THCA : Thyroid carcinoma;

UCEC : Uterine Corpus Endometrial Carcinoma;

LGG : Brain lower grade glioma;

OV : ovarian serous cystadenocarcinoma;

THYM : Thymoma;

UCS : uterine carcinosarcoma;

SKCM : skin cutaneous melanoma;

TGCT : Testicular germ cell tumors;

STAD : stomach and esophageal carcinoma;

THCA : Thyroid carcinoma;

BRCA : breast cancer;

SARC : sarcoma;

UVM : Uveal Melanoma;

ACC : Adrenocortical Carcinoma;

LIHC : Liver Hepatocellular Carcinoma;

CESC : Cervical Squamous Cell Carcinoma;

UCEC : Uterine Corpus Endometrial Carcinoma;

ESCA : Esophageal Carcinoma;

THYM : Thymoma;

PAAD : Pancreatic Adenocarcinoma;

READ : Rectum Adenocarcinoma;

GBM : Glioblastoma Multiforme;

PCPG : Pheochromocytoma and Paraganglioma;

DSS : disease-specific survival;

DFS : disease-free survival;

PFS : progression-free survival;

DMFS : distant metastasis-free survival;

MESO : Mesothelioma;

NLRC3 : Nucleotide-oligomerization domain-like receptor subfamily C3;

SPN : solid-pseudopapillary neoplasm;

ITGA4 : integrin alpha4;

PTPN7 : Protein tyrosine phosphatase nonreceptor type 7;

CBFA2T3 : CBFA2/RUNX1 partner transcriptional co-repressor 3;

TD : T cell-dependent;

GO : Gene ontology;

KEGG : Kyoto encyclopedia of genes and genomes;

STRING : Search Tool for the Retrieval of Interaction Gene/Proteins;

CPTAC : Clinical Proteomic Tumor Analysis Consortium;

Declarations

Ethics approval and consent to participate

This research was retrospective analysis of existing patient data based on the datasets of TCGA and GEO. Therefore, patient consent was not required.

Consent for publication

Not applicable.

Availability of data and materials

Publicly available datasets were analyzed in this study. The datasets analyzed during the current study are available in the TCGA data portal, https://portal.gdc.cancer.gov and GEO datasets, https://www.ncbi.nlm.nih.gov/geo/ . This data can be found here: TIMER (http:// cistrome.org/TIMER/)", "Oncomine (www.oncomine.org), "PrognoScan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html)", "Kaplan–Meier Plotter (https://kmplot.com/analysis/)", "GEPIA (http://gepia.cancer- pku.cn)", "UALCAN(http://ualcan.path.uab.edu/index.html)", cBioPortal web (https://www.cbioportal.org/), DNMIVD web (http://119.3.41.228/dnmivd/index/), (STRING) website (https://string-db.org/) and xiantao tools (https://www.xiantao.love/).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LLY analyzed the data. LYF was a major contributor in writing the manuscript. Both authors read and approved the final manuscript.

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Figures

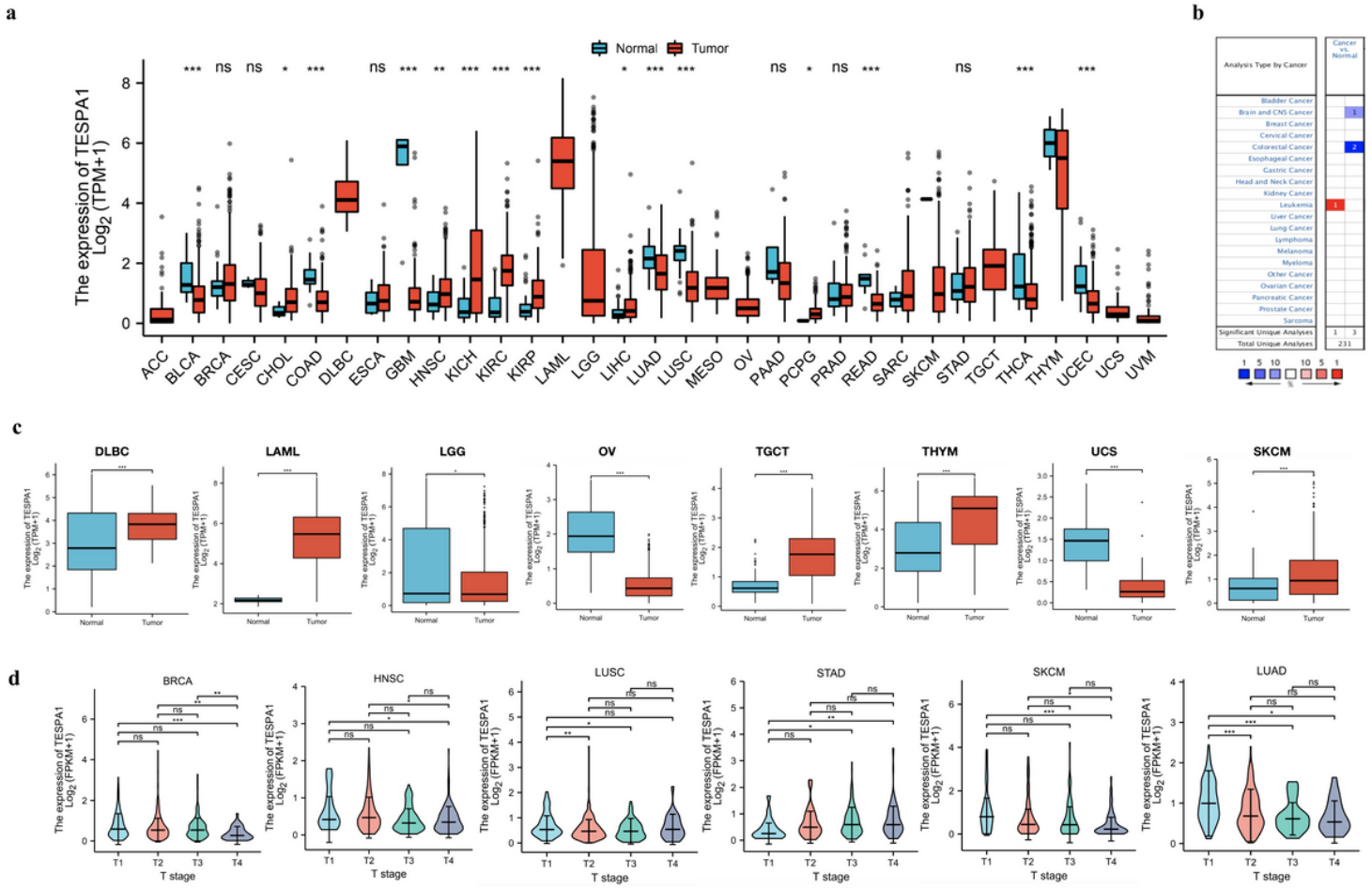

Figure 1

Expression level of TESPA1 gene across pan-cancers and pathological stages. (a)Expression level of TESPA1 gene in different cancers or specific cancer subtypes analyzed via xiantao. (b)Expression status of TESPA1 gene in different cancers analyzed by Oncomine. The redder the red, the higher the expression. (c) Expression status of TESPA1 gene in DLBC, LAML, LGG, OV, TGCT, THYM, UCS and SKCM compared with corresponding normal tissues of the GTEx database in the TCGA project. (d) Expression levels of TESPA1 gene analyzed by the main pathological stages (stage 0, stage 1, stage 2, stage 3, and stage 4) of BRCA, HNSC, LUSC, LUAD, SKCM, STAD. * P <0.05; ** P < 0.01; *** P < 0.001.

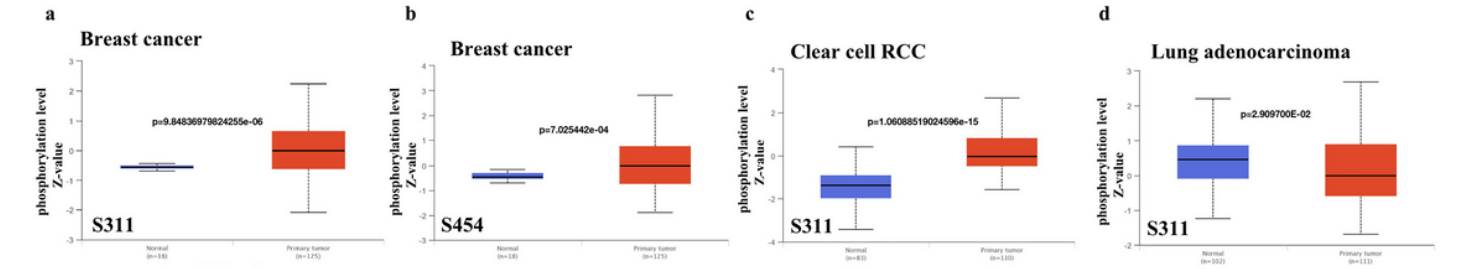

Figure 2

Phosphorylation analysis of TESPA1 protein in different cancers. Phosphoprotein level of TESPA1 (NP_001092285.1, S311 and S454 sites) between normal tissue and primary tissue of selected tumors based on the CPTAC dataset, via the UALCAN, including (a, b) breast cancer, (c)clear cell RCC, (d)LUAD.

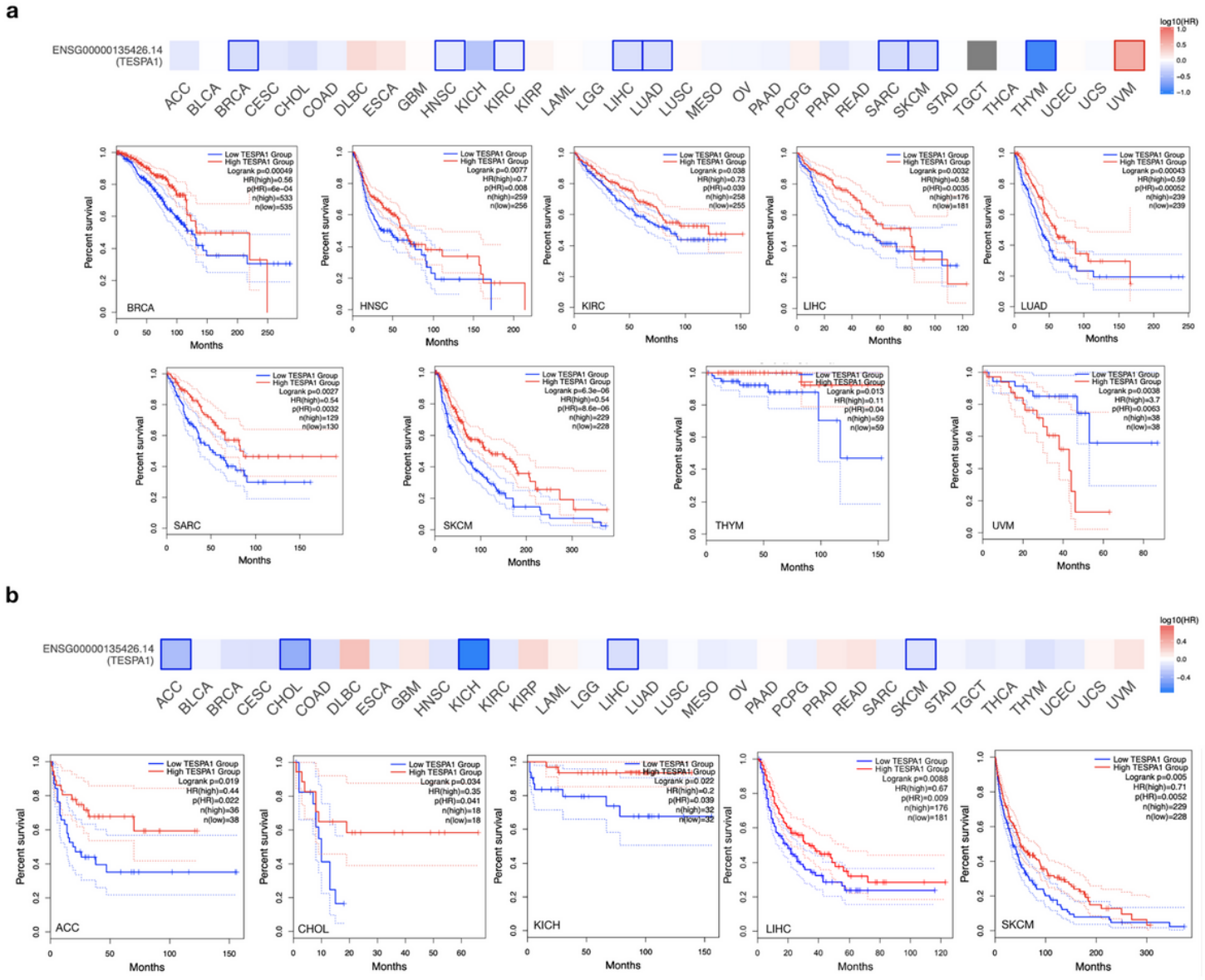

Figure 3

Survival map and Kaplan-Meier curves shown correlation between TESPA1 gene expression and survival prognosis including (a) overall survival and (b) disease-free survival of cancers in TCGA.

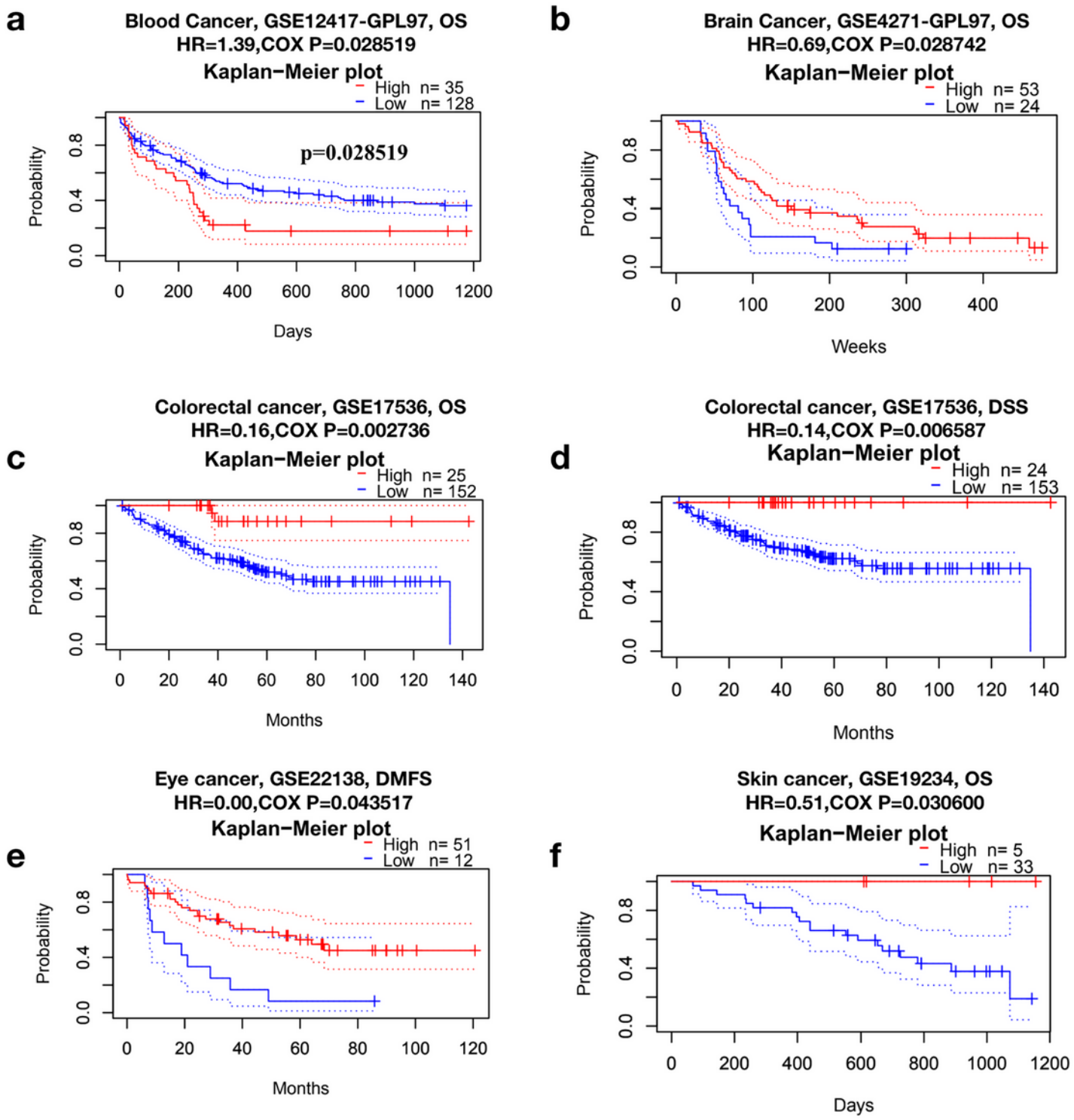

Figure 4

Correlation between expression level of TESPA1 and survival in different cancer types analyzed by Kaplan–Meier survival curves via PrognoScan. (a) OS of blood Cancer. (b) OS of Brain Cancer. (c,d)OS and RFS of colorectal cancer. (e) DMFS of eye cancer. (f) OS of skin Cancer. OS, overall survival; DMFS, distant metastasis-free survival; DSS, disease-specific survival.

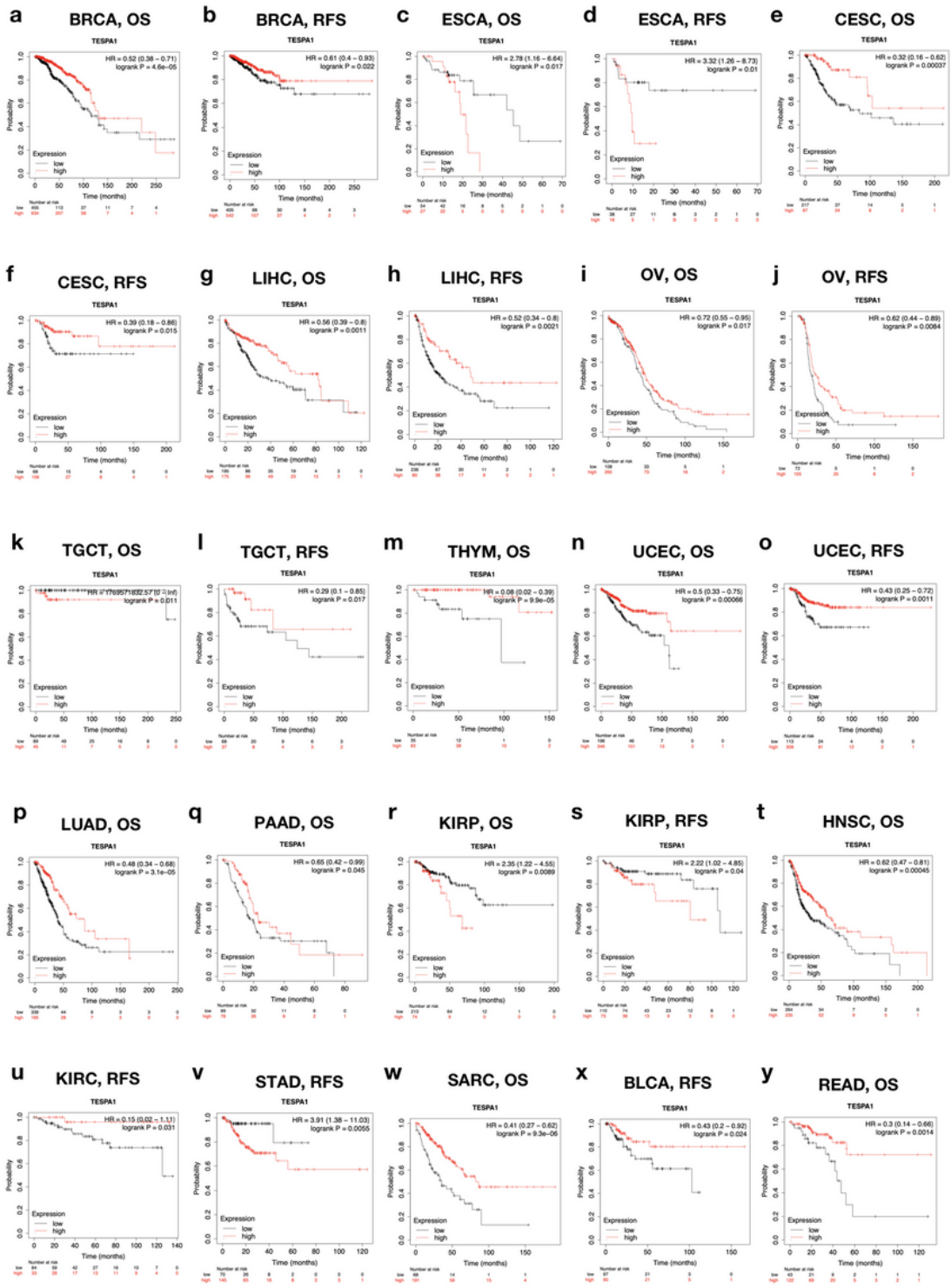

Figure 5

Correlation between expression level of TESPA1 and survival prognosis in different types of cancer analyzed by Kaplan–Meier survival curves via Kaplan–Meier Plotter. OS and RFS of (a, b) BRCA, (c, d) ESCA, (e, f) CESC, (g, h) LIHC, (i,j) OV, (k,l) TGCT, (n,o) UCEC, (r,s) KIRP and OS of (m) THYM, (p) LUAD, (q)PAAD, (t)HNSC, (w)SARC, (y)READ and RFS of (u)KIRC, (v)STAD, (x)BLCA. OS, overall survival; RFS, relapse-free survival.

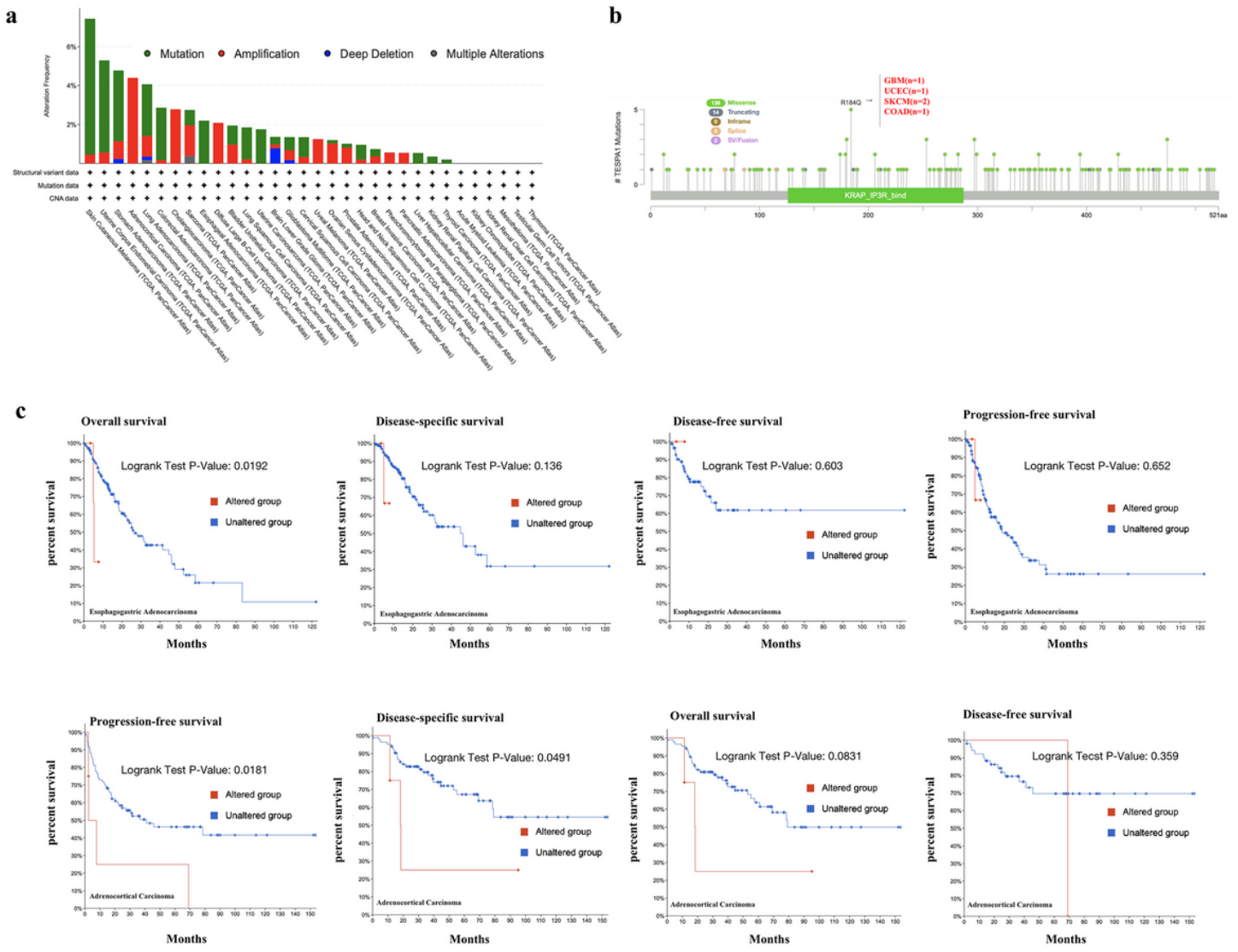

Figure 6

Mutation characters of TESPA1 in different cancers of TCGA. Alteration frequency with (a) mutation type and (b) mutation site of TESPA1 of cancers in TCGA using the cBioPortal tool. (c) Correlation between mutation status and overall, disease-specific, disease-free and progression-free survival of esophageal adenocarcinoma and adrenocortical carcinoma analyzed using the cBioPortal tool.

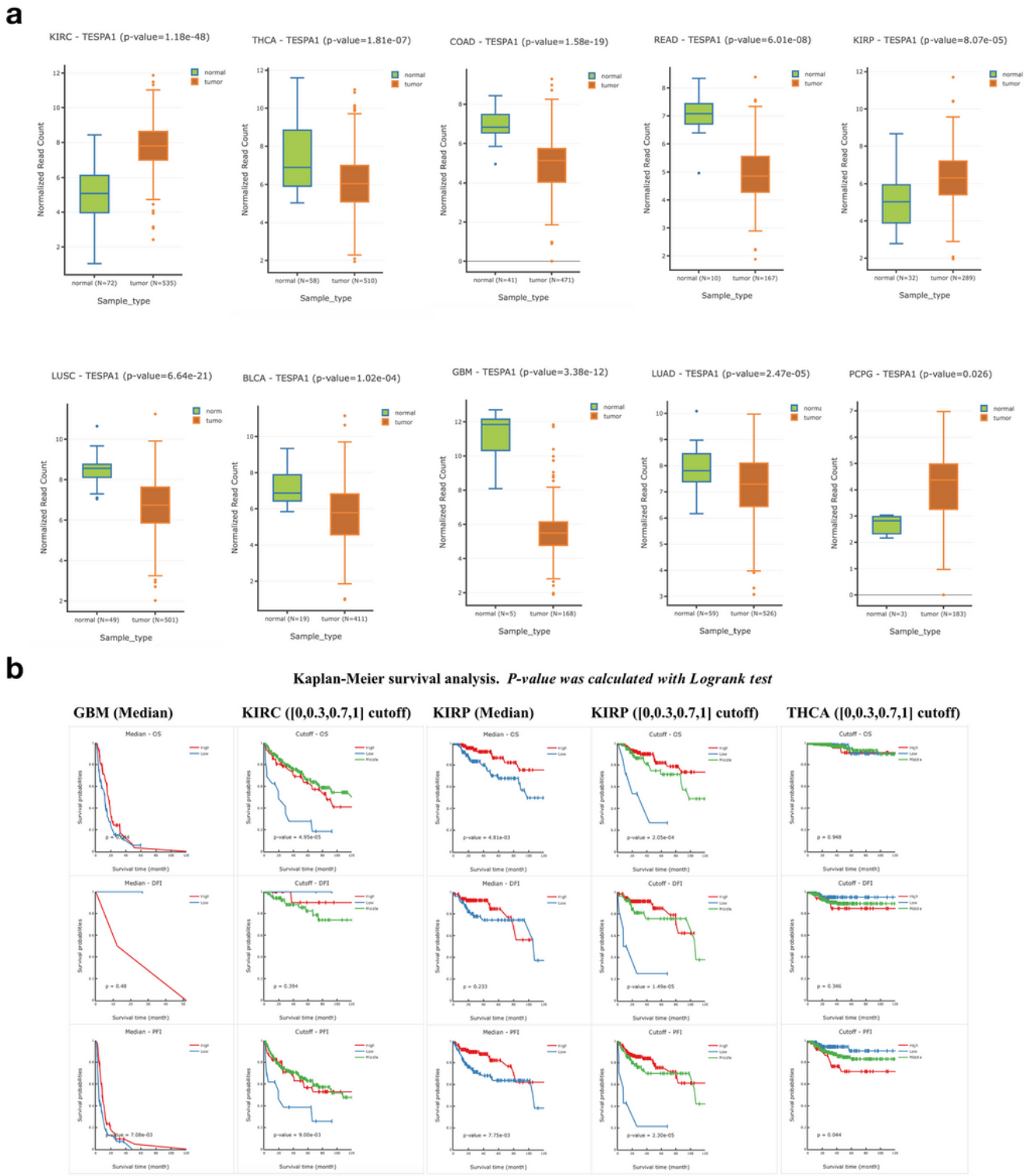

Figure 7

Correlation analysis between TESPA1 methylation and survival. (a) Methylation level of TESPA1 between normal tissue and primary tissue of selected tumors. (b) Correlation between methylation level of TESPA1 and survival analyzed by DNMIVD dataset.

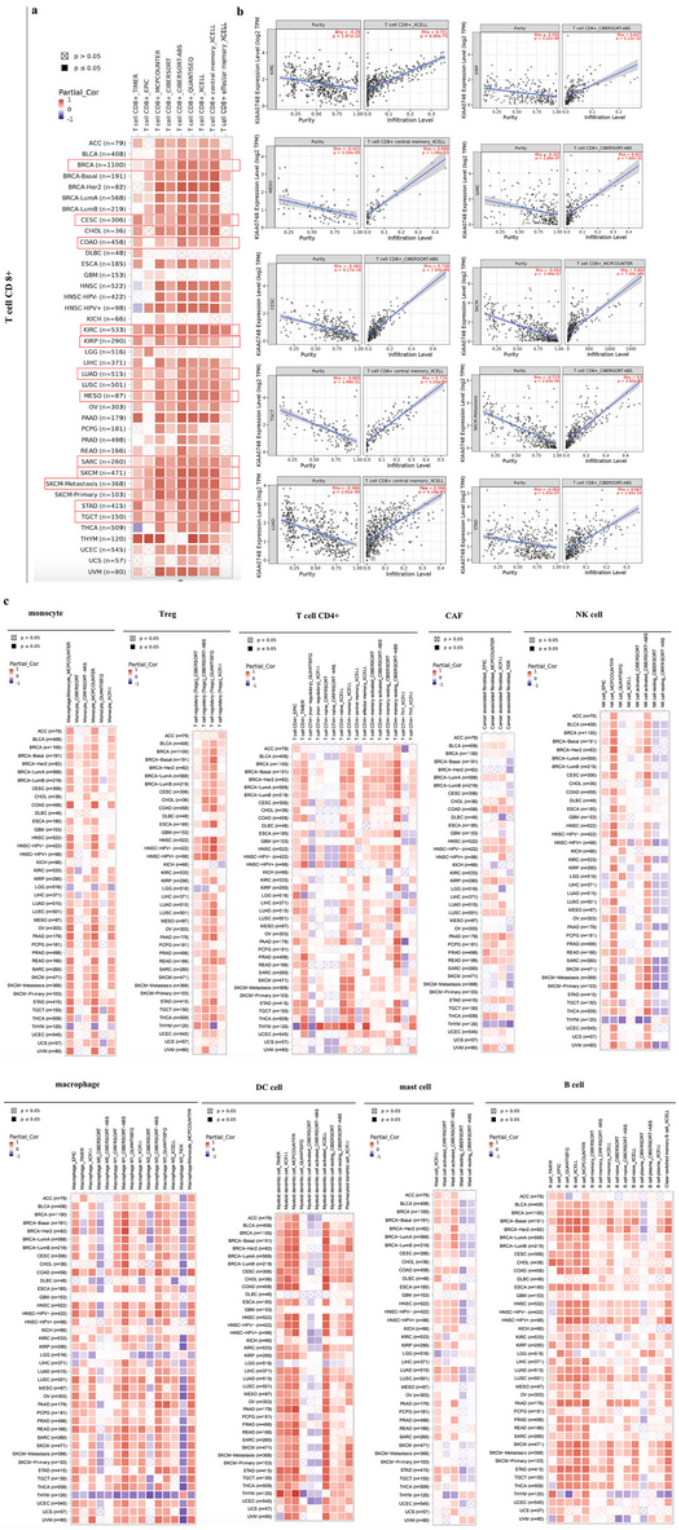

Figure 8

Correlation between TESPA1 expression and immune infiltration. (a) Correlation between TESPA1 expression and immune infiltration of T cell CD8+. (b) Correlation between the expression level of the TESPA1 gene and the infiltration level of T cell CD8+ analyzed with different algorithms across in various of cancer in TCGA. (c) Correlation analysis between TESPA1 expression and immune infiltration of monocyte, Treg, T cell CD4+, CAF, NK cell, macrophage, DC cell, mast cell and B cell.

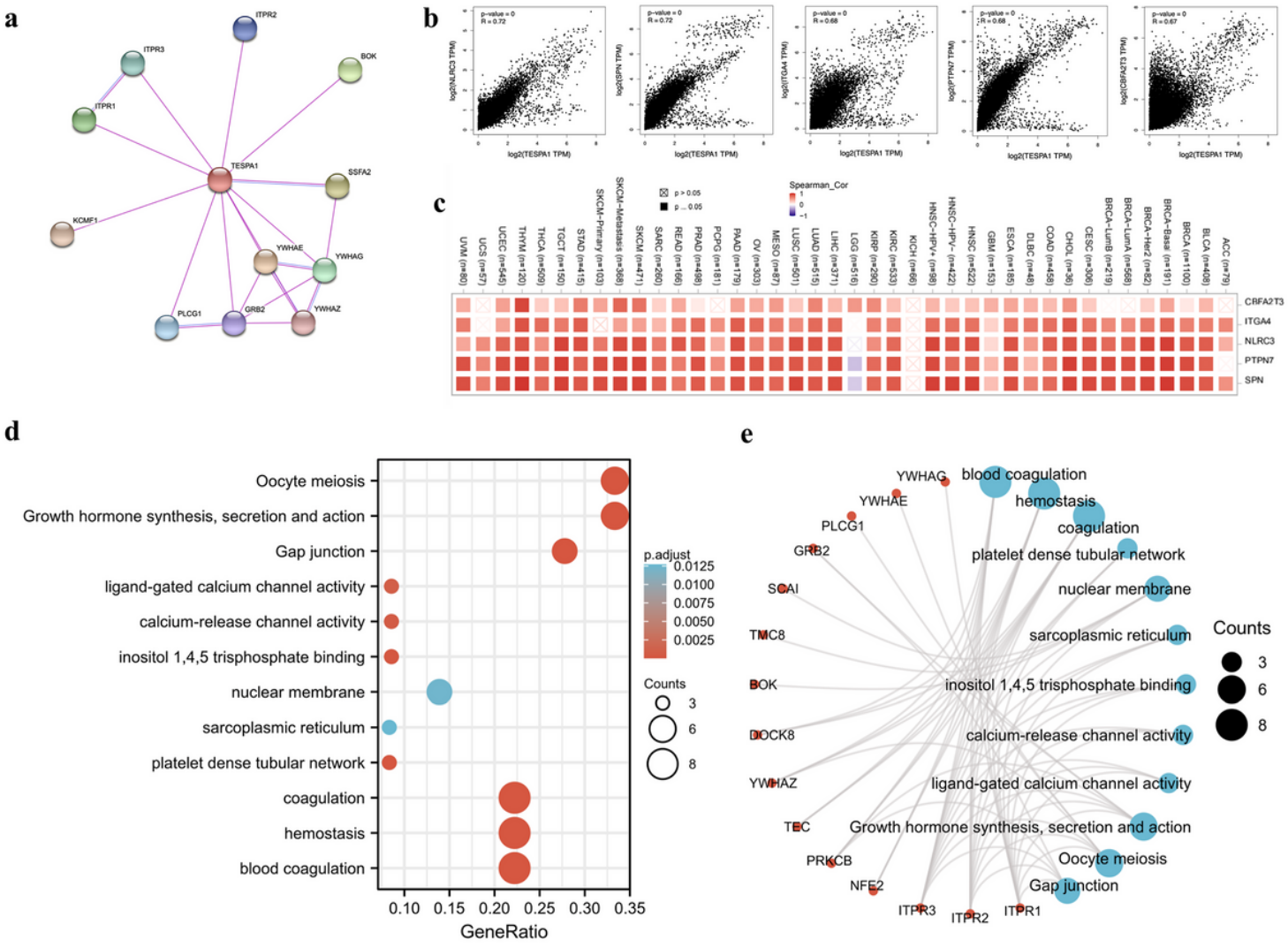

Figure 9

TESPA1-related gene enrichment analysis. (a) TESPA1-binding proteins obtained by STRING tool. (b) Expression correlation between TESPA1 and 5 targeting genes selected from top 100 TESPA1-correlated genes, including NLRC3, SPN, ITGA4, PTPN7, and CBFA2T3 using the GEPIA2 approach in dot plot. (c) The corresponding heatmap data in the detailed cancer types. (d) Combined the TESPA1-correlated and TESPA1-interacted genes, KEGG pathway analysis was performed. (e) The cnetplot for the molecular function data in GO analysis is also shown.