Identification of TUBA1C as a prognostic biomarker and associated with immune cells infiltration in human tumors

Xin Fan
Ningbo First Hospital
Bingjiang Lin
Ningbo First Hospital
Xiudi Wu
Ningbo First Hospital
Yingzhe Yu
Ningbo First Hospital
Lingyi Lu
Ningbo First Hospital
Shuyu Fang
Ningbo First Hospital
Yanqing Liu (liuyanqing1989@126.com)
Ningbo First Hospital

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Abstract

Background: It is reported that TUBA1C contributed to the development and progression of several tumors. However, the role of TUBA1C in most cancers remains unclear. The study aimed to assess the prognostic values, potential biological functions, and immunity of TUBA1C in pan-cancer.

Methods: TUBA1C expression was assessed using pan-cancer data of 33 cancer types from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) database. The clinical features and prognostic roles of TUBA1C were assessed using TCGA cohort. cBioPortal database was used to examine TUBA1C mutations in pan-cancer. The relationship between TUBA1C expression and patients' prognosis was performed by univariate Cox hazard regression. Gene set enrichment analysis (GSEA) of TUBA1C was conducted using the R software. we applied ESTIMATE and CIBERSORT algorithm to assess the stromal score, immune score, and the infiltrating levels of immune cells from published study and ImmuCellAI database.

Results: Our results showed that TUBA1C was highly expressed in most tumors. High TUBA1C expression was associated with poor overall survival (OS) and disease-specific survival (DSS) in various tumors. GSEA analysis showed that TUBA1C expression was closely related to immune regulation-related signaling pathways. Besides, tumor-associated macrophages (TAMs) were the dominating immune cell positively correlated with TUBA1C expression in most tumors. In addition, TUBA1C expression was positively associated with tumor mutation burden (TMB), microsatellite instability (MSI), and mainly immunosuppressive genes such as PD-L1, PD-1, CD244, TIGIT, TGFB1, and TGFBR1 in several tumors.

Conclusion: Our results indicated that TUBA1C was associated with poor prognosis in tumors. TUBA1C might be a valuable prognostic biomarker and a potential target for immunotherapy.

Introduction

Cancer still ranks as a leading cause of death and a major economic and public health burden worldwide [1]. In China, the burden of cancer incidence and mortality is rapidly increasing, despite great advances in cancer prevention and treatment have been made [2]. In recent years, molecular targeted therapy including cancer immunotherapy has rapidly developed in cancer treatment for a variety of cancers, such as lung cancer and breast cancer [3, 4]. The tumor microenvironment (TME) is reported to play an important role in outcomes and immunotherapy responses for cancers. The tumor-infiltrating immune cells (TIICs) included in the TME profoundly influence the development and progression of tumors and the outcomes of anti-cancer immunotherapy [5, 6]. Therefore, it is important to discover new immunotherapeutic targets for cancers and elucidate the corresponding roles involved in prognosis, pathways, TME, and tumor immunity.

Microtubules, an important component of the eukaryotic cytoskeleton, are assembled from conservatively globular α/β tubulin heterodimers [7]. Microtubules play a critical role in depolymerization and dynamic aggregation through cell division and intracellular transport [8, 9]. Besides, it is reported that microtubules

could impact cell proliferation, differentiation, and apoptosis via the regulation of mitotic apparatus [10]. Evidence have shown an emerging link between alterations of tubulin isotypes correlated with modifying enzymes in certain cancers, such as hepatocellular carcinoma (HCC), breast cancer, and prostate cancer [11, 12]. On this occasion, TUBA1C, a subtype of α-tubulin, has been indicated poor prognosis in gliomas, lung adenocarcinoma, and HCC [13-15]. However, no previous study has explored the roles of TUBA1C in most tumor types.

Currently, there has been no pan-cancer research on the associations between TUBA1C and various cancers. Therefore, multiple databases including the TCGA, GTEx, and cBioPortal were used to analyze TUBA1C expression level and its influences on prognosis for a variety of tumors. Besides, we also explored the related signaling pathways, TME, and levels of immune cell infiltration for TUBA1C across 33 types of cancer. Moreover, we assessed the potential correlations between TUBA1C expression and TMB, MSI, and immunosuppressive genes in TCGA pan-cancer samples. Our results showed that TUBA1C could be used to predict the prognosis in various cancers, and TUBA1C might play a vital role in TME tumor immunity via affecting tumor-infiltrating immune cells, especially macrophages. Furthermore, TUBA1C expression was positively associated with TMB, MSI, and immunosuppressive genes such as PD-L1 (CD274), PD-1 (PDCD1), CD244, TIGIT, TGFB1 in pan-cancer samples. The present study indicated the roles of TUBA1C in tumor prognosis and tumor immunotherapy.

Materials And Methods

Data collection

TCGA and GTEx expression and clinical data were downloaded from the UCSC Xena database (https://xenabrowser.net/datapages/). DNA copy number and methylation data were downloaded from the cBioPortal database (https://www.cbioportal.org/). The number of samples used in this study was shown in Table 1.

Survival and mutation analysis

The results of TUBA1C expression between tumor and normal tissues were shown in the form of $\log_2$ fold change ($\log_2$FC). Survival analyses including OS, DSS, and disease-free interval (DFI) was also carried out by using R software ('survminer' and 'survival' package). Patients were divided into high expression group and low expression group based on the optimal TUBA1C expression level. Then the Kaplan-Meir method was used to draw survival curves and the hazard ratio (HR) was calculated by univariate Cox proportional hazard regression analysis. A $p < 0.05$ was considered to be statistically significant. Analysis of DNA copy number alteration (CNA) and methylation for TUBA1C in pan-cancer samples was performed based on cBioPortal database.

Functional and pathway enrichment analysis

The Pearson correlation coefficient was used to calculate the correlations between TUBA1C and co-expression genes in TCGA database. We selected the top 300 genes, most positively correlated with TUBA1C, as a gene set for Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis to predict the functional pathway of TUBA1C. GSEA was performed by using the R software with 'clusterProfiler' package. A $p < 0.05$ was considered to be significant enrichment.

TME and immune cell infiltration analysis

We applied the ESTIMATE algorithm to calculate the stromal and immune scores by using the R software with 'estimate' package [16]. The tumor purity of patients was indicated by the ESTIMATE algorithm and consensus measurement of purity estimations (CPE) algorithms [17]. Pearson correlation analysis was used to evaluate the correlations among immune score, stromal score, and tumor purity. Immune cell infiltration analysis was estimated using the CIBERSORT algorithm downloaded from the published study conducted by Thorsson et al. [18] and ImmuCellAI database (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/), respectively. The level of immune cell infiltration was compared between the high TUBA1C group and the low TUBA1C group, which was based on the median expression of TUBA1C in each tumor type. Moreover, the correlations between 26 TIICs and the expression of TUBA1C in each tumor were also evaluated.

Tumor mutation burden analysis

Tumor mutation burden (TMB) is defined as the number of base mutations per million bases, calculated by using TCGA mutation data. TMB is a marker of the efficacy of immune checkpoint inhibitors. The higher TMB is, the more neoantigens that can be recognized by T cells will be produced, and the better the immunotherapy effect will be. TCGA mutation data of 33 tumors from the GDC TCGA was downloaded from the UCSC XENA database (http://xena.ucsc.edu/).

Statistical analysis

All the statistical analyses were performed by R software (version 4.0.5). Data were shown as the mean ± standard deviation (SD). Mann–Whitney *U* test was used to compare different variables between two groups. Kruskal-Wallis *H* tests were used for comparisons of more than two groups. A two-sided $p < 0.05$ was considered to be statistically significant. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; and $^{****}p < 0.0001$.

Results

TUBA1C expression in different tumors

TUBA1C gene expression was evaluated in pan-cancer data from tumor tissue samples from TCGA and normal tissue samples from TCGA and the GTEx database. Results showed that TUBA1C expression was higher in the following 28 types of tumors: ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM,

HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, PRAD, READ, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS (Figure 1A). Besides, TUBA1C mean expression was highest in HNSC and lowest in LGG among 33 tumor tissues from TCGA database (Figure 1B). TUBA1C mean expression was highest in bone marrow (BM) and lowest in the brain among 31 normal tissues from GTEx database (Figure 1C). TUBA1C was overexpressed in tumor tissues of BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, READ, STAD, and UCEC (Figure 2A–N) in paired tumors and normal tissues from TCGA database. Moreover, TUBA1C expression was also statistically significant in the different clinical stages of various tumors (Supplement Figure 1A-K).

TUBA1C gene mutations in tumors

Figure 3 showed TUBA1C gene mutations in TCGA pan-cancer samples from cBioPortal database. Results indicated higher gene alteration frequency in DLBC and UCEC (Figure 3A). Amplifications mainly occurred in DLBC and ACC, and mutations mainly occurred in UCEC and SKCM (Figure 3A). In addition, we also analyzed the status of copy number alteration (CNA) and methylation of TUBA1C in TCGA pan-cancer and performed a correlation analysis. The results suggested that CNA was positively correlated with TUBA1C expression in various tumor types (Figure 3B), while methylation was negatively correlated with TUBA1C expression in several tumor types (Figure 3C).

Prognostic value of TUBA1C in tumors

Based on the data from the TCGA database, univariate Cox regression analysis and Kaplan-Meier survival analysis were used to assess the correlations between TUBA1C expression and OS, DSS, and DFI in tumors. Cox regression analysis revealed that TUBA1C was a prognostic risk factor for OS and DSS in LGG, LUAD, LIHC, PAAD, SKCM, BRCA, GBM, KICH, MESO, and KIRP (Supplement Figure 2A-B), while TUBA1C was favorable for OS in patients with READ and COAD, and DSS in patients with PRAD (Supplement Figure 2A-B). Besides, TUBA1C expression a risk factor for LGG, LUAD, and PAAD (Supplement Figure 2C). Kaplan-Meier survival analysis indicated that higher expression of TUBA1C was related to worse OS and DSS in LGG, LUAD, LIHC, PAAD, SKCM, BRCA, GBM, KICH, MESO, and KIRP (Figure 4 and Figure 5). In contrast, higher expression of TUBA1C was significantly associated with longer OS in COAD and READ (Figure 4C and 4L). For DFI, higher expression of TUBA1C contributed to short DFI in LGG, LUAD, and PAAD (Figure 5K-M).

TUBA1C-related signaling pathways based on GSEA

GSEA based on the Reactome database was applied to predict pathways in which TUBA1C may be involved in pan-cancer. The results indicated that TUBA1C was involved in immune regulation-related pathways in pan-cancer, such as neutrophil degranulation, transcriptional regulation by TP53, cytokine signaling in the immune system, and the adaptive immune system, and the innate immune system. The pathways associated with TUBA1C were shown in BRCA, GBM, LGG, LIHC, LUAD, and PAAD (Figure 6A-F).

Correlations between TUBA1C expression and TME

We used the ESTIMATE algorithm to analyze the correlation between TUBA1C expression and the scores of both stromal cells and immune cells in 33 types of cancer. Our results indicated that TUBA1C expression was significantly positively correlated with the stromal score, immune score, and estimate score in LGG, GBM, DLBC, PAAD, PCPG and so on (Figure 7A). However, TUBA1C expression was significantly negatively correlated with the stromal score, immune score, and estimate score in other tumors, such as ESCA, STAD, UCEC, LUSC. (Figure 7A). The three tumors with the highest positive and negative correlation coefficients and significant *p* values were shown in Figure 7B.

Correlations between TUBA1C and infiltrating immune cells

We next explored the correlations between TUBA1C expression and levels of infiltrating immune cells in tumors. Based on the previously published database [18], we found that infiltrating levels of M1 macrophages and macrophages were positively associated with TUBA1C expression in most tumors, especially in BLCA, BRCA, CESC, DLBC, LGG, LUAD, STAD, and THCA (Figure 8A). Nevertheless, TUBA1C expression levels were negatively associated with infiltrating levels of lymphocytes and memory B cells in most tumors, mainly in BLCA, BRCA, HNSC, KIRC, LGG, LUAD, LUSC, SARC, SKCM, STAD, and THCA (Figure 8A).

To further evaluate the TIICs in the TME in pan-cancer, the correlations between TUBA1C expression and the infiltrating levels of 24 immune cell subtypes were also analyzed by using the ImmuCellAI database. As shown in Supplement Figure 3, the correlations between infiltration score and TUBA1C expression were significant in BRCA, HNSC, LGG, TGCT, THYM, and UCEC. The results further demonstrated that TUBA1C expression was positively associated with the filtrating levels of macrophages and negatively with the filtrating levels of B cells in BLCA, BRCA, CESC, LGG, LUAD, LUSC, and STAD (Supplement Figure 3 and Figure 8B). Besides, we revealed that TUBA1C expression was positively associated with filtrating levels of regulatory T cells (natural regulatory T cells (naturally occurring Tregs, nTregs) and induced-to-adjust T cells (inducible Tregs, iTregs)) and dendritic cells (DCs) in most tumors, while it was negatively related to $CD8^+$ T cells, gamma delta T cells (Tgd), and $CD4^+$ T cells in most tumors (Figure 8B).

Correlations between TUBA1C expression and immunosuppressive genes, TMB, and MSI

We further analyzed the relationship of TUBA1C expression with immunosuppressive genes, TMB, and MSI using TCGA pan-cancer data. TUBA1C expression was positively associated with main immunosuppressive genes, such as PD-L1 (CD274), PD-1 (PDCD1), CD244, TIGIT, TGFB1, and TGFBR1 in most tumors (Figure 9A). Besides, TUBA1C expression was positively associated with TMB in LUAD, KICH, SKCM, SARC, STAD, and BRCA (Figure 9B). TUBA1C expression was positively associated with MSI in SARC and STAD, while was negative with HNSC and PRAD (Figure 9C). The results indicated that TUBA1C might play a key role in mediating immune evasion in the development of several tumors.

Discussion

TUBA1C, a subtype of a-tubulin, is a multifunctional cytoskeleton protein, which is correlated with cellular elements of the microtubule and is involved in the progress of cellular mitosis and division, including cell shape, cell motility, and intracellular trafficking [19, 20]. Wang and colleagues indicated that TUBA1C could promote cell migration and proliferation in hepatocellular carcinoma involving in cell cycle signaling pathway and predicted a poor prognosis [15]. Besides, several studies also indicated that high TUBA1C expression might lead to poor prognosis in osteosarcomas, breast cancer, and lung adenocarcinoma [14, 21, 22]. Recently, Gui et al. showed that knocking down TUBA1C suppressed glioma cell proliferation via cell cycle arrest and high TUBA1C expression was correlated with poor outcomes in glioma patients [13]. The above results indicated TUBA1C might be an oncogene and a prognostic biomarker for several tumors. However, no previous studies reported the roles of TUBA1C gene in systematic pan-cancer analysis. Therefore, in the current study, we investigated the potential prognostic and immune-related roles of TUBA1C gene in systematic pan-cancer analysis.

In the present study, the data obtained from TCGA and GTEx databases revealed that the TUBA1C expression was upregulated in 28 types of tumor tissues compared to normal tissues. Besides, the mutations of TUBA1C gene were detected in 21 tumors, such as DLBC, UCEC, SKCM, ADC, ESCA, and so on and the most common type of mutation was amplification. Next, we explored the prognostic values of TUBA1C in pan-cancer analysis. Following the results afore-mentioned studies, higher TUBA1C expression was not only related to worse OS and DSS in LGG, LUAD, LIHC, and BRCA, but predicted an unfavorable OS and DSS in PAAD, SKCM, GBM, KICH, MESO, and KIRP. In contrast, high TUBA1C expression was correlated with a good prognosis in READ, COAD, and PRAD. Our results suggested that TUBA1C might be used as a routinely prognostic biomarker for several tumors in clinical practice in the future.

Besides, the enrichment analyses showed that TUBA1C was involved in the tumor development process through multiple immune-related pathways, including such as neutrophil degranulation, cytokine signaling in the immune system, the adaptive immune system, and the innate immune system. Consequently, the above results strongly indicated that TUBA1C might play a vital role in the development and progression of tumors via mediating immune-related pathways. Then immune cell infiltration analysis showed that TUBA1C expression was positively correlated with infiltrating levels of macrophages in BLCA, BRCA, CESC, DLBC, LGG, LUAD, STAD, and THCA. It is well known that macrophages derived from bone marrow, circulate into the blood and differentiate in various tissues [23]. Macrophages are the major immune cells mainly participating in the tumor microenvironment, that is TAMs. TAMs, consisting of M1 type macrophages (M1) and M2 type macrophages (M2), play vital roles in promoting tumor growth, invasion, metastasis, as well as drug resistance [24, 25]. Numerous evidence revealed that TAMs contributed to tumor cell proliferation and survival via secreting a variety of cytokines, including transforming growth factor-β1 (TGF-β1), epithelial growth factor (EGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF) [26, 27]. Besides, TAMs could also promote tumor angiogenesis and metastasis by directly producing several soluble factors, such as vascular endothelial growth factor (VEGF), interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), matrix metalloproteinases (MMPs), and serine proteases [28, 29]. The relationship between TUBA1C expression and macrophages

infiltration was also validated using ImmuCellAI databases. Moreover, TUBA1C expression was positively associated with filtrating levels of Treg cells but was negatively related to $CD8^+$ T cells and $CD4^+$ T cells in most tumors. Under the TME, Tregs were induced and differentiated to play an immunosuppressive function, inhibit antitumor immunity, and facilitate the occurrence and development of tumors [30]. Besides, Tregs can also suppress antitumor immunity antigen presentation by DCs, $CD4^+$ T helper (Th) cell function, and generate tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) via secreting TGF-β, IL-10, and IL-35 to promote immune escape [31, 32]. $CD4^+$ T1 cells and activated $CD8^+$ T cells can induce type I immune responses, which was associated with a good prognosis in tumors [33]. Therefore, our results indicated that TUBA1C might be involved in tumor development and progress through mediating TIICs-related immune regulation either directly or indirectly.

Recently, immune checkpoint blockade (ICB) therapy has improved the OS rates in patients with a variety of malignant tumors [34]. Treatment with anti-PD-1 and/or anti-CTLA-4 has reinvigorated T cells and the adaptive immune system in tumor treatment [35]. TMB is an independent biomarker to predict the ICB efficacy and evaluate the benefits of immunotherapy in tumor patients [34, 36]. TMB is usually defined as the total number of base substitutions and somatic mutations in the exon coding region of targeted genes. Therefore, the tumors with the higher TMB mean greater mutation load, which might increase the likelihood of recognition by neoantigen-reactive T cells. In our study, TUBA1C expression was positively associated with higher TMB in LUAD, KICH, SKCM, SARC, STAD, and BRCA. Therefore, TUBA1C might be used as a potential target for immunotherapy in those tumors.

In conclusion, we found that TUBA1C was found to be highly expressed in most tumors, and its high expression is associated with poor survival in several tumors. Besides, TUBA1C expression was significantly related to infiltrating levels of immune cells, especially macrophages. TUBA1C expression was positively correlated with TMB, MSI, and dominating immunosuppressive genes in certain tumors. Our results provided novel insights into the potential role of TUBA1C involving in tumor development and progress. Finally, our findings showed that TUBA1C might be a valuable prognostic biomarker and a potential target for immunotherapy.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

YQL and XDW conceived and designed the study. XF and BJL analyzed the data and XF wrote the manuscript. YZY, LYL, and SYF collected the data. XF and BJL contributed equally to the study. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All the datasets used in the study were open access datasets that could be found online. The TCGA and GTEx database used in this study were available from UCSC XENA website (https://xenabrowser.net/datapages/). DNA copy number and methylation data were downloaded from the cBioPortal database (https://www.cbioportal.org/). Immune cell infiltration analysis was estimated using the CIBERSORT algorithm downloaded from the ImmuCellAI database (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/).

Ethics approval and consent to participate

All analyses of human data conducted in this study were in accordance with the Helsinki Declaration. The study was approved by the Ethics Committee of Ningbo First Hospital. Written informed consent was obtained from all participants according to the publication guidelines provided by TCGA, GTEx, and ImmuCellAI database.

Competing interests

The authors declare that they have no conflict of interest.

Consent for publication

Not applicable.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.

2. Sun D, Li H, Cao M, He S, Lei L, Peng J, Chen W Cancer burden in China: trends, risk factors and prevention. Cancer Biol Med. 2020;17(4):879-95.

3. Schwaederle M, Zhao M, Lee JJ, Lazar V, Leyland-Jones B, Schilsky RL, Mendelsohn J, Kurzrock R Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. JAMA Oncol. 2016;2(11):1452-59.

4. Ribas A, Wolchok JD Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350-55.

5. Fridman WH, Pagès F, Sautès-Fridman C, Galon J The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298-306.

6. Chen DS, Mellman I Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321-30.

7. Nieuwenhuis J, Brummelkamp TR The Tubulin Detyrosination Cycle: Function and Enzymes. Trends Cell Biol. 2019;29(1):80-92.

8. Kim ND, Park ES, Kim YH, Moon SK, Lee SS, Ahn SK, Yu DY, No KT, Kim KH Structure-based virtual screening of novel tubulin inhibitors and their characterization as anti-mitotic agents. Bioorg Med Chem. 2010;18(19):7092-100.

9. Janke C, Magiera MM The tubulin code and its role in controlling microtubule properties and functions. Nat Rev Mol Cell Biol. 2020;21(6):307-26.

10. Glotzer M The 3Ms of central spindle assembly: microtubules, motors and MAPs. Nat Rev Mol Cell Biol. 2009;10(1):9-20.

11. Xue X, Gao W, Sun B, Xu Y, Han B, Wang F, Zhang Y, Sun J, Wei J, Lu Z, et al. Vasohibin 2 is transcriptionally activated and promotes angiogenesis in hepatocellular carcinoma. Oncogene. 2013;32(13):1724-34.

12. Lee YS, Lim KH, Guo X, Kawaguchi Y, Gao Y, Barrientos T, Ordentlich P, Wang XF, Counter CM, Yao TP The cytoplasmic deacetylase HDAC6 is required for efficient oncogenic tumorigenesis. Cancer Res. 2008;68(18):7561-9.

13. Gui S, Chen P, Liu Y, Chen Q, Cheng T, Lv S, Zhou T, Song Z, Xiao J, He W, et al. TUBA1C expression promotes proliferation by regulating the cell cycle and indicates poor prognosis in glioma. Biochem Biophys Res Commun. 2021;577(130-38.

14. Bian T, Zheng M, Jiang D, Liu J, Sun H, Li X, Liu L, Zhang J, Liu Y Prognostic biomarker TUBA1C is correlated to immune cell infiltration in the tumor microenvironment of lung adenocarcinoma. Cancer Cell Int. 2021;21(1):144.

15. Wang J, Chen W, Wei W, Lou J Oncogene TUBA1C promotes migration and proliferation in hepatocellular carcinoma and predicts a poor prognosis. Oncotarget. 2017;8(56):96215-24.

16. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, Treviño V, Shen H, Laird PW, Levine DA, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun. 2013;4(2612.

17. Aran D, Sirota M, Butte AJ Systematic pan-cancer analysis of tumour purity. Nat Commun. 2015;6:8971.

18. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, et al. The Immune Landscape of Cancer. Immunity. 2018;48(4):812-30.e14.

19. Tangutur AD, Kumar D, Krishna KV, Kantevari S Microtubule Targeting Agents as Cancer Chemotherapeutics: An Overview of Molecular Hybrids as Stabilizing and Destabilizing Agents. Curr Top Med Chem. 2017;17(22):2523-37.

20. Jordan MA, Wilson L Microtubules as a target for anticancer drugs. Nat Rev Cancer. 2004;4(4):253-65.

21. Li Y, Liang Q, Wen YQ, Chen LL, Wang LT, Liu YL, Luo CQ, Liang HZ, Li MT, Li Z Comparative proteomics analysis of human osteosarcomas and benign tumor of bone. Cancer Genet Cytogenet. 2010;198(2):97-106.

22. Wang CCN, Li CY, Cai JH, Sheu PC, Tsai JJP, Wu MY, Li CJ, Hou MF Identification of Prognostic Candidate Genes in Breast Cancer by Integrated Bioinformatic Analysis. J Clin Med. 2019;8(8).

23. Biswas SK, Allavena P, Mantovani A Tumor-associated macrophages: functional diversity, clinical significance, and open questions. Semin Immunopathol. 2013;35(5):585-600.

24. Salmaninejad A, Valilou SF, Soltani A, Ahmadi S, Abarghan YJ, Rosengren RJ, Sahebkar A Tumor-associated macrophages: role in cancer development and therapeutic implications. Cell Oncol (Dordr). 2019;42(5):591-608.

25. DeNardo DG, Ruffell B Macrophages as regulators of tumour immunity and immunotherapy. Nat Rev Immunol. 2019;19(6):369-82.

26. Pan Y, Yu Y, Wang X, Zhang T Tumor-Associated Macrophages in Tumor Immunity. Front Immunol. 2020;11(583084.

27. Yin M, Li X, Tan S, Zhou HJ, Ji W, Bellone S, Xu X, Zhang H, Santin AD, Lou G, et al. Tumor-associated macrophages drive spheroid formation during early transcoelomic metastasis of ovarian cancer. J Clin Invest. 2016;126(11):4157-73.

28. Annamalai RT, Turner PA, Carson WFt, Levi B, Kunkel S, Stegemann JP Harnessing macrophage-mediated degradation of gelatin microspheres for spatiotemporal control of BMP2 release. Biomaterials. 2018;161(216-27.

29. Hao S, Meng J, Zhang Y, Liu J, Nie X, Wu F, Yang Y, Wang C, Gu N, Xu H Macrophage phenotypic mechanomodulation of enhancing bone regeneration by superparamagnetic scaffold upon magnetization. Biomaterials. 2017;140(16-25.

30. Toker A, Ohashi PS Expression of costimulatory and inhibitory receptors in FoxP3(+) regulatory T cells within the tumor microenvironment: Implications for combination immunotherapy approaches. Adv Cancer Res. 2019;144(193-261.

31. Li C, Jiang P, Wei S, Xu X, Wang J Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Mol Cancer. 2020;19(1):116.

32. Sawant DV, Yano H, Chikina M, Zhang Q, Liao M, Liu C, Callahan DJ, Sun Z, Sun T, Tabib T, et al. Adaptive plasticity of IL-10(+) and IL-35(+) T(reg) cells cooperatively promotes tumor T cell exhaustion. Nat Immunol. 2019;20(6):724-35.

33. Nesseler JP, Lee MH, Nguyen C, Kalbasi A, Sayre JW, Romero T, Nickers P, McBride WH, Schaue D Tumor Size Matters-Understanding Concomitant Tumor Immunity in the Context of Hypofractionated Radiotherapy with Immunotherapy. Cancers (Basel). 2020;12(3).

34. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, Peters S Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30(1):44-56.

35. Pardoll DM The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-64.

36. Kim SI, Cassella CR, Byrne KT Tumor Burden and Immunotherapy: Impact on Immune Infiltration and Therapeutic Outcomes. Front Immunol. 2020;11:629722.

Table

Table 1 Number of 33 tumors from TCGA database for pan-cancer analysis

TCGA ID	Cancer	Normal	Tumor
ACC	Adrenocortical carcinoma	0	77
BLCA	Bladder urothelial carcinoma	19	407
BRCA	Breast invasive carcinoma	113	1098
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	3	306
CHOL	Cholangiocarcinoma	9	36
COAD	Colon adenocarcinoma	41	288
DLBC	Lymphoid neoplasm diffiuse large B-cell lymphoma	0	47
ESCA	Esophageal carcinoma	13	82
GBM	Glioblastoma multiforme	0	165
HNSC	Head and neck squamous cell carcinoma	44	520
KICH	Kidney chromophobe	25	66
KIRC	Kidney clear cell carcinoma	72	531
KIRP	Kidney papillary cell carcinoma	32	289
LAML	Acute myeloid leukemia	0	173
LGG	Brain lower grade glioma	0	522
LIHC	Liver hepatocellular carcinoma	50	371
LUAD	Lung adenocarcinoma	59	515
LUSC	Lung squamous cell carcinoma	50	498
MESO	Mesothelioma	0	87
OV	Ovarian serous cystadenocarcinoma	0	427
PAAD	Pancreatic adenocarcinoma	4	179
PCPG	Pheochromocytoma and paraganglioma	3	182
PRAD	Prostate adenocarcinoma	52	496
READ	Rectum adenocarcinoma	10	92
SARC	Sarcoma	2	262
SKCM	Skin cutaneous melanoma	1	469
STAD	Stomach adenocarcinoma	36	414

TGCT	Testicular germ cell tumors	0	137
THCA	Thyroid carcinoma	59	512
THYM	Thymoma	2	119
UCEC	Uterine corpus endometrial carcinoma	13	181
USC	Uterine carcinosarcoma	0	57
UVM	Uveal melanoma	0	79
Total		712	9684

Figures

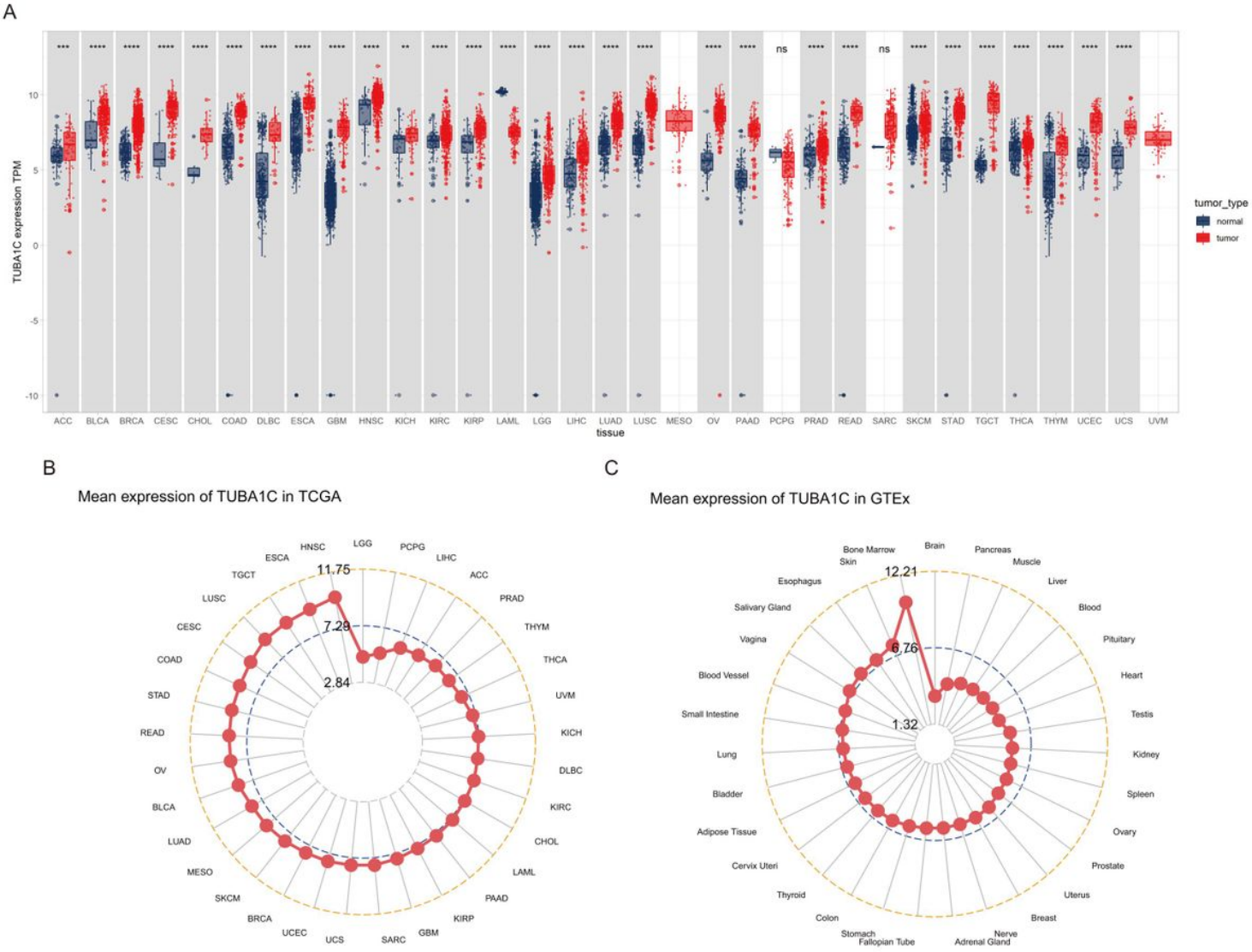

Figure 1

TUBA1C expression in pan-cancer. (A) Pan-cancer TUBA1C expression between tumor tissues from TCGA database and normal tissues from TCGA and GTEx database. (B) Mean TUBA1C expression levels in tumor tissues from TCGA database. (C) Mean TUBA1C expression levels in normal tissues from the GTEx database. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

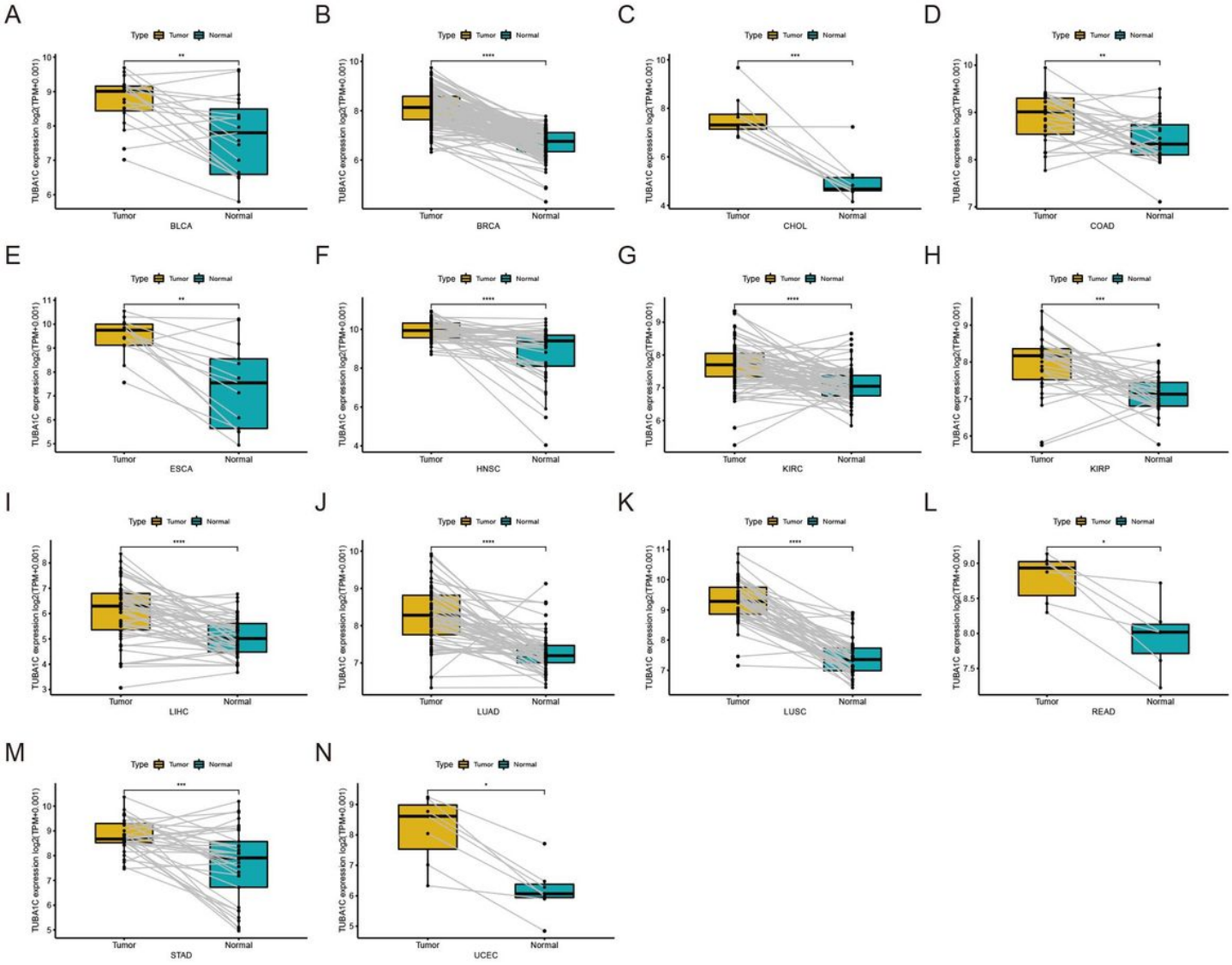

Figure 2

Pan-cancer TUBA1C expression in paired tumor and normal tissues. (A–N) TUBA1C expression in paired tumor and normal tissues in pan-cancer data from TCGA database. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Figure 3

TUBA1C gene alterations in pan-cancer. (A) Mutated types of TUBA1C in pan-cancer samples from TCGA database. (B) The correlations between TUBA1C gene expression and CNA in pan-cancer samples from TCGA database. (C) The correlations between TUBA1C gene expression and methylation levels of TUBA1C promoter in pan-cancer samples from TCGA database. The pearson's correlation coefficient test was used for testing.

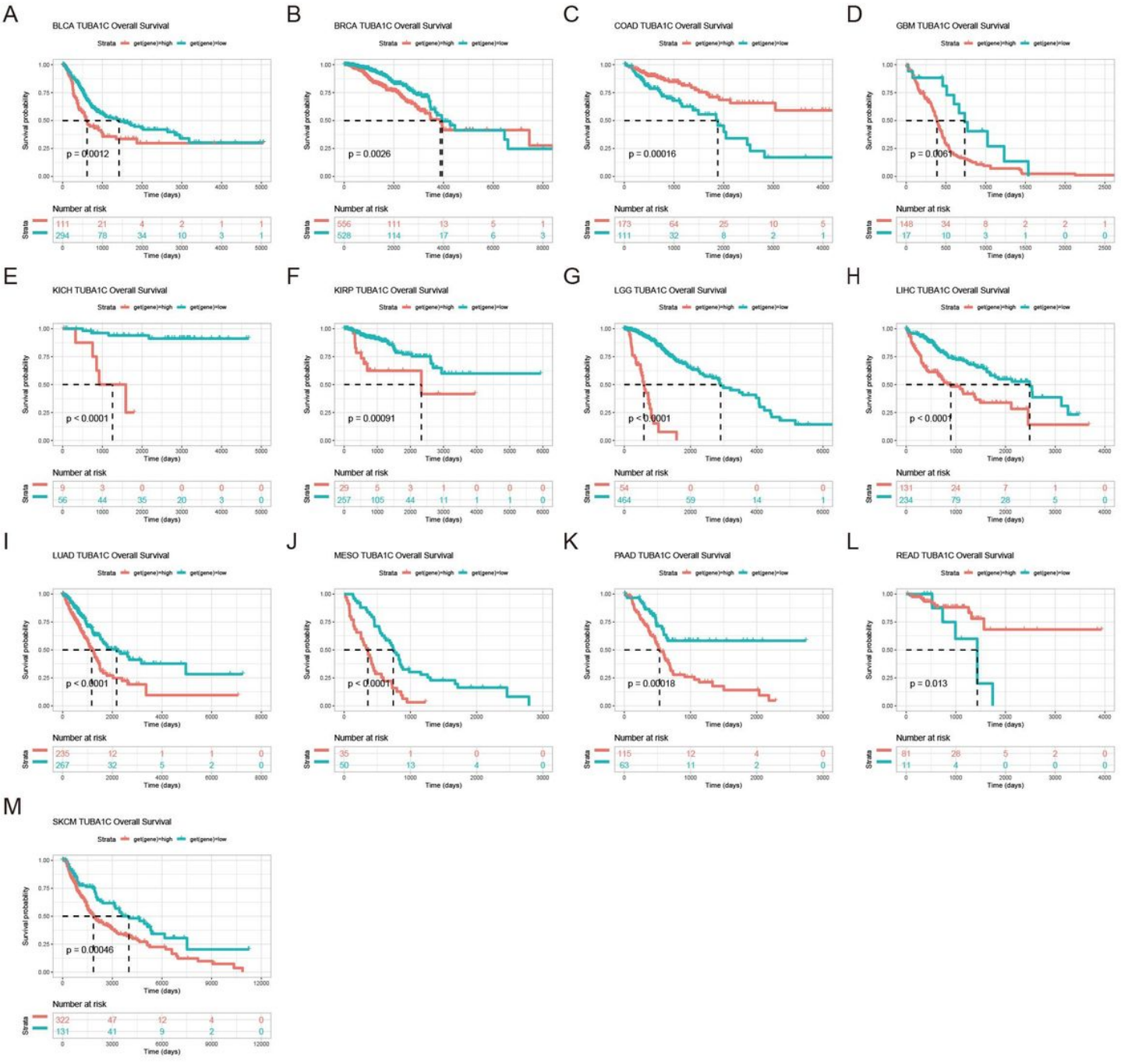

Figure 4

Associations between TUBA1C expression and OS. (A-M) Kaplan-Meier analysis of the association between TUBA1C expression and OS. The division of high TUBA1C expression and low TUBA1C

expression group was according to the optimal TUBA1C expression level.

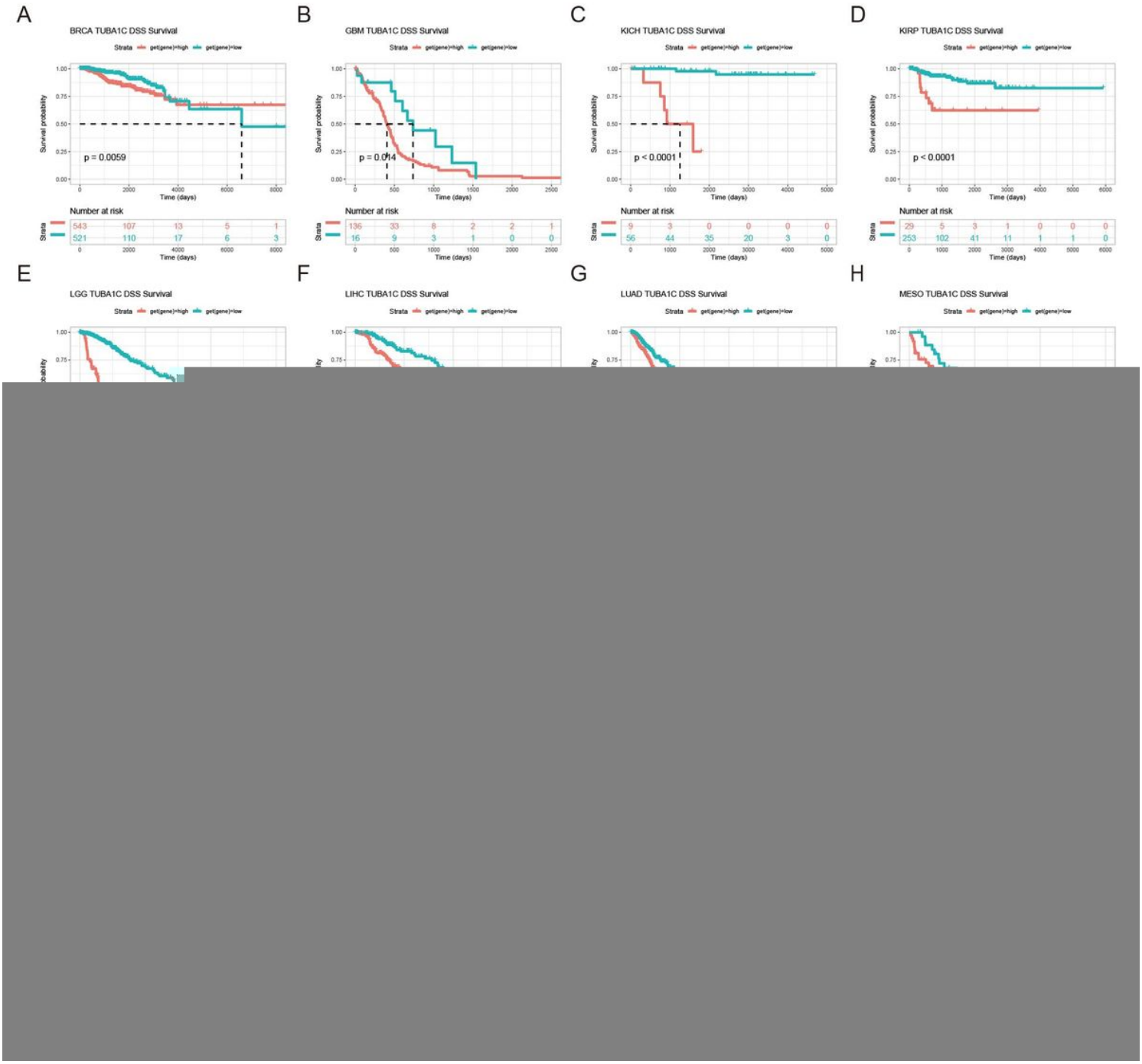

Figure 5

Associations between TUBA1C expression and DSS and DFI. (A–J) Kaplan–Meier analysis of the association between TUBA1C expression and DSS as well as (K-M) DFI. The division of high TUBA1C expression and low TUBA1C expression group was according to optimal TUBA1C expression level.

Figure 6

GSEA analysis of TUBA1C in pan-cancer. (A–F) The top 20 Reactome results of TUBA1C GSEA in six tumor types (adjusted *p*-value < 0.05). Red indicates the pathways involved in cell cycle, TP53, or immune regulation.

Figure 7

Correlation of scores with TUBA1C expression in cancers. (A) ESTIMATE analysis for the correlations between TUBA1C and stromal score, ESTIMATE score, and immune score in 33 TCGA tumor types. (B) The top three tumors with the highest positive or negative correlation coefficients and significant *p* values for stromal score, ESTIMATE score, and immune score, respectively. The pearson's correlation coefficient test was used for testing. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Figure 8

Immune cell infiltration analysis. (A) The correlation between TUBA1C expression and infiltrating levels of 26 immune cells in TCGA pan-cancer samples using the previously published database. (B) The correlation between TUBA1C expression and infiltrating levels of eight immune cells (macrophage, DC, nTreg, iTreg, CD8+ T cells, Tgd, B cells, and CD4+ T cells) in TCGA pan-cancer samples using data from the ImmuCellAI database. The pearson's correlation coefficient test was used for testing. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Figure 9

Correlations between TUBA1C expression and immunosuppressive genes, TMB, MSI in various tumors in 33 TCGA tumor types. (A) Correlation between TUBA1C expression and immunosuppressive genes. (B) Correlation between TMB and TUBA1C expression. (C) Correlation between MSI and TUBA1C expression. The pearson's correlation coefficient test was used for testing. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Supplementary Files

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- SupplementFigure1.tif
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- SupplementFigure3.tif