Original Article

A comprehensive pan-cancer analysis on the immunological role and prognostic value of *TYMP* in human cancers

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Background: The *TYMP* gene encodes an important nucleoside metabolism enzyme which is a rate-limiting enzyme for chemotherapeutic drug metabolism. Previous studies have shown that *TYMP* is highly expressed in many different tumors, promoting invasiveness and progression, and that it helps to predict the response to chemotherapeutic drugs. However, the role of *TYMP* in tumor immunity and prognosis remains largely unclear. The purpose of this pan-cancer analysis was to acquire more data on the function of *TYMP* function and its clinical significance.

Methods: To access the *TYMP* expression, we accessed datasets from The Cancer Genome Atlas (TCGA), Oncomine, Gene Expression Profiling Interactive Analysis (GEPIA), Cancer Cell Line Encyclopedia (CCLE) databases, and analyzed its differential expression between paired tumor and normal samples. We employed PrognoScan and Kaplan-Meier plotter for survival analyses. *TYMP* mutations were analyzed using cBioPortal. Correlations of *TYMP* with tumor stage, tumor mutational burden (TMB), microsatellite instability (MSI), immune checkpoint genes (ICGs), and immune cell infiltration were estimated via bioinformatics tools and methods. The CellMiner database was used to predict drug response. Gene set enrichment analysis (GSEA) was applied to explore the biological functions of *TYMP* in different tumors.

Results: Our results indicated that *TYMP* was overexpressed and also significantly associated with a worse prognosis in several human cancers, such as kidney clear cell carcinoma (KIRC) and lower grade glioma (LGG). *TYMP* was also associated with TMB, MSI, and ICGs across a variety of malignancies. *TYMP* was most significantly correlated with immune cell infiltration in five tumors, namely, breast cancer (BRCA), cervical cancer (CESC), KIRC, skin cutaneous melanoma (SKCM), and stomach adenocarcinoma (STAD). Moreover, *TYMP* expression predicted sensitivity to chemotherapy drugs and also influenced relevant biological pathways, according to enrichment analysis.

Conclusions: According to the results of this comprehensive analysis, *TYMP* is associated with prognosis and tumor immunology, which might make it be a potential therapeutic target for cancer treatment.

Keywords: *TYMP*; pan-cancer analysis; prognosis; tumor mutational burden (TMB); immune infiltration

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Introduction

Cancer is major cause of death worldwide, the incidence and mortality rates of which are rapidly rising globally. According to Global Cancer Statistics (GLOBOCAN 2020), in 2020 there were 19.3 million new cancer diagnoses and about 10 million cancer deaths (1). Most cancer deaths are due to metastasis and no effective treatment currently exists for cancer (2). Fortunately, in recent years, cancer

immunotherapy including immune checkpoint inhibitors has become an increasingly successful strategy (3,4). Therefore, new therapeutic targets and sensitive tumor biomarkers are required for the diagnosis and treatment of cancer.

The *TYMP* gene encoding thymidine phosphorylase (TP) is located on chromosome 22q13.33 (5). TP is an enzyme involved in nucleoside metabolism in the salvage pathway of pyrimidine nucleosides. It is also known as platelet-derived endothelial cell growth factor (PD-ECGF), which plays an important role in cell proliferation and angiogenesis (6). Mutations in *TYMP* have been found to cause mitochondrial neurogastrointestinal encephalopathy (MNGIE) (7). Also, much evidence has suggested that *TYMP* is expressed in several human tumors, such as in lung cancer (8), head and neck cancer (HNSC) (9), esophageal squamous cell carcinoma (10), gastric carcinoma (11), and colorectal cancer (12). Moreover, *TYMP* is also suggested to play multiple roles in tumor progression and response to therapy. On the one hand, *TYMP* promotes cell proliferation, enhances the epithelial to mesenchymal transition, as well as angiogenesis, activities which all accelerate tumor invasiveness and progression (13). On the other hand, because the *TYMP* gene encodes TP which is an important rate-limiting enzyme in thymidine catabolism involved in the 5-fluorouracil (5-FU) activation pathway, quantification of *TYMP* expression will be extremely useful in predicting the effects of chemotherapeutic drugs, such as 5-FU and capecitabine (14). In addition, *TYMP* is a target of anti-cancer drugs to inhibit its expression and promote the suppression of angiogenesis and enhance apoptosis.

Several studies have reported that increased *TYMP* expression in different cancers is linked to poor outcomes (11,15,16). Furthermore, tumor cells and stromal cells express *TYMP* in the tumor microenvironment (TME) (13) and a previous study suggested that *TYMP* may be a target for immunotherapy in solid tumors (17). Taken together, available evidence suggests that *TYMP* influences cancer prognosis, and may potentially represent a useful prognostic biomarker.

Nevertheless, the significance of *TYMP* for tumor immunity and prognosis in many diverse malignancies remains unclear, and a comprehensive pan-cancer study on the relationship between *TYMP* expression and cancer biology has not yet been conducted. Therefore, we performed such a pan-cancer analysis to better understand the role of *TYMP* in cancer. In our investigation, we examined the *TYMP* expression profiles in tumors and cancer cell lines by leveraging the The Cancer Genome Atlas (TCGA), Oncomine, Gene Expression Profiling Interactive Analysis (GEPIA), and Cancer Cell Line Encyclopedia (CCLE) databases. Additionally, the expression of *TYMP* and its connection with cancer prognosis was investigated using PrognoScan and Kaplan-Meier Plotter. We also performed a genetic mutation analysis, and microsatellite instability (MSI), tumor mutational burden (TMB), and immune scores were calculated to study their relationships with *TYMP* expression in tumors. Subsequently, co-expression analyses of ICGs and *TYMP*, drug sensitivity analysis, and gene set enrichment analyses (GSEAs) were carried out. Our findings suggest that *TYMP* may be a predictive factor in a variety of malignancies and a critical in tumor immunity, but further laboratory evidence is needed in future. We present the following article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-502/rc).

Methods

Data acquisition and TYMP expression analysis

Gene expression data was downloaded using the UCSC Xena platform (https://xena.ucsc.edu) including RNA sequencing, somatic mutation, and related clinical data for 33 cancer types from TCGA. *TYMP* gene expression data were obtained from datasets and converted into a gene expression matrix for analysis using a Perl script written in Strawberry Perl for Windows (version 5.32.1; available at https://strawberryperl.com/). The Oncomine (https://www.oncomine.org/) database was searched for differences in *TYMP* expression between around 20 cancer types and paired normal tissue. *TYMP* expression was compared between human malignant tumors and associated normal tissue using the GEPIA database (http://gepia.cancer-pku.cn/). Further, we accessed *TYMP* expression levels in 33 different cancer cell lines, through the CCLE database (https://sites.broadinstitute.org/ccle). *TYMP* expression data from TCGA were compared in normal tissue and tumor tissue among 33 cancer entities by Wilcoxon testing, and a P value of <0.05 was regarded as significant. R software v4.05 (https://www.r-project.org/) was used to perform the statistical analysis, and box plots was drawn with the "ggpubr" package of R software. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Analysis of correlations between TYMP expression and prognosis of tumor patients

Survival data of patients using 10,121 tumor samples from 33 tumors were downloaded from TCGA. The relationships between *TYMP* expression and patient prognosis, including overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) were investigated. Cox proportional hazard regression analysis and the Kaplan-Meier method were used for survival analysis, presented as forest plots and Kaplan-Meier survival curves, using the R packages "survival" and "forestplot", survival" and "survminer" packages, respectively.

Genetic alteration analysis

Genetic alterations of *TYMP* were analyzed in cancers via cBioPortal (https://www.cbioportal.org/) using data from TCGA (18). Applying cBioPortal, we investigated the frequency, mutation type, and copy number alteration (CNA) of *TYMP* in all TCGA cancers. These results can be obtained from the 'Cancer Types Summary' tab. We explored the *TYMP* mutation sites in pan-cancer and also compared clinical outcomes in TCGA cancer samples with or without *TYMP* gene mutations.

Associations analysis of TYMP expression with tumor stage, TMB and MSI

Clinical information on pathological tumor stage of all the tumor samples derived from TCGA was downloaded and associations of *TYMP* with tumor stage analyze using the R-packages "limma" and "ggpubr". The total amount of mutations in tumor cells is referred to as TMB, and is used to predict responsiveness to immune checkpoint inhibitor therapy (19). MSI in tumor cells is related to defects in the DNA mismatch repair system (20). We downloaded mutation data from TCGA of 33 tumors from 10,114 samples and then calculated TMB scores for each sample. TCGA provided data on somatic mutations of tumor samples and the calculated MSI scores. Finally, the link between *TYMP* expression and TMB, as well as MSI, was investigated using Spearman's rank correlation testing. TMB and MSI correlation radar maps were created using the R-package "fmsb".

Correlations between TYMP expression and immunity

In all tumor samples, immune scores and stromal scores were estimated by the ESTIMATE (estimation of stromal and immune cells in malignant tumor tissues using expression) algorithm method. This can assist in determining the level of stromal and immune cell infiltration (21). To assess the association between *TYMP* expression and these two scores, we further applied the R packages "estimate" and "limma". Moreover, we used CIBERSORT, a computational approach, to study tumor cell infiltration abundance among 33 human malignancies (22). We also used "ggplot2", "ggpubr", and "ggExtra" to examine associations between *TYMP* expression and the degree of infiltration of immune cells into each cancer. Finally, we generated a heatmap of connections between *TYMP* expression and 47 ICGs across 33 human cancers using the R-package "limma", "reshape2" and "RColorBrewer".

Drug sensitivity analysis

The CellMiner database (https://discover.nci.nih.gov/cellminer/) contains molecular and pharmacological datasets for the 60 different National Cancer Institute (NCI) human tumor cell lines from nine cancer types (NCI-60 cell lines) (23). Relationships between *TYMP* mRNA expression and drug sensitivity were also investigated using Pearson correlations. Data processing and correlation analysis visualization were conducted through the "impute", "limma", "ggplot2", and "ggpubr" packages, with significance set to $P<0.05$.

GSEA based on TYMP expression in tumors

GSEA (https://www.gsea-msigdb.org/gsea/index.jsp) of *TYMP* was performed to gain insight into tumor biology. Enrichment analyses were carried out via the "limma", "org.Hs.eg.db", "clusterProfiler" and "enrichplot" packages.

Statistical analysis

TYMP expression was $\log_2$-transformed and *TYMP* expression was analyzed using the "limma" package and the Student's *t*-test. Survival analyses adopted the Kaplan-Meier method, log-rank testing, and Cox proportional hazard

regression analysis. Correlations between two variables were determined using Pearson's or Spearman's correlation test. R software (version 4.0.5) was used to conduct statistical analysis and data visualizations. P<0.05 is regarded as statistically significant.

Results

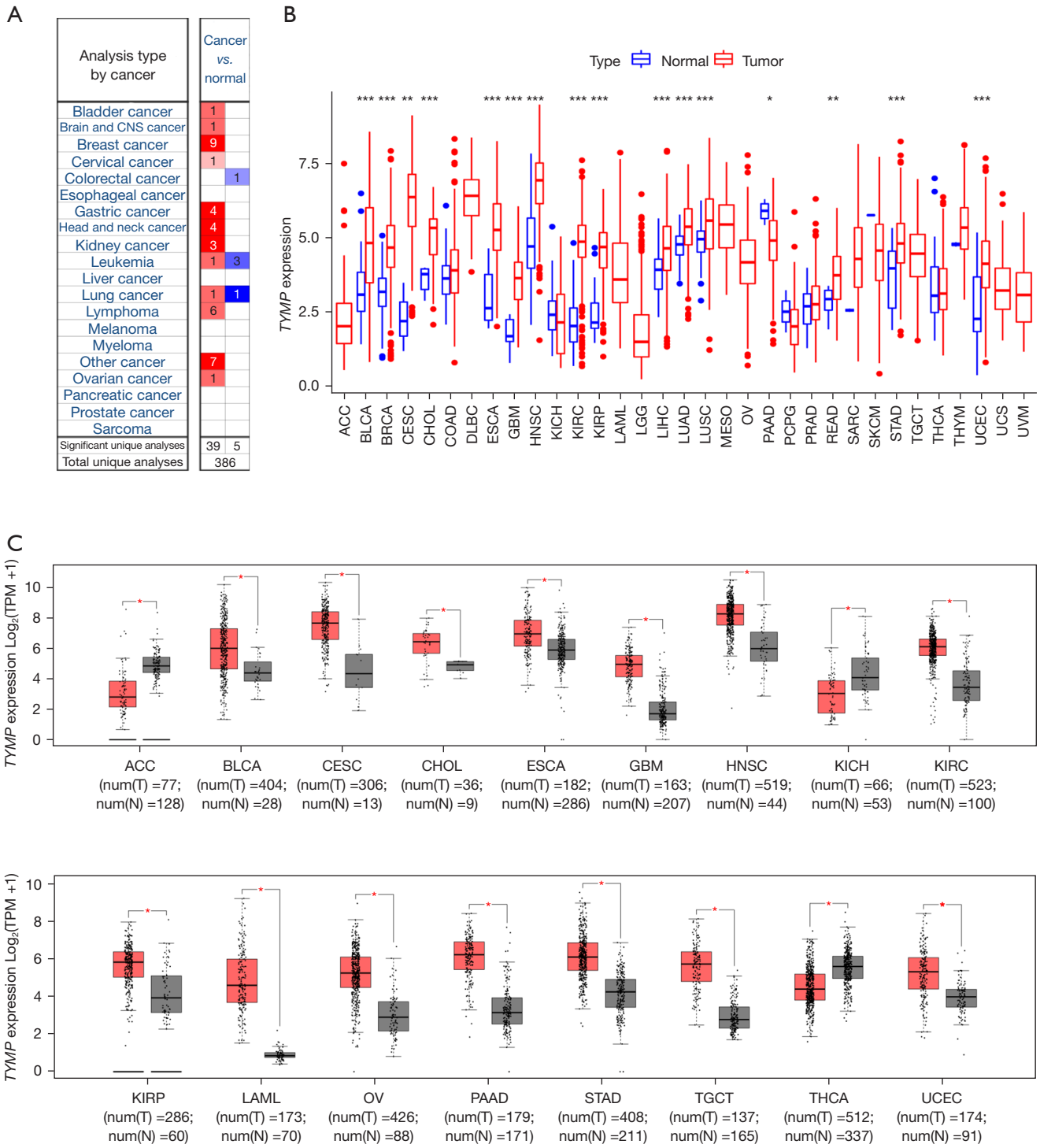
Pan-cancer analysis of expression profiles of TYMP from different databases

To determine *TYMP* expression in different cancer types, *TYMP* levels in tumor and normal samples from the Oncomine database were analyzed. This revealed that *TYMP* expression was significantly increased in bladder cancer (BLCA), brain and central nervous system (CNS) cancer, breast cancer (BRCA), cervical cancer (CESC), gastric cancer, HNSC, kidney cancer, leukemia, lung cancer, lymphoma, ovarian cancer (OV), and other cancer types (*Figure 1A*). We next analyzed *TYMP* expression levels in all 33 cancers in the TCGA database. *TYMP* expression was detectable in different cancers and matched normal tissues (*Figure 1B*). Compared with matched non-tumor tissue, differential *TYMP* expression was present in 16 of the 33 cancer types. *TYMP* expression was higher in cancer tissues versus normal tissues in BLCA, BRCA, CESC, bile duct cancer (CHOL), esophageal cancer (ESCA), glioblastoma (GBM), HNSC, kidney clear cell carcinoma (KIRC), kidney papillary cell carcinoma (KIRP), liver cancer (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), rectal cancer (READ), stomach adenocarcinoma (STAD), and endometrioid cancer (UCEC). The expression of *TYMP* in CESC showed the greatest difference between tumor tissues and non-tumor tissues. In contrast to most tumors, lower levels of *TYMP* were also observed in tumor tissues versus normal tissues in pancreatic cancer (PAAD). Finally, no significant differences in *TYMP* levels were discernible between colon cancer (COAD), prostate adenocarcinoma (PRAD), pheochromocytoma and paraganglioma (PCPG), and thyroid cancer (THCA). Furthermore, *TYMP* expression profiles were analyzed using the GEPIA database, confirming that the majority of tumor types exhibited significant upregulation of *TYMP* expression, except adrenocortical cancer (ACC), kidney chromophobe (KICH), and THCA (*Figure 1C*). *Figure 1D* displays gene expression of *TYMP* across human cell lines and ranks them from low to high based on CCLE data. The results of differential expression analysis across cancer types further confirmed that *TYMP* expression was generally upregulated in cancer, suggesting that it may play an important role in carcinogenesis, and warranting more detailed analyses in further.

The prognostic signature of TYMP in human cancers

We investigated the relationships of *TYMP* expression and survival in terms of OS, DSS, DFI, and PFI in each cancer type using the datasets from TCGA. Initially, we employed Cox proportional hazards modeling to evaluate associations between *TYMP* expression and patient prognosis (OS, DSS, DFI, and PFI) across different tumors. Notably, analysis of OS showed that *TYMP* could classified as a high-risk gene in ACC, GBM, KIRC, lower grade glioma (LGG), PAAD, thymoma (THYM), and uveal melanomas (UVM). However, it could be classified as a low-risk gene in patients with BRCA and skin cutaneous melanoma (SKCM), as shown in *Figure 2A*. From the forest plot in *Figure 2B*, in the DSS survival analysis, elevated *TYMP* expression was correlated with poor clinical outcome in patients with COAD (P=0.033), GBM (P=0.027), KIRC (P=0.001), LGG (P<0.001), PAAD (P=0.042), and UVM (P<0.001). In contrast, gene expression of *TYMP* was positively correlated with patient prognosis in BRCA (P=0.010) and SKCM (P<0.001). Furthermore, an association between *TYMP* expression and DFI was only seen in patients with PRAD (P=0.016; hazard ratio =1.610) as shown in *Figure 2C*. Regarding associations between *TYMP* expression and PFI, Cox regression analysis indicated that high levels of *TYMP* were related to poor PFI in GBM (P=0.005), KIRC (P=0.031), LGG (P<0.001), PAAD (P=0.036), PRAD (P=0.002), THYM (P=0.025), and UVM (P=0.009) (*Figure 2D*).

Next, we employed Kaplan-Meier survival analysis to investigate associations between *TYMP* expression and cancer patient prognosis. Kaplan-Meier survival estimates showed that high *TYMP* expression was associated with worse OS than low levels of *TYMP* in 5 malignancies, namely, ACC (P=0.013), LGG (P<0.001), KIRC (P=0.005), THYM (P=0.049), and UVM (P<0.001), while SKCM patients with high levels of *TYMP* survived longer (P=0.009) (*Figure 2E*). Low *TYMP* expression correlated with poor DSS in patients with BRCA (P=0.009) and SKCM (P<0.001). However, for patients with KIRC (P=0.020), LGG (P<0.001), ACC (P=0.014), THYM (P=0.021), and UVM (P<0.001), increased *TYMP* expression was



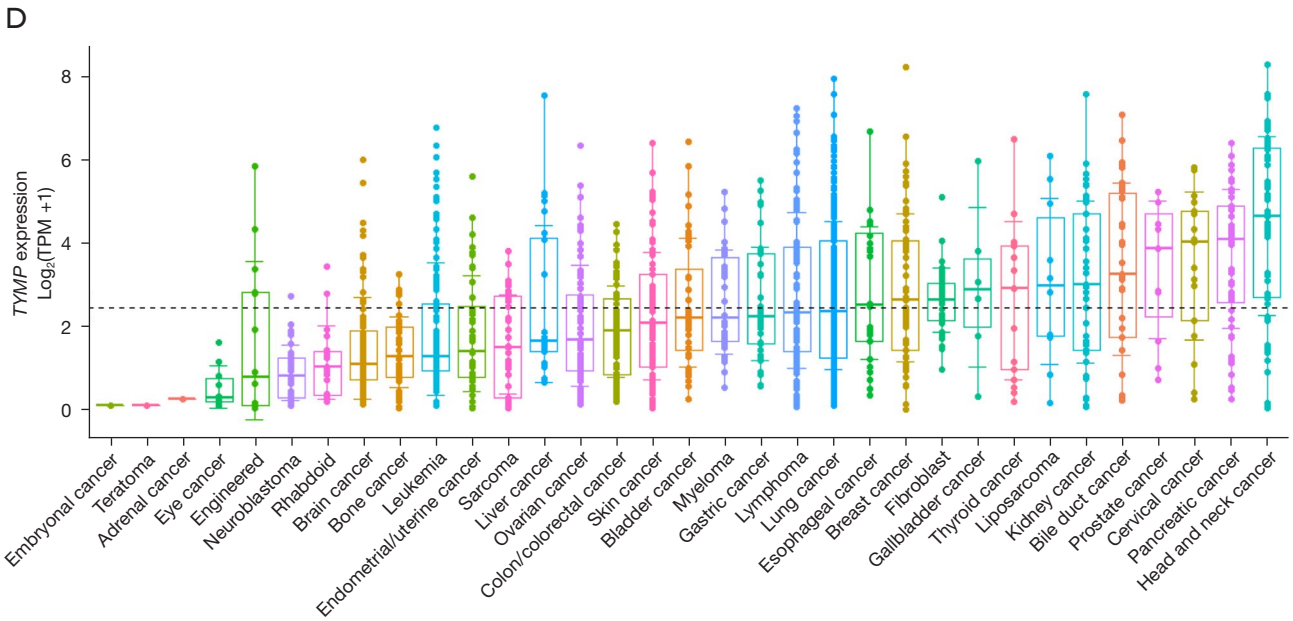
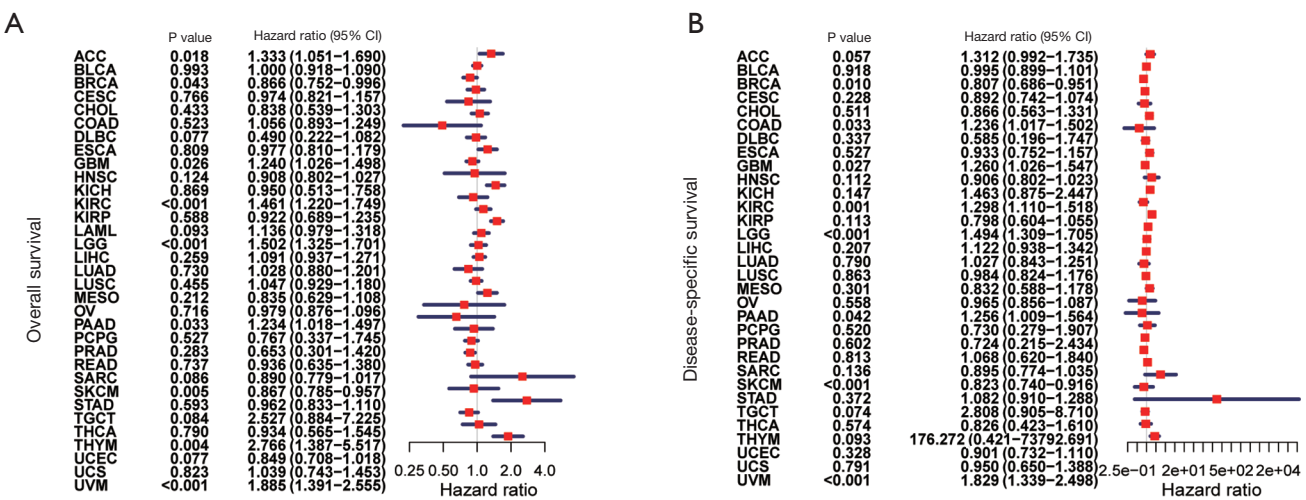


Figure 1 *TYMP* gene expression levels in normal tissues, tumor tissues, and cancer cell lines (A) *TYMP* expression in different cancers and matched normal tissues, assessed using the Oncomine database. (B) Differential expression of *TYMP* in 33 human cancer types based on the TCGA database. (*P<0.05; **P<0.01; ***P<0.001). (C) The level of *TYMP* expression in different cancers and paired normal tissues from the GEPIA database (*P<0.05). (D) *TYMP* expression in different cancer lines, from the CCLE database. CNS, central nervous system; ACC, adrenocortical cancer; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical cancer; CHOL, bile duct cancer; COAD, colon cancer; DLBC, large B-cell lymphoma; ESCA, esophageal cancer; GBM, glioblastoma; HNSC, head and neck cancer; KICH, kidney chromophobe; KIRC, kidney clear cell carcinoma; KIRP, kidney papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic cancer; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal cancer; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular cancer; THCA, thyroid cancer; THYM, thymoma; UCEC, endometrioid cancer; UCS, uterine carcinosarcoma; UVM, uveal melanomas; TPM, transcripts per million; TCGA, The Cancer Genome Atlas; GEPIA, Gene Expression Profiling Interactive Analysis; CCLE, Cancer Cell Line Encyclopedia.



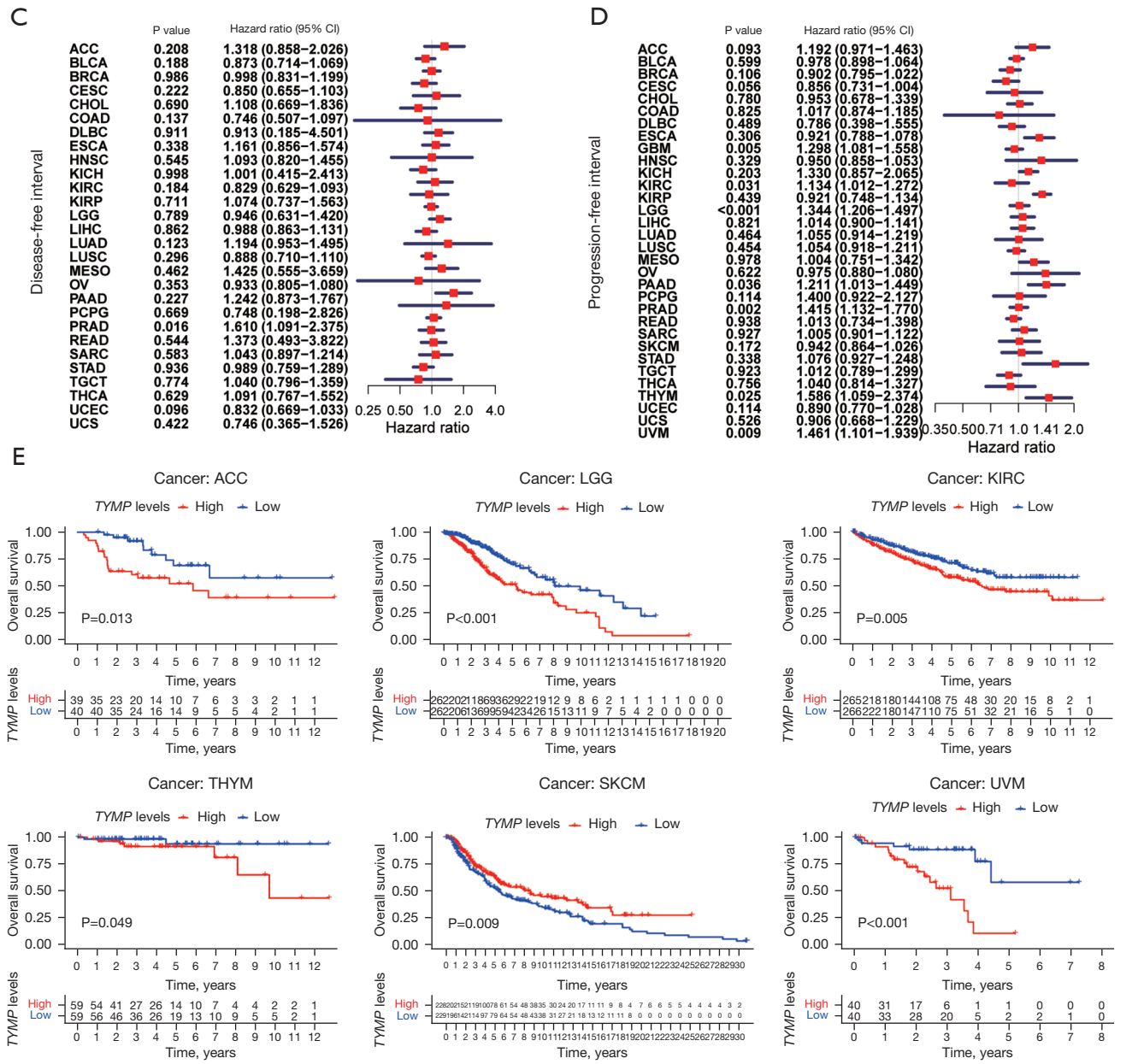


Figure 2 The correlation between the expression of *TYMP* and patient prognosis using R software. (A) Forest plot of OS across cancers. (B) Forest plot of DSS in pan-cancer. (C) Forest plot of DFI in pan-cancer. (D) Forest plot of PFI in pan-cancer. (E) Kaplan-Meier survival analysis for OS and *TYMP* expression. The P value of each tumor: ACC: P=0.013; LGG: P<0.001; KIRC: P=0.005; THYM: P=0.049; SKCM: P=0.009; UVM: P<0.001. ACC, adrenocortical cancer; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical cancer; CHOL, bile duct cancer; COAD, colon cancer; DLBC, large B-cell lymphoma; ESCA, esophageal cancer; GBM, glioblastoma; HNSC, head and neck cancer; KICH, kidney chromophobe; KIRC, kidney clear cell carcinoma; KIRP, kidney papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic cancer; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal cancer; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular cancer; THCA, thyroid cancer; THYM, thymoma; UCEC, endometrioid cancer; UCS, uterine carcinosarcoma; UVM, uveal melanomas; OS, overall survival; DSS, disease-specific survival; DFI, disease-free interval; PFI, progression-free interval.

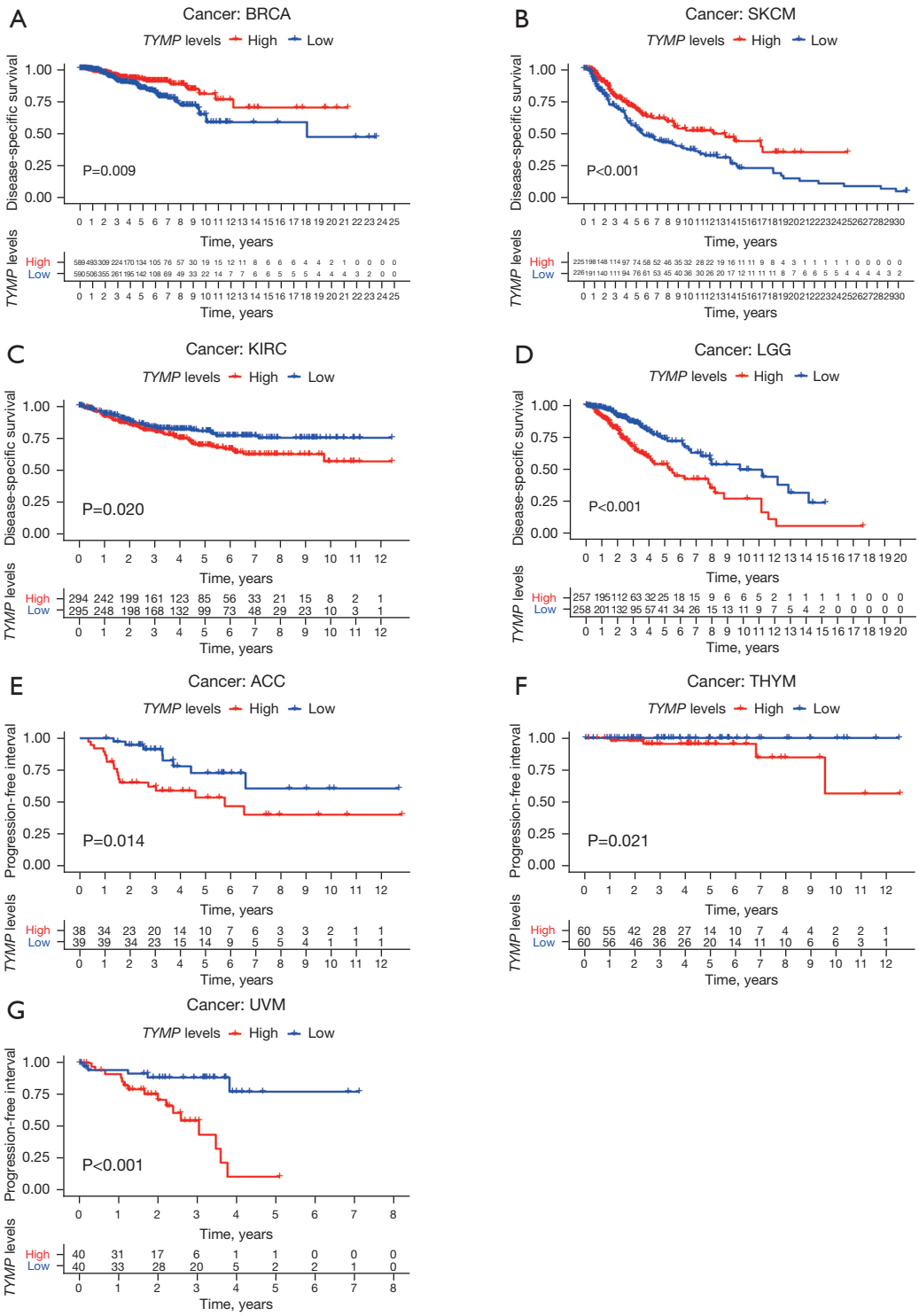


Figure 3 Correlations of *TYMP* expression and DSS determined by Kaplan-Meier analysis using R software. (A-G) Kaplan-Meier survival analysis for DSS and *TYMP* expression. The P value of each tumor: BRCA: P=0.009; SKCM: P<0.001; KIRC: P=0.020; LGG: P<0.001; ACC: P=0.014; THYM: P=0.021; UVM: P<0.001. BRCA, breast cancer; SKCM, skin cutaneous melanoma; KIRC, kidney clear cell carcinoma; LGG, lower grade glioma; ACC, adrenocortical cancer; THYM, thymoma; UVM, uveal melanomas; DSS, disease-specific survival.

significantly related to shorter DSS (*Figure 3*). In addition, estimates indicated a negative association between TYMP expression and DFI in LUAD (P=0.045) and PRAD (P=0.003) (*Figure 4A*,*4B*). An analysis of TYMP and PFI showed that in patients with ACC (P=0.032), GBM (P=0.022), LGG (P<0.001), KIRC (P=0.020), PRAD (P=0.017) and UVM (P=0.005), high *TYMP* expression had poor PFI (*Figure 4C-4H*). Collectively, these results imply that upregulation of the *TYMP* gene in multiple but not all cancers predicts a poor prognosis.

TYMP mutations in tumors

Mutations of the *TYMP* gene in different cancers were investigated further in the TCGA. Ovarian serous cystadenocarcinoma had the highest frequency of TYMP mutations (>10%), which was linked to "deep deletion" (*Figure 5A*). Not only in ovarian serous cystadenocarcinoma, but also in diffuse large B-cell lymphoma, UCEC, stomach adenocarcinoma, bladder urothelial carcinoma, LUAD, sarcoma (SARC), LUSC, and others, the most common type of CNA was "deep deletion". *Figure 5B* depicts the types, sites, and numbers of cases with *TYMP* genetic alterations. However, *TYMP* mutations had no significant influence on OS and DSS, but there were some statistically significant differences of DFS and PFS between patients with tumors harboring *TYMP* gene mutants versus wild-type *TYMP* (*Figure 5C*).

Associations between TYMP expression levels and clinicopathology, TMB, and MSI in pan-cancer

We subsequently examined *TYMP* expression among tumor at different stages. *TYMP* expression was linked to clinical tumor stage in 12 different cancers, as shown in *Figure 6A*. We also found that changes in *TYMP* expression were not consistent across tumor stages in different tumors. Notably, *TYMP* expression increased from early stages (stage I/II) to advanced stages (stage III/IV) in ACC, BLCA, KIRC, and STAD, as shown in *Figure 6A* (P<0.05). For instance, *TYMP* expression levels in ACC at stage IV were higher than at stage II. Conversely, *TYMP* levels decreased from early stages (stage I/II) to advanced stages (stage III/IV) in KIRP, LUAD, and testicular cancer (TGCT) (P<0.05). For example, *TYMP* expression was generally higher in stage II LUAD than in stage III LUAD. We also probed any possible links between *TYMP* expression and TMB and MSI in these tumor samples. *TYMP* expression was significantly correlated with TMB in 15 tumors such as BRCA, COAD, ESCA, and KIRC (*Figure 6B*) and was also closely linked with MSI in 10 tumors, such as THCA, TGCT, READ, and PAAD (*Figure 6C*).

Associations between TYMP expression and the TME

The TME is composed of tumor cells, immune cells and stromal cells. Its constitution affects therapeutic responses and clinical outcomes (24). Hence, in the present study, we analyzed relationships between immune and stromal scores, and *TYMP* expression. Using the ESTIMATE algorithm, scores for stromal and immune cells were calculated for 33 tumor types, and correlations between them and *TYMP* expression were analyzed. In KICH, OV, PCPG, SARC, and TGCT, *TYMP* and immune scores were positively correlated according to this analysis. The most significant associations between TYMP expression and stromal scores were observed in GBM, KICH, LGG, PCPG, and UVM. *Figure 7* displays the results for the top 5 tumor types with the highest correlation coefficients, and results of the remaining cancers can be found in Figures S1,S2.

Associations between TYMP expression and immune cell infiltration and immune checkpoint gene (ICG) expression

We explored *TYMP* expression in relation to the degree of infiltration of 22 immune cell types. *TYMP* expression was closely linked with the levels of immune cell infiltration in numerous malignancies according to this analysis (Table S1). As presented in *Table 1*, we selected five tumors that had the most meaningful correlation between *TYMP* expression and infiltrating immune cells for the next analysis, namely BRCA (n=13), CESC (n=13), KIRC (n=13), SKCM (n=14), and STAD (n=13). In these five tumors, infiltrating CD8 T cells, activated CD4 memory T cells, activated NK cells, and M1 macrophages all had a substantial positive correlation with *TYMP* expression, while *TYMP* expression was found to be inversely linked with infiltrating resting CD4 memory T cells. There was also no link between naïve CD4 T cell infiltration and *TYMP* expression. *TYMP* expression was also linked to a variety of macrophage subgroups. For instance, infiltrating M1 macrophages were positively associated with *TYMP* expression in these five tumors, but infiltrating M0 macrophages (except in BRCA) and infiltrating M2 macrophages (except in CESC and STAD) were negatively correlated with *TYMP* expression. Additionally, differential associations between

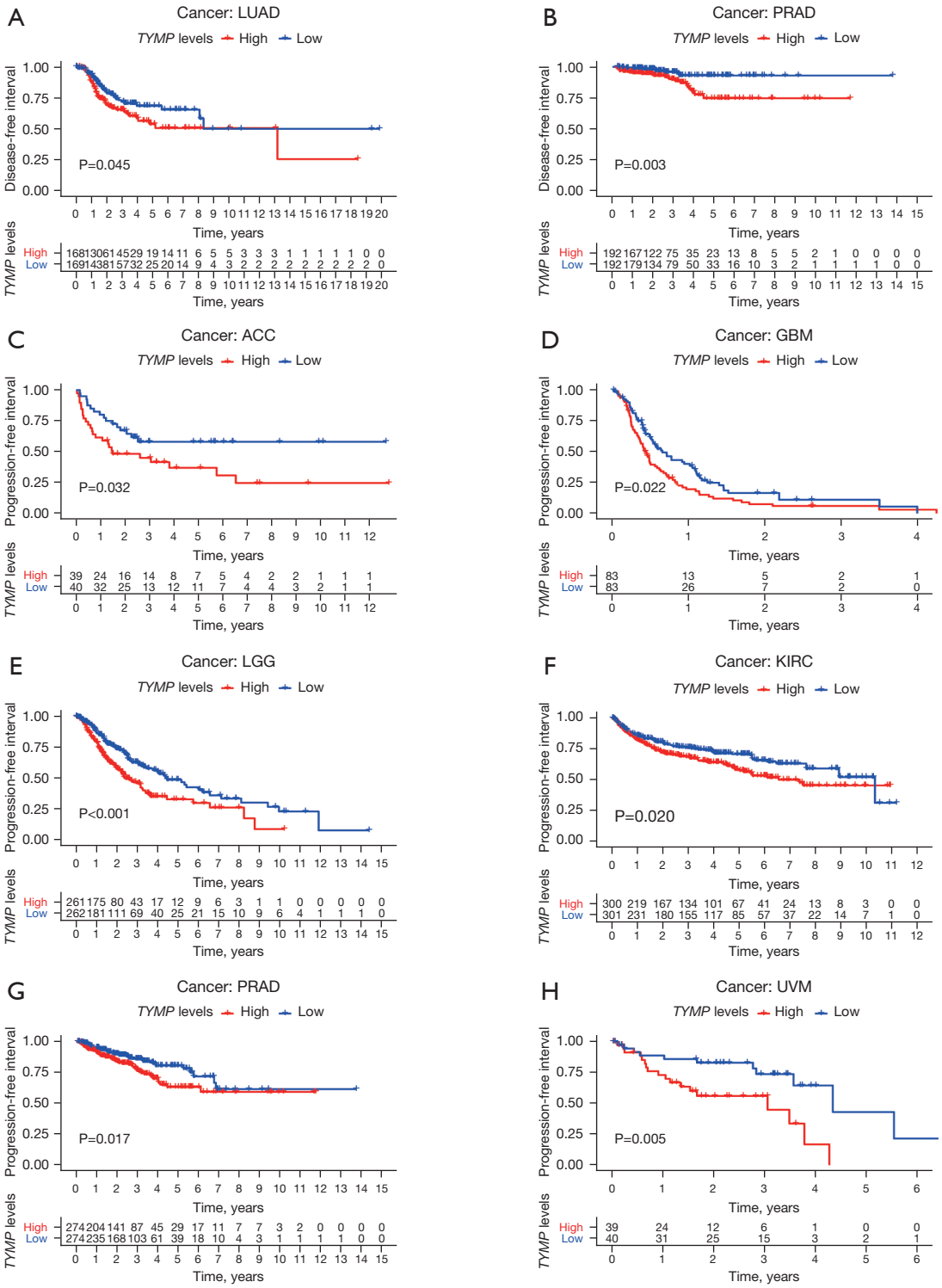


Figure 4 Correlations between *TYMP* expression and DFI and PFI by Kaplan-Meier analysis using R software. (A,B) Kaplan-Meier survival analysis for DFI and *TYMP* expression. (C-H) Kaplan-Meier survival analysis for PFI and expression. The P value of each tumor: LUAD: P=0.045; PRAD: P=0.003; ACC: P=0.032; GBM: P=0.022; LGG: P<0.001; KIRC: P=0.020; PRAD: P=0.017; UVM: P=0.005. LUAD, lung adenocarcinoma; PRAD, prostate adenocarcinoma; ACC, adrenocortical cancer; GBM, glioblastoma; LGG, lower grade glioma; KIRC, kidney clear cell carcinoma; UVM, uveal melanomas; DFI, disease-free interval; PFI, progression-free interval.

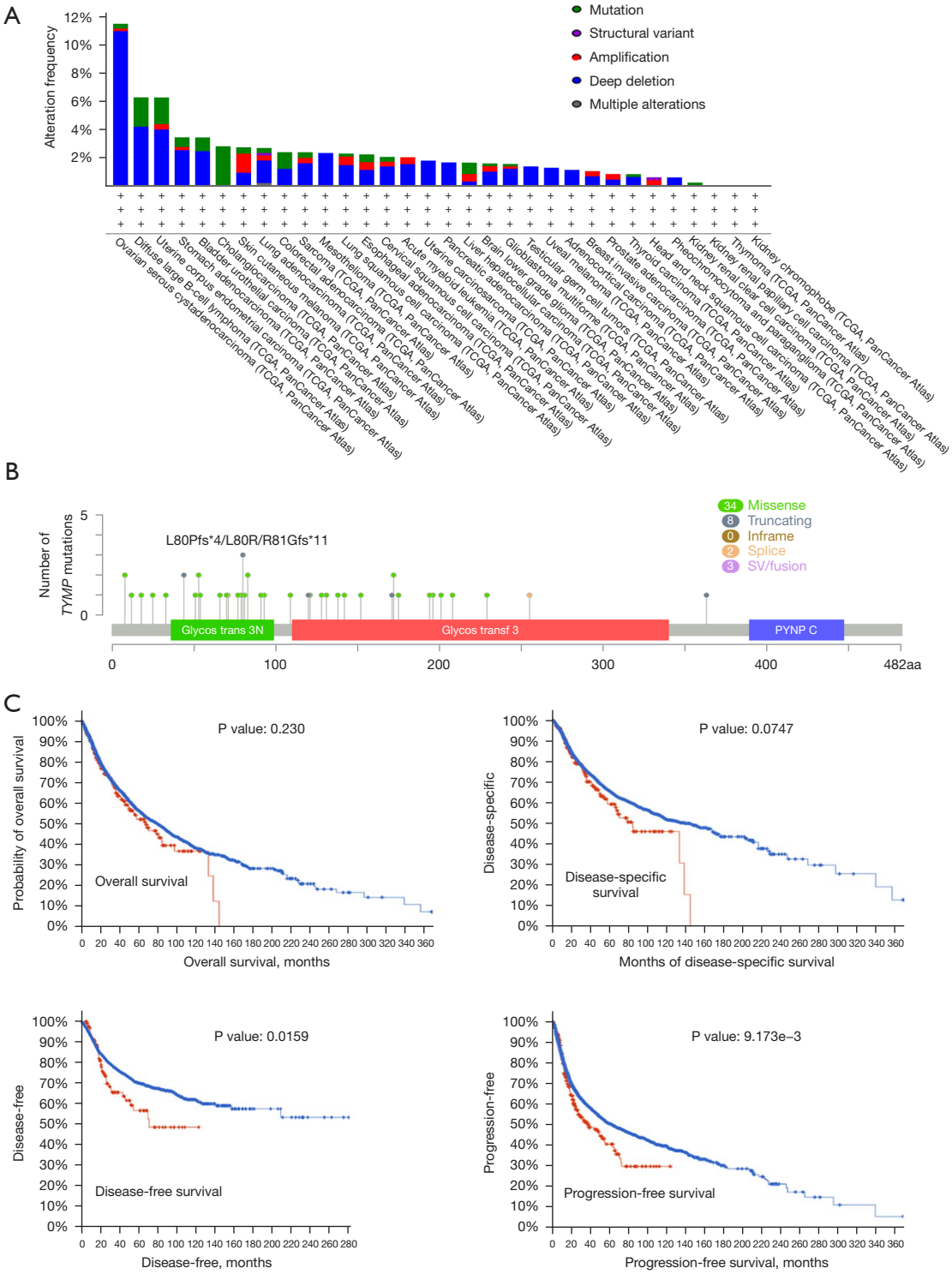
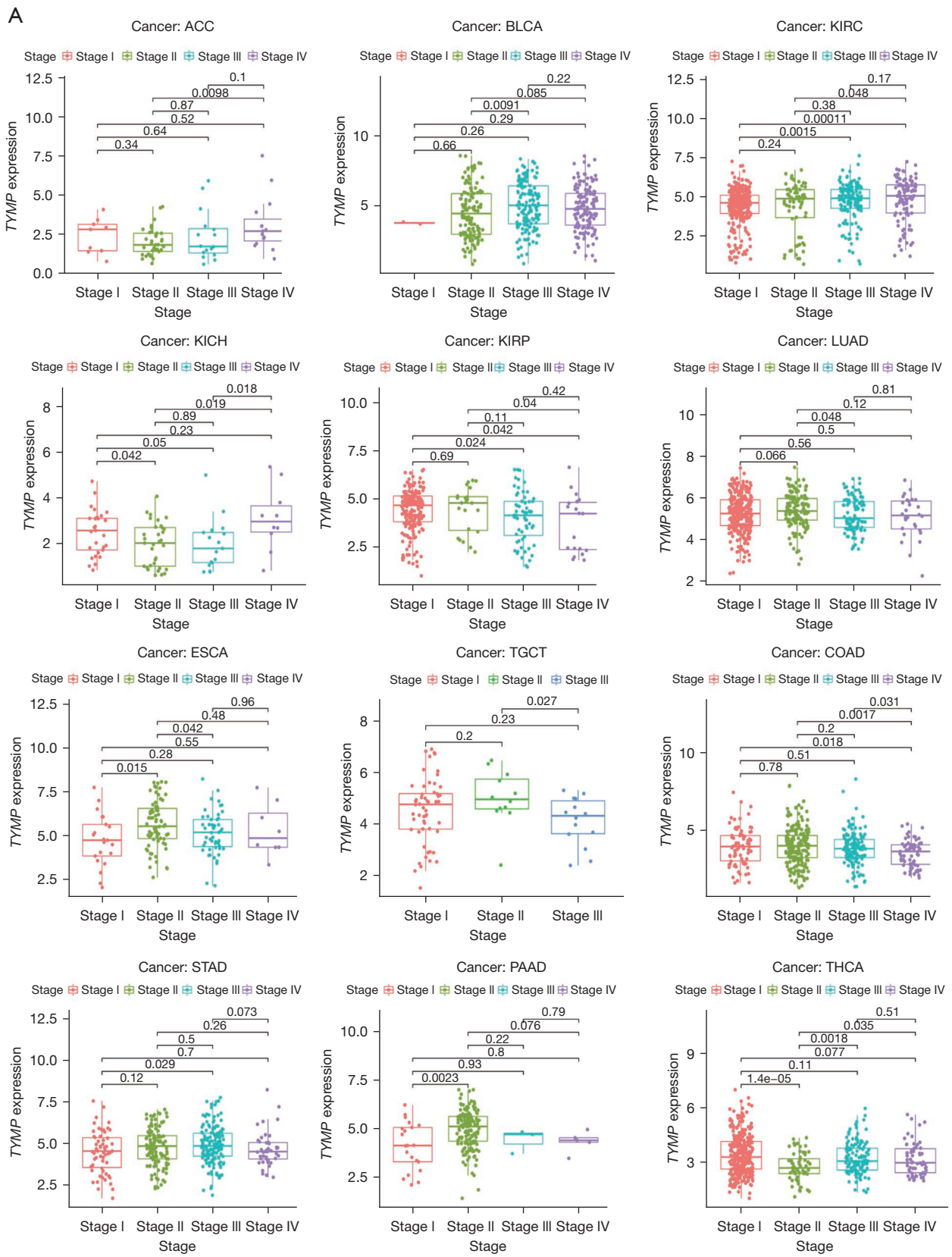


Figure 5 Genetic mutations of *TYMP* and survival across different cancers as assessed by cBioPortal tool analysis. (A) Frequency of *TYMP* mutations in different tumor types. (B) Types, sites and number of case with *TYMP* genetic alterations in pan-cancer from cBioPortal. (C) Kaplan-Meier curves of OS, DSS, DFS, and PFS in cancers with genetic alterations in *TYMP*. TCGA, The Cancer Genome Atlas; OS, overall survival; DSS, disease-specific survival; DFS, disease-free survival; PFS, progress-free survival.



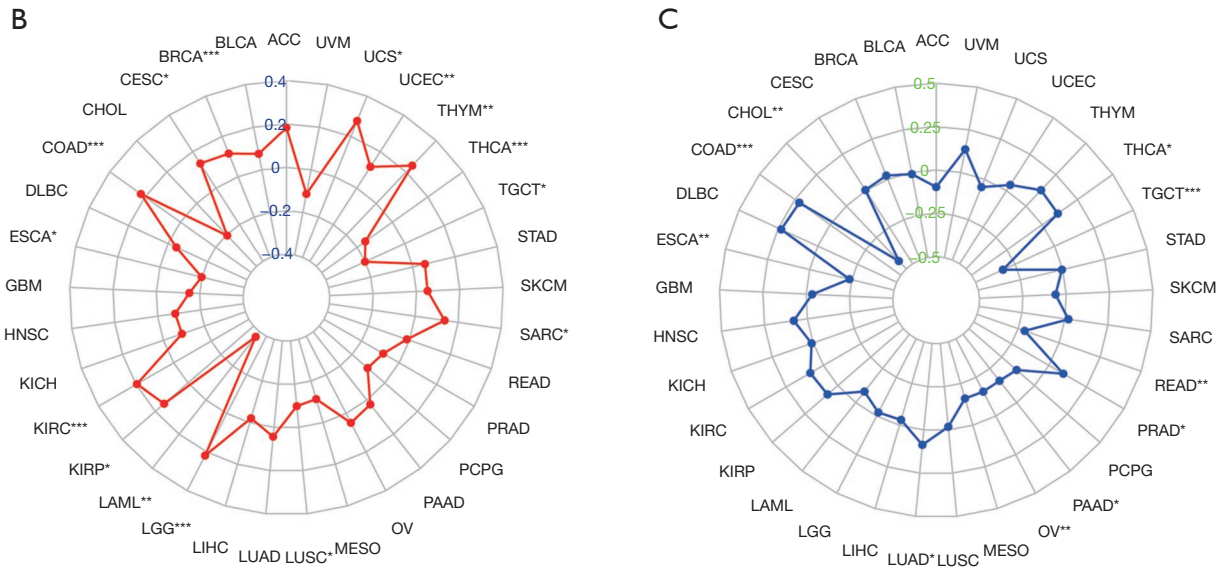


Figure 6 Relationship between *TYMP* expression and tumor stage in cancer patients based on TCGA represented by stage plots (A), relationship between *TYMP* expression TMB (B) in cancer patients based on TCGA represented by radar maps (*P<0.05; **P<0.01; ***P<0.001; meaningful P values in cancers, BRCA: P=0.0003, CESC: P=0.023; COAD: P=6.99372605004755e-06; ESCA: P=0.012; KIRC: P=0.0003; KIRP: P=0.0147; LAML: P=0.0028; LGG: P=7.80820021923056e-07; LUSC: P=0.0308; SARC: P=0.036; TGCT: P=0.0150; THCA: P=0.0007; THYM: P=0.0090; UCEC: P=0.0076; UCS: P=0.0386). Relationship between TYMP expression MSI (C) in cancer patients based on TCGA represented by radar maps. (*P<0.05; **P<0.01; ***P<0.001; meaningful P values in cancers, CHOL: P=0.0080; COAD: P=5.22958938728024e-06; ESCA: P=0.0027; LUAD: P=0.0498; OV: P=0.0092; PAAD: P=0.0346; PRAD: P=0.0326; READ; P=0.0091; TGCT: P=5.22958938728024e-06; THCA: P=0.0140). ACC, adrenocortical cancer; BLCA, bladder cancer; KIRC, kidney clear cell carcinoma; KICH, kidney chromophobe; KIRP, kidney papillary cell carcinoma; LUAD, lung adenocarcinoma; ESCA, esophageal cancer; TGCT, testicular cancer; COAD, colon cancer; STAD, stomach adenocarcinoma; PAAD, pancreatic cancer; THCA, thyroid cancer; BRCA, breast cancer; CESC, cervical cancer; CHOL, bile duct cancer; DLBC, large B-cell lymphoma; GBM, glioblastoma; HNSC, head and neck cancer; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver cancer; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal cancer; SARC, sarcoma; SKCM, skin cutaneous melanoma; THYM, thymoma; UCEC, endometrioid cancer; UCS, uterine carcinosarcoma; UVM, uveal melanomas; TCGA, The Cancer Genome Atlas; TMB, tumor mutational burden; MSI, microsatellite instability.

TYMP expression levels and distinct subgroups of tumor-infiltrating NK cells were also observed. The level of infiltrating activated NK cells was strongly associated with *TYMP* expression. Except in patients with BRCA, *TYMP* expression was found to be negatively correlated with invading resting NK cells. Finally, co-expression analysis was conducted to explore the relationships between *TYMP* expression and ICGs across 33 tumors. As illustrated in *Figure 8*, we assessed 47 ICGs and found that among all the significant associations, *TYMP* expression was positively correlated with *CTLA4*, *CD200R1*, *HAVCR2*, *PDCD1LG2*, *VSIR*, *CD86*, and *TNFRSF9* in multiple cancer types, but negatively associated with *VTCN1* and *CD200* in multiple cancers.

Associations of TYMP with drug sensitivity in pan-cancer

A Pearson correlation analysis was performed to explore relationships between *TYMP* expression in the NCI-60 tumor cell lines and susceptibility to 263 antitumor drugs, in order to evaluate potential drug responsiveness. As shown in *Figure 9*, displaying all significant results, we found that *TYMP* expression correlated positively with susceptibility to 8 antitumor drugs, namely SCH-900776, EPZ-015666, alectinib, VE-822, LDK-378, vismodegib, CCT-251545, and itraconazole. However, there was a significant negative association with sensitivity to ARQ-087. Taken together, these findings suggest that *TYMP* could be useful for predicting drug response.

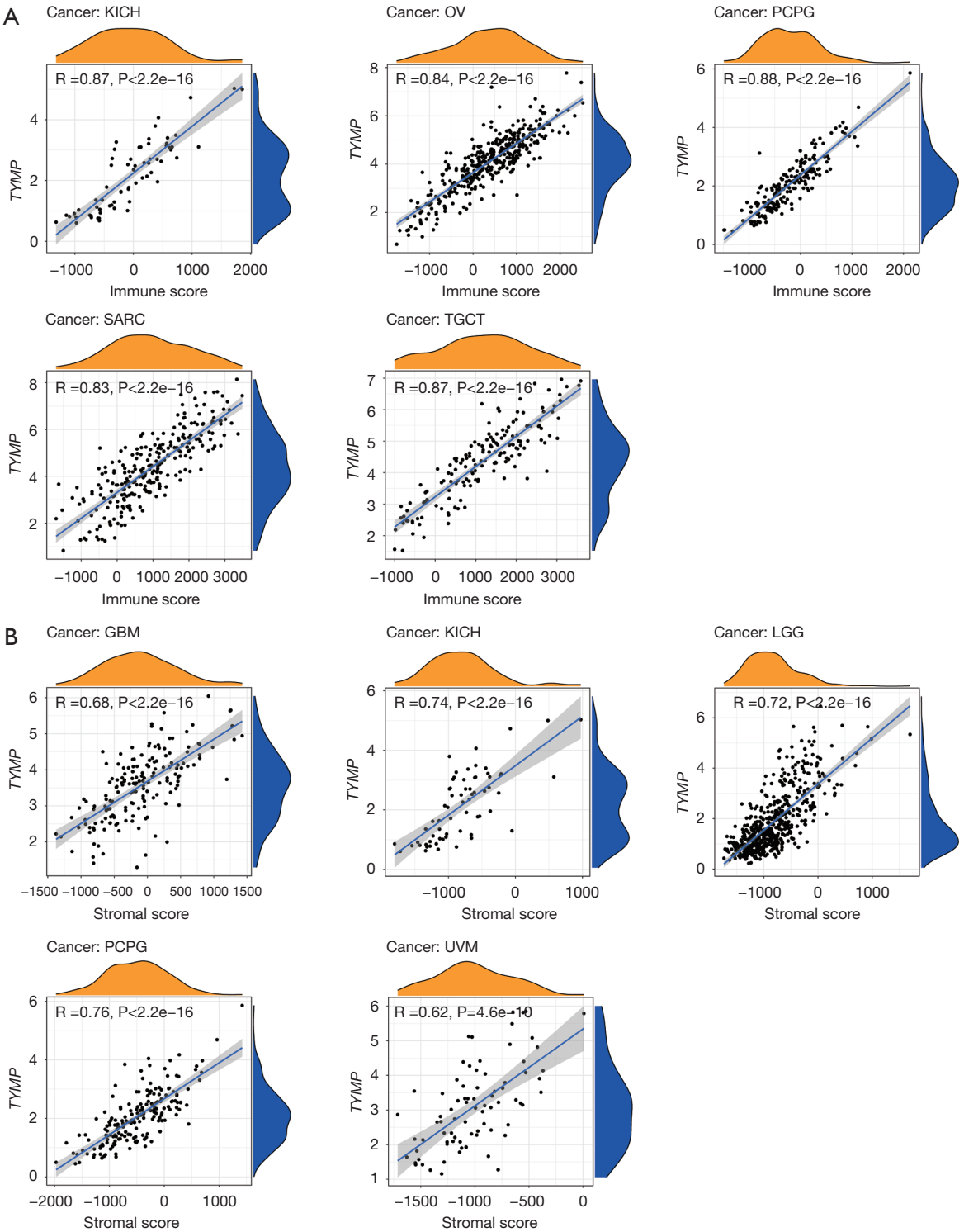


Figure 7 Relationships between *TYMP* expression and the tumor immune microenvironment. The top 5 tumors with the highest immune cell scores or stromal cell scores calculated by the ESTIMATE algorithm method. (A) Correlations of *TYMP* expression and immune cell scores in KICH, OV, PCPG, SARC, and TGCT. (B) Correlations of TYMP expression and stromal cell scores in GBM, KICH, LGG, PCPG, and UVM. KICH, kidney renal clear cell carcinoma; OV, ovarian cancer; PCPG, pheochromocytoma and paraganglioma; SARC, sarcoma; TGCT, testicular; GBM, glioblastoma; LGG, lower grade glioma; UVM, uveal melanomas.

Table 1 Association between *TYMP* expression and immune cell infiltration in human cancers

Cell type	BRCA (P value/Cor)	CESC (P value/Cor)	KIRC (P value/Cor)	SKCM (P value/Cor)	STAD (P value/Cor)
Naïve B cells	***/–0.10	***/–0.26	***/–0.22	0.012	***/–0.18
Memory B cells	***/0.17	**/0.17	***/0.17	0.047	*/0.11
Plasma cells	0.053	–0.065	***/0.24	**/0.15	**/0.16
CD8 T cells	***/0.26	***/0.22	***/0.45	***/0.43	***/0.3
Naïve CD4 T cells	0	0	0	0	0
Resting CD4 memory T cells	***/–0.31	***/–0.31	***/–0.32	***/–0.29	***/–0.48
Activated CD4 memory T cells	***/0.24	***/0.24	***/0.23	***/0.32	***/0.31
Follicular T helper cells	**/0.081	***/0.21	***/0.37	0.076	***/0.26
Regulatory T cells	***/0.22	0.027	***/0.36	***/0.26	**/0.14
Gamma delta T cells	0.019	–0.000007	0.015	**/–0.14	0
Resting NK cells	0	–0.058	***/–0.27	*/–0.12	–0.084
Activated NK cells	***/0.34	***/0.21	***/0.29	***/0.27	***/0.27
Monocytes	**/0.09	0.095	0.014	*/0.12	0.074
M0 macrophages	0.034	***/–0.20	–0.069	***/–0.21	–0.044
M1 macrophages	***/0.20	***/0.40	***/0.16	**/0.15	***/0.27
M2 macrophages	***/–0.13	***/0.22	***/–0.39	***/–0.29	0.054
Resting dendritic cells	–0.043	**/0.18	–0.02	0.059	–0.026
Activated dendritic cells	***/0.13	–0.011	–0.029	0.061	***/0.27
Resting mast cells	***/–0.12	*/0.13	***/–0.34	*/–0.11	0.047
Activated mast cells	0	*/–0.13	0	–0.08	***/–0.19
Eosinophils	0	0	0	0	*/–0.13
Neutrophils	0.0087	–0.04	–0.038	*/0.12	0.087

*P<0.05; **P<0.01; ***P<0.001. BRCA, breast cancer; CESC, cervical cancer; KIRC, kidney clear cell carcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma.

GSEA

Finally, a GSEA analysis of *TYMP* expression in 33 types of tumor tissues was performed to determine its biological role. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to explore the functional equivalents of high or low *TYMP* expression in cancers. *Figure 10* depicts the major pathways identified by GO and KEGG analyses for eight different cancer types. GO enrichment terms revealed that *TYMP* positively regulated biological processes including immune response regulating signaling pathways and leukocyte migration in ACC, GBM, KICH, and LGG. *TYMP* expression was mainly associated with immunity-related activities in PRAD, as shown in *Figure 10A*. Additionally, *TYMP* was associated with intermediate filament, intermediate filament cytoskeleton, RNA polymerase binding, and RNA polymerase core enzyme binding in LIHC (*Figure 10A*). At the same time, KEGG enrichment terms showed that *TYMP* expression was mainly connected to immune-related pathways, metabolic-related activities, and tumor biological activity. Cytokine receptor interactions and chemokine signaling pathways were positively regulated by *TYMP* in ACC, LIHC, KICH, and GBM, and *TYMP* was involved in the signaling pathways of T cell receptors in ACC, LGG, and PCPG, *TYMP* also participated in the B cell receptor signaling pathway in PCPG. However, *TYMP* expression

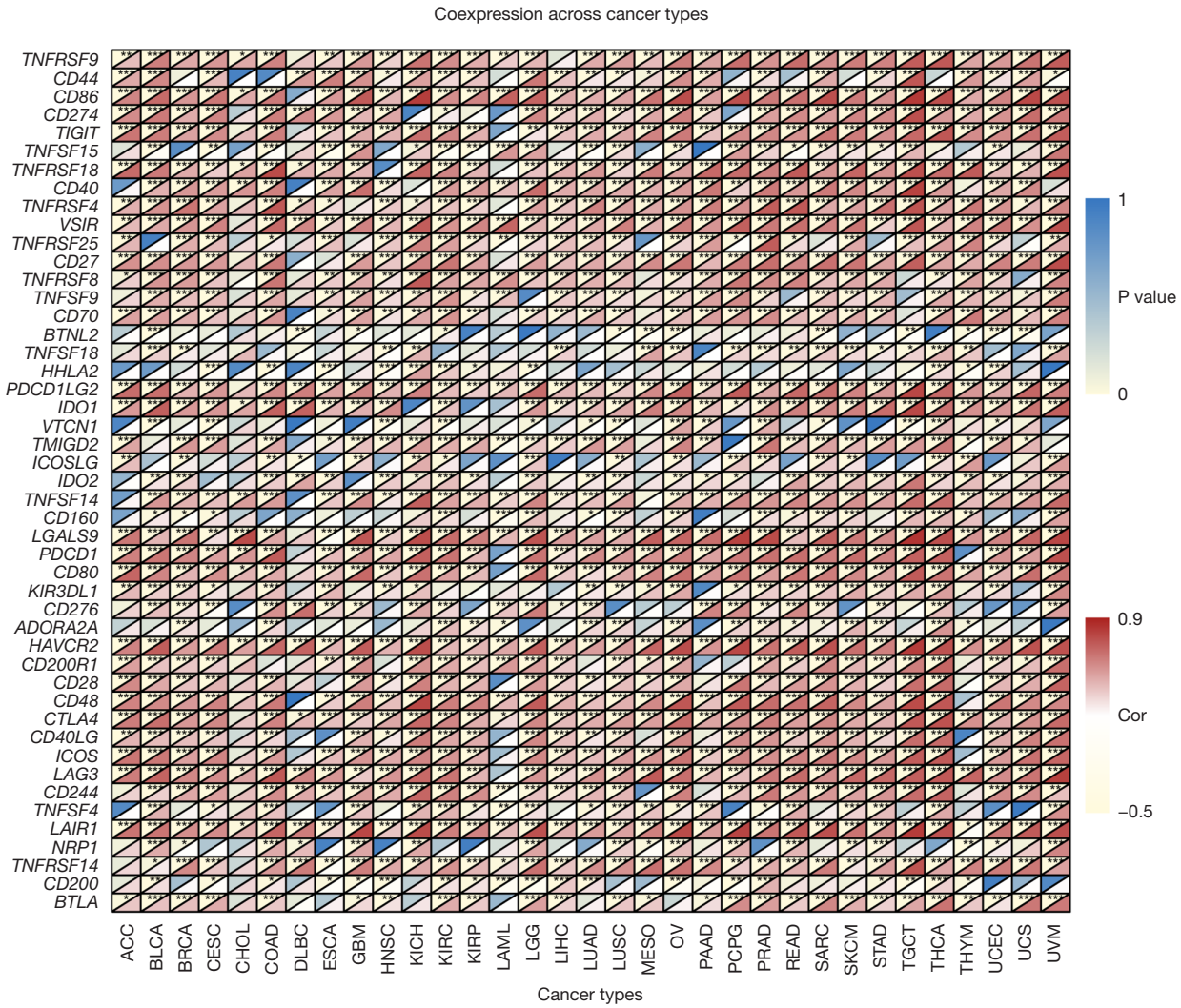


Figure 8 Relationship between *TYMP* expression and 47 ICGs depicted in a heatmap. *P<0.05; **P<0.01; ***P<0.001. ACC, adrenocortical cancer; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical cancer; CHOL, bile duct cancer; COAD, colon cancer; DLBC, large B-cell lymphoma; ESCA, esophageal cancer; GBM, glioblastoma; HNSC, head and neck cancer; KICH, kidney chromophobe, KIRC, kidney clear cell carcinoma; KIRP, kidney papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic cancer; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal cancer; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular cancer; THCA, thyroid cancer; THYM, thymoma; UCEC, endometrioid cancer; UCS, uterine carcinosarcoma; UVM, uveal melanomas; ICGs, immune checkpoint genes.

was negatively correlated with several drug metabolic enzymes in HNSC. In parallel, *TYMP* expression was positively correlated with the JAK/STAT signaling pathway in LGG and PRAD, and in LIHC, PCPG, and KICH, *TYMP* expression was enriched in the Nod-like receptor signaling pathway (*Figure 10B*).

Discussion

Based on available studies, *TYMP* participates in the metabolism of chemotherapeutic drugs such as 5-FU and capecitabine, and is associated with chemoresistance, as well possibly assisting in predicting susceptibility to chemotherapeutic drugs (25-27). *TYMP* plays a vital role

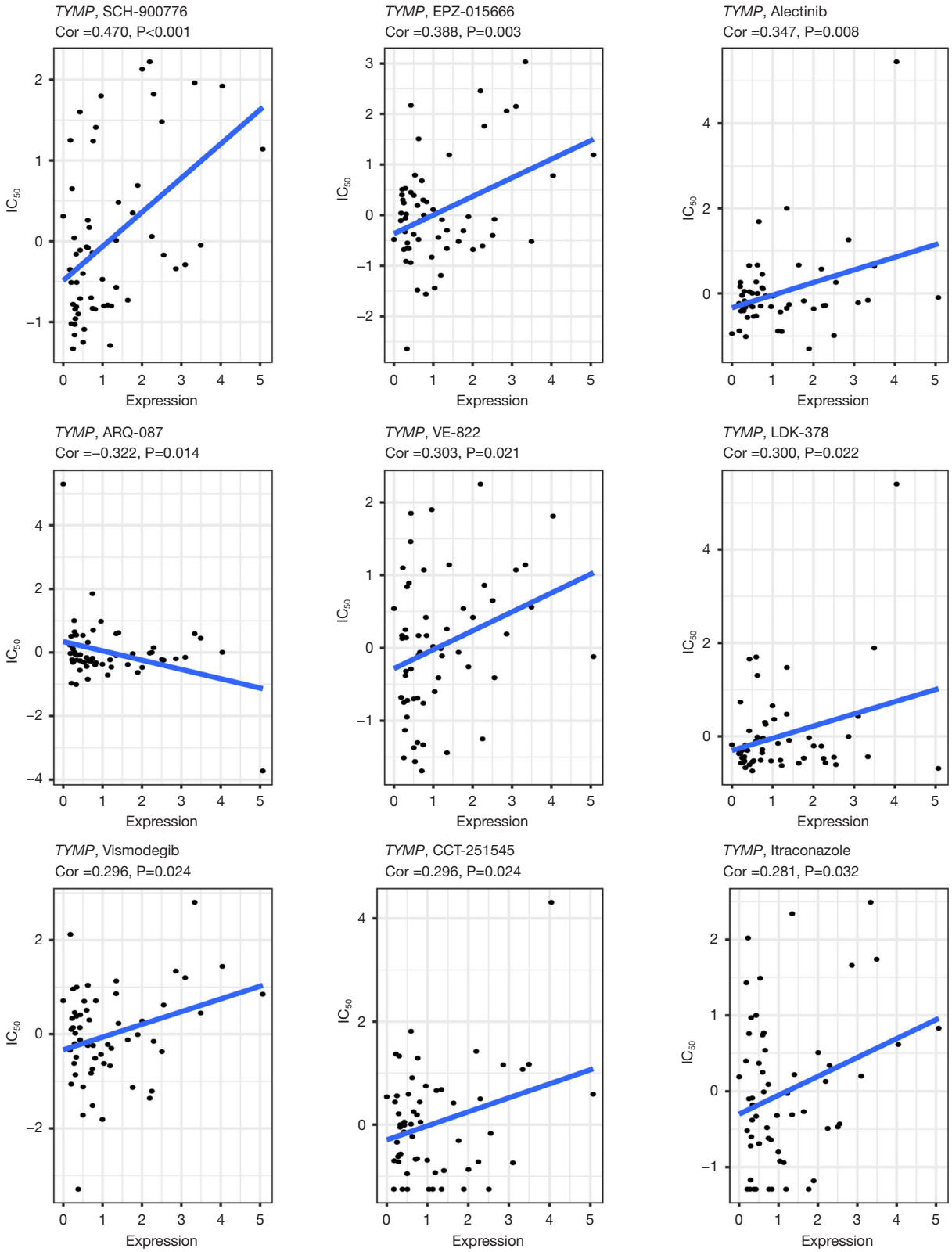


Figure 9 Associations of *TYMP* gene expression with sensitivity to chemotherapy (IC_{50}) based on the CellMiner database using Pearson correlation analysis. IC_{50}, half maximal inhibitory concentration.

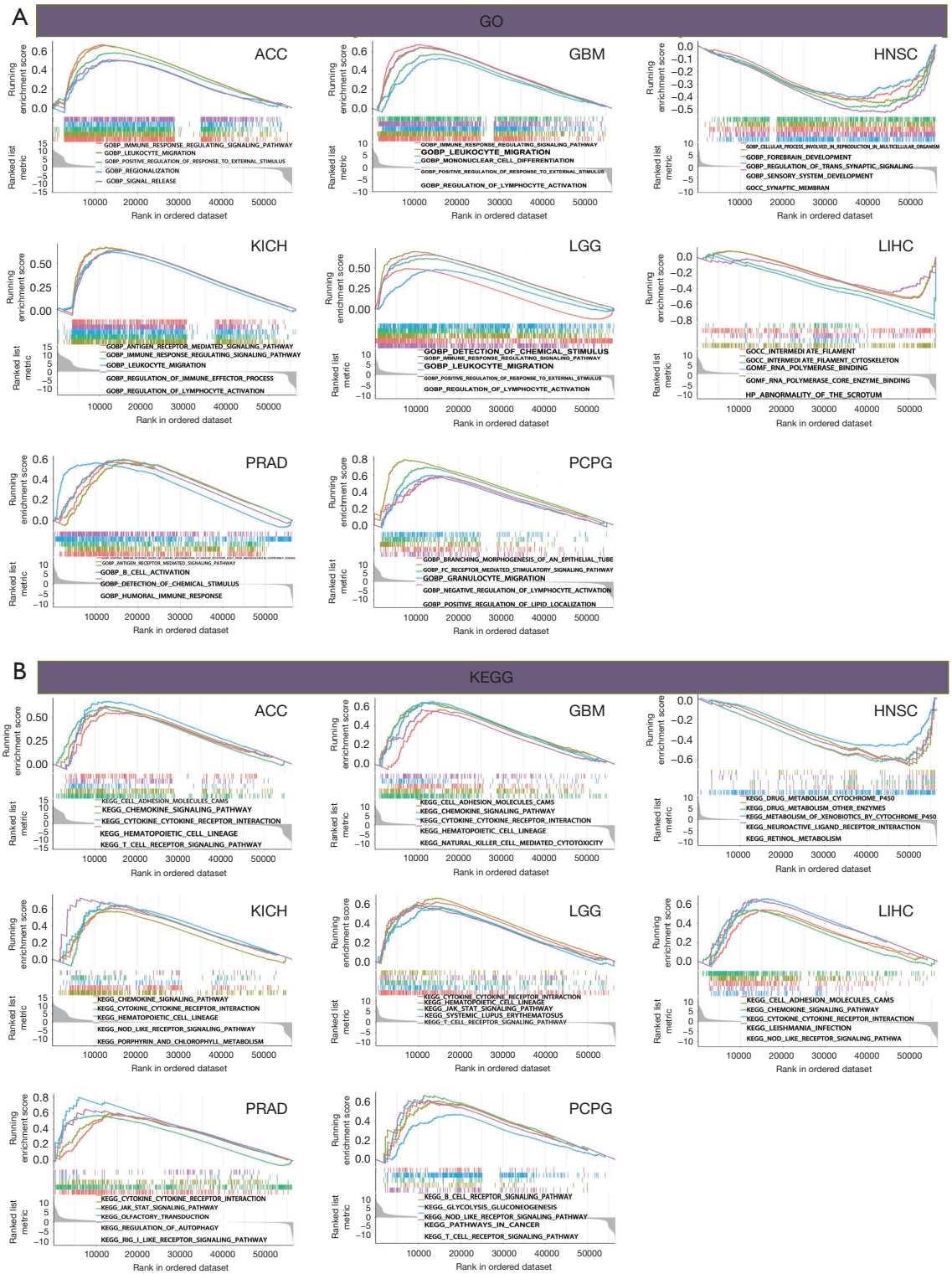


Figure 10 GSEA of *TYMP* expression. (A) The top 8 results from the GO analysis of *TYMP* in cancers. (B) The top 8 results from KEGG pathway enrichment analysis of *TYMP* in cancers. GO, Gene Ontology; ACC, adrenocortical cancer; GBM, glioblastoma; HNSC, head and neck cancer; KICH, kidney chromophobe; LGG, lower grade glioma; LIHC, liver cancer; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis.

in promoting tumor progression, and overexpression of TP enhanced viability and decreased apoptosis in human cholangiocarcinoma cell lines, as well as promoting angiogenesis in intrahepatic cholangiocarcinoma (28). Furthermore, *TYMP* itself serves as a therapeutic target in cancers and has been utilized in cancer treatment as a TP inhibitor (13). However, its connection to the tumor immune microenvironment remains unclear. Therefore, the role of *TYMP* across human cancers in a pan-cancer analysis was comprehensively explored here. We report that *TYMP* expression is significantly increased in 15 types of cancer in TCGA database, and is also highly expressed in multiple tumor cells, especially in the PRAD, cervical, pancreatic, and HNSC cells. *TYMP* expression levels were higher in BLCA, BRCA, ESCA, KIRC, and LIHC, consistent with the results of previous studies (16,29-32). *TYMP* is a major enzyme in the 5-FU pathway and is frequently mutated in gastric cancer (33). Upregulation of *TYMP*, one of the most commonly altered genes leading to 5-FU resistance, was found to be the origin of this increased vasculogenic potential (34). Our Cox proportional hazards analysis demonstrated that PAAD patients with higher *TYMP* expression had a poorer OS, while in BRCA patients it tended to be associated with a better outcome. Similarly, high expression of *TYMP* predicted a longer survival time in BRCA patients, and low *TYMP* expression a higher OS in pancreatic adenocarcinoma, although not statistically significant. These data are also similar to those reported in previous studies, and are further supported by assessments of prognostic value in our work (35,36). Moreover, prognosis is also affected by *TYMP* expression among multiple cancers reported in previous studies, including gastric carcinoma, CESC, renal cell carcinoma, and hepatocellular carcinoma (11,15,37,38). Overall, in many types of cancer, *TYMP* expression is associated with a poor prognosis. Additionally, *TYMP* expression was shown to be associated with tumor stage among 12 types of cancer. Of these, *TYMP* expression was associated with the tumor stage in most malignant tumors, with a notable difference between early and advanced cancers. In ACC, BLCA, KIRC, the three types of cancer exhibited a trend that *TYMP* expression was at a higher level in patients who were in advanced stages. In contrast, *TYMP* expression was higher in patients at the early stage of COAD, ESCA, and KIRP. This information may help to improve therapies of tumors based on the clinical characteristics of each cancer type.

The TMB represents the number of mutations identified in a tumor, with a higher TMB implying greater numbers of potential tumor neoantigens, which correlates with a better response to immune checkpoint inhibitors (39). Recent studies demonstrated that the TMB can serve as a biomarker to predict the effectiveness of immunotherapy in colorectal cancer and gastric cancer (40,41). In addition, the TMB predicts the prognosis of patients treated by immunotherapy across multiple cancer types (42). Currently, MSI also functions as a biomarker for PD1/PD-L1-blockade. A previous review contended that MSI status was helpful for deciding on cancer treatment because of its predictive value (43). Colorectal cancer patients with high MSI had a longer PFI when treated with pembrolizumab than with chemotherapy (44). *TYMP* expression was linked to the TMB in 15 tumor types and MSI in 10 tumor types, according to our findings. This implies that *TYMP* expression influences the TMB and MSI of tumors and affects how patients respond to immune checkpoint inhibitors, and may thus represent a novel prognostic biomarker for immunotherapies in many different tumors.

Features of the TME can be used to assess immune responses to cancer, and assess influence on clinical outcomes (24). Our results suggest that *TYMP* is crucial for cancer immunity. Regarding immune scores and stromal scores, positive correlations between the former and *TYMP* expression were identified in 32 tumor types, and for stromal scores in 25 tumor types. The degree of immune cell infiltration contributes to predicting cancer patient prognosis (45,46). We also examined associations between *TYMP* and immune cell infiltration in 33 different tumor types, and found that *TYMP* expression was positively associated with the presence of memory B cells and CD8 T cells. An earlier study had identified *TYMP*-positive macrophages as promoters of angiogenesis and metastasis in gastric cancer (47). In BRCA patients, *TYMP* expression in macrophages had also been linked to tumor angiogenesis and prognosis (48). Hence, we further conducted co-expression analyses of *TYMP* and ICGs, most of which were significantly related to *TYMP*. Results showed that *TYMP* expression and tumor immunity were closely associated, influencing cancer progression and the patient prognosis.

Moreover, our findings showed that *TYMP* expression was correlated to responses to a number of chemotherapeutic drugs, such as SCH-900776, EPZ-015666, and alectinib. A previous study had demonstrated that *TYMP* expression was substantially linked to the capecitabine response (26). Finally, our GSEA indicated that *TYMP* influenced many biological processes in different tumors through pathways

involving cell adhesion, immune response regulating signaling pathway, leukocyte migration, T cell, and B cell receptor pathways. Nonetheless, the molecular mechanisms of *TYMP* involvement in tumorigenesis remain poorly understood. GSEA can help uncover some important biological processes.

In sum, we conclude that the *TYMP* gene expressed at a higher level in the majority of tumors relative to the respective normal tissues. We explored correlations between *TYMP* expression, clinical prognosis and tumor stage. We suggest that *TYMP* could be employed as a prognostic marker for a range of malignancies. Moreover, in various types of cancers, *TYMP* expression has been related to TMB, MSI, and immune cell infiltration. Furthermore, *TYMP* expression is associated with the effectiveness of several chemotherapeutic drugs. These findings reveal something of *TYMP*'s function in cancer etiology, pathology and progression, and further contribute to immunotherapeutic strategies. Validation will require future experimental work.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-502/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-502/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-502/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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