

Research Article

Comprehensive Analysis of YTH Domain Family in Lung Adenocarcinoma: Expression Profile, Association with Prognostic Value, and Immune Infiltration

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Background. All YTH domain family members are m⁶A reader proteins accounting for the methylation modulation involved in the process of tumorigenesis and tumor progression. However, the expression profiles and roles of the YTH domain family in lung adenocarcinoma (LUAD) remain to be further illustrated. **Methods.** GEPIA2 and TNMplot databases were used to generate the expression profiles of the YTH family. Kaplan-Meier plotter database was employed to analysis the prognostic value of the YTH family. Coexpression profiles and genetic alterations analysis of the YTH family were undertaken using the cBioPortal database. YTH family protein-associated protein-protein interaction (PPI) network was identified by using STRING. Functional enrichment analysis was performed with the help of the WebGestalt database. The correlation analysis between the YTH family and immune cell infiltration in LUAD was administrated by using the TIMER2.0 database. **Results.** mRNA expression of YTHDC1 and YTHDC2 was significantly lower in LUAD, whereas YTHDF1, YTHDF2, and YTHDF3 with apparently higher expression. YTHDF2 expression was observed to be the highest in the nonsmoker subgroup, and its expression gradually decreased with the increased severity of smoking habit. LUAD patients with low expression of YTHDC2, YTHDF1, and YTHDF2 were correlated with a better overall survival (OS) time. The YTHDF1 genetic alteration rate was 26%, which was the highest in the YTH family. The major cancer-associated functions of YTH family pointed in the direction of immunomodulation, especially antigen processing and presentation. Most of the YTH family members were significantly correlated with the infiltration of CD4+ T cells, CD8+ T cells, macrophages, and neutrophils, indicating the deep involvement of the YTH domain family in the immune cell infiltration in LUAD. **Conclusion.** The molecular and expression profiles of the YTH family were dysregulated in LUAD. YTH family members (especially YTHDC2) were promising biomarkers and potential therapeutic targets that may bring benefit for the patients with LUAD.

1. Introduction

As the leading cause of cancer-related deaths in global, lung cancer is composed of two major subtypes lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD), with LUAD exhibits relatively higher incidence and mortality [1–3]. While tremendous efforts have been made in drug

discovery against LUAD, the clinical outcomes of most patients with LUAD remain to be poor [4]. Hence, exploring novel biomarkers and molecular targets is of great value for the development of the LUAD therapeutic strategy.

Dysregulation of RNA methylation has frequently been reported to be implicated in the initiation and progression of cancer [5–7]. N⁶-Methyladenosine (m⁶A) modification,

as the most common RNA methylation, has received intensive attention these days and is expected to be a promising therapeutic target against cancer [8]. With the function of recognizing m6A-modified mRNA and regulating the expression of target genes, the YT521-B homology (YTH) family as the major reading proteins (known as “readers”) is composed of five proteins that carry the highly conserved YTH domain in common. These proteins are further classified into three categories: YTH domain-containing 1 (YTHDC1), YTH domain-containing 2 (YTHDC2), and YTH m6A-binding protein (YTHDF) including YTHDF1-3. Accumulative studies have shown the robust association between YTH family members and various types of cancer [9]. For instance, there was proved to be a positive correlation between YTHDF1 overexpression and poor prognosis in patients with liver cancer [10]. In addition, the silence of YTHDF2 resulted in the increased invasion of tumor cells in pancreatic cancer [11]. Furthermore, it was reported that YTHDC2 could promote radiotherapy resistance of nasopharyngeal carcinoma via activation of the AKT signal pathway [12]. However, the expression signatures and function of YTH family proteins in LUAD initiation and progression is still lacking.

In the present study, we aimed to further broaden the understanding of the role of the YTH family in LUAD through various public databases, thereby providing novel insights into the diagnosis and treatment of LUAD. The expression profiles, prognostic value, and functional enrichment analysis of YTH family members in LUAD were evaluated, and the correlation of immune cell infiltration with the YTH family was also discussed.

2. Materials and Methods

2.1. GEPIA2 and TNMplot. Gene Expression Profiling Interactive Analysis (GEPIA) 2.0 and TNMplot are two databases that support comprehensive expression analyses based on TCGA and GTEx data [13–15]. The expression profiles of YTH family members in LAUD and normal lung tissue were retrieved from these two databases. A cutoff of 0.05 in p value was set as statistical significance. The relative expression of each YTH in LUAD was compared using GEPIA2. The color density of each block represents the median expression value of each YTH member in LUAD tumor tissue, normalized by the maximum median expression value across all blocks. All the databases used in our study were summarized in Supplementary Table S1.

2.2. UALCAN. UALCAN is a portal for tumor subgroup analysis of different gene expressions [16]. In this study, UALCAN database was also applied to analysis the different expressions of YTH family members based on smoking habits. A cutoff of 0.05 in p value was set as statistical significance.

2.3. Kaplan-Meier Plotter. Kaplan-Meier plotter database is used for evaluating the prognostic role of the expression level of specific gene [17]. In this study, overall survival (OS), first progression (FP), and postprogression survival (PPS) of LUAD patients with different expressions of each

YTH family member were compared, respectively, through the Kaplan-Meier plotter database. A cutoff of 0.05 in p value was set as statistical significance.

2.4. cBioPortal. cBioPortal is a database of cancer genomics which provides download and analysis of genomic alteration data of diverse types of cancer [18, 19]. A dataset of 230 patients with LUAD (TCGA, Firehose Legacy) from cBioPortal was used for the analyses of coexpression and genetic alterations of the YTH family.

2.5. STRING and Cytoscape. Probable protein-protein interactions (PPIs) among YTH family members were predicted using STRING, and a database provides an interactive network among interested proteins [20, 21]. In addition, 152 YTH-associated genes were selected from cBioPortal, then, Cytoscape was used to generate the molecular interaction networks [22].

2.6. WebGestalt. WebGestalt is a comprehensive tool online that supports gene set enrichment analysis and network topology analysis [23]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses were performed by using WebGestalt to get the enrichment pathways correlated to the YTH family in LUAD.

2.7. TIMER2.0. TIMER2.0 is a public resource that offers comprehensive analyses of immune cells infiltration in various types of cancer [24]. Here, we conduct the “immune association” module to obtain the scatterplots which show the association between the expression of YTH family proteins and different types of infiltrated immune cells (CD4+ T cells, CD8+ T cells, B cells, dendritic cells, macrophages, and neutrophils).

3. Results

3.1. Expression Profiles of YTH Domain Family in Patients with LUAD. Data returned from both GEPIA2 and TNMplot databases demonstrated that the mRNA expression of YTH domain family members in LUAD tissues was of widely divergence comparing with that in adjacent normal tissues, especially in the cases of YTHDC2 with significantly lower expression, and YTHDF1, YTHDF2, and YTHDF3 with apparently higher expression only in TNMplot database (Figures 1(a) and 1(b)). Moreover, the expression abundance of each YTH member in LUAD varied. The results showed that the relative expression of YTHDC2 was the lowest in the YTH domain family, while the expression of YTHDF3 was the highest (Figure 1(c)).

There was no significant difference in the expression of YTH family molecules in the four clinical stage subgroups of LUAD (data no shown). While intriguingly, based on the classification of the status of smoking habits (nonsmoker, smoker, reformed smoker1 (who are current reformed smokers for ≤ 15 years) and reformed smoker2 (who are current reformed smokers for > 15 years)) in LUAD patients, YTHDC2 expression was observed to be the highest in nonsmoker subgroup, and its expression

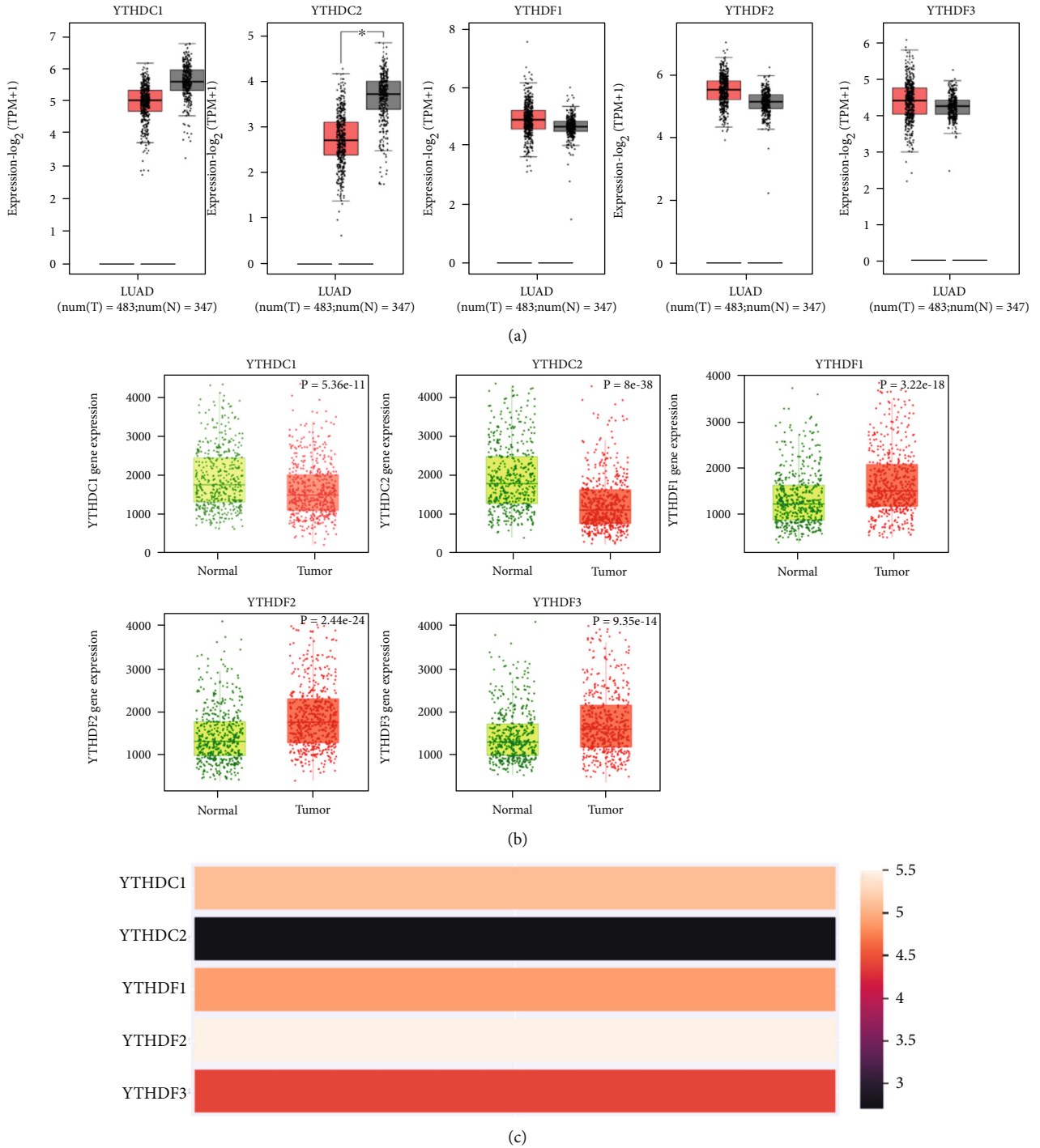


FIGURE 1: The mRNA expression levels of the YTH family in LUAD were compared with that in normal lung tissues. (a) Data were retrieved from the GEPIA2 database. (b) Data were retrieved from the TNMplot database. (c) The relative expression levels of each YTH family member in LUAD patients (GEPIA2). * $p < 0.05$.

gradually decreased with the increased severity of smoking habit, suggesting that the smoking habits might reflect the expression of YTHDC2 level, and even for the LUAD patient with smoking, smoking cessation can effectively reduce YTHDC2 expression (Figure 2). Moreover, we made the logistic regression analysis using LUAD data from TCGA, and the results showed that the relationship between

smoking and YTHDC2 expression was not statistically significant, indicating that more samples are needed to verify the relationship between them (Supplementary Table S3). Furthermore, we analyzed the prognostic effect of YTHDC2 on both smoking and nonsmoking LUAD patients and found that both smoking and nonsmoking patients with high expression of YTHDC2 showed better

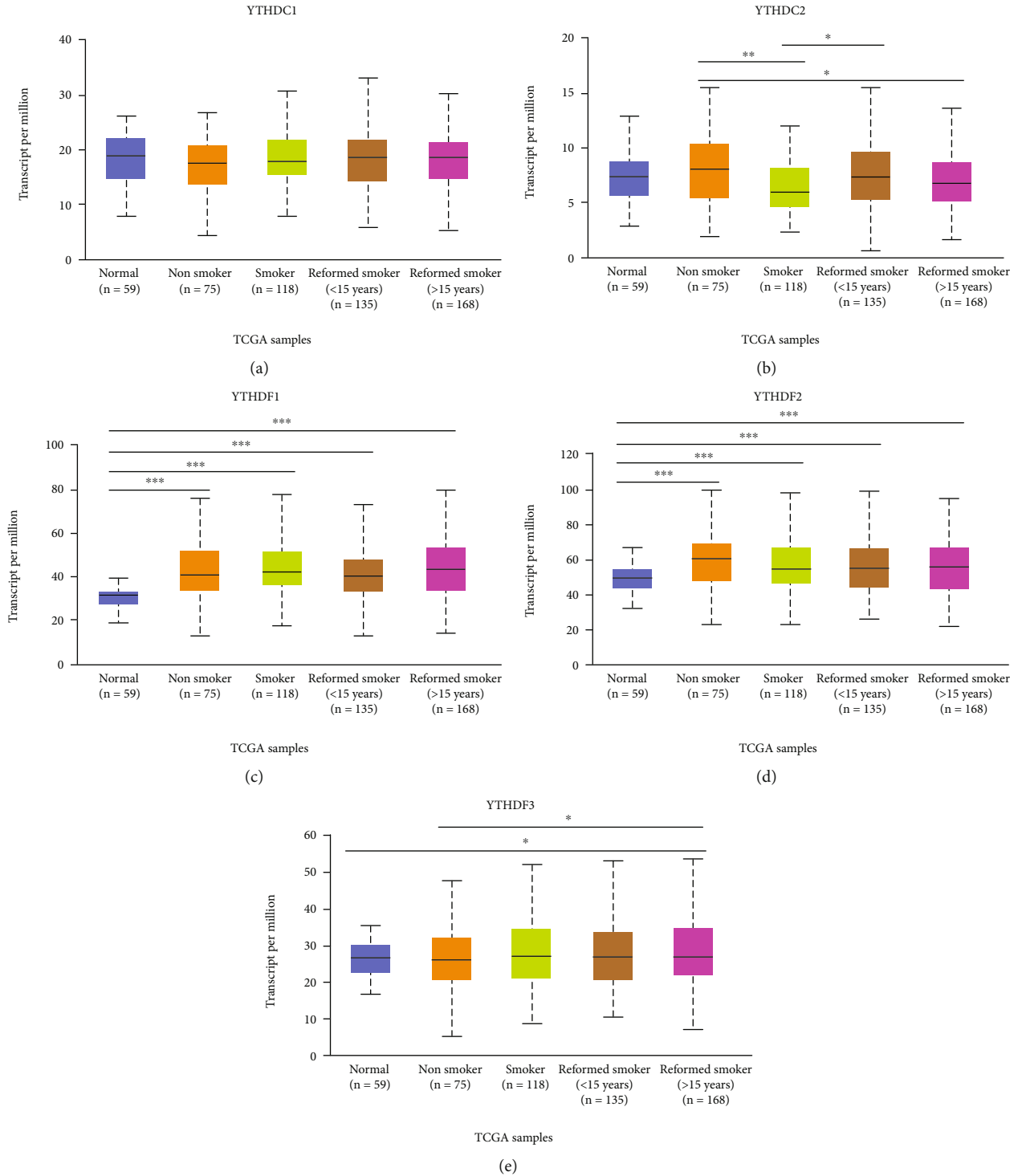


FIGURE 2: The association between the expression of YTH family members and different smoking habits from UALCAN (nonsmoker, smoker, reformed smoker). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

prognosis (HR = 0.65, $p = 0.0011$; HR = 0.19, $p = 5.8e - 08$, respectively) (Supplementary Figure S1).

3.2. YTH Domain Family in Prognosis of LUAD Patients. To assess the prognostic value of the YTH domain family in LUAD, the correlations between the expression of YTH family members and survival endpoints like overall survival

(OS), first progression (FP), and postprogression survival (PPS) were further analyzed through Kaplan-Meier plotter website. As a consequence, LUAD patients with low expression of YTHDC2 ($p < 0.001$), YTHDF1 ($p < 0.05$), and YTHDF2 ($p < 0.01$) were correlated with a better OS time (Figure 3). Similarly, low expression of YTHDC1 ($p < 0.001$), YTHDC2 ($p < 0.001$), YTHDF2 ($p < 0.001$),

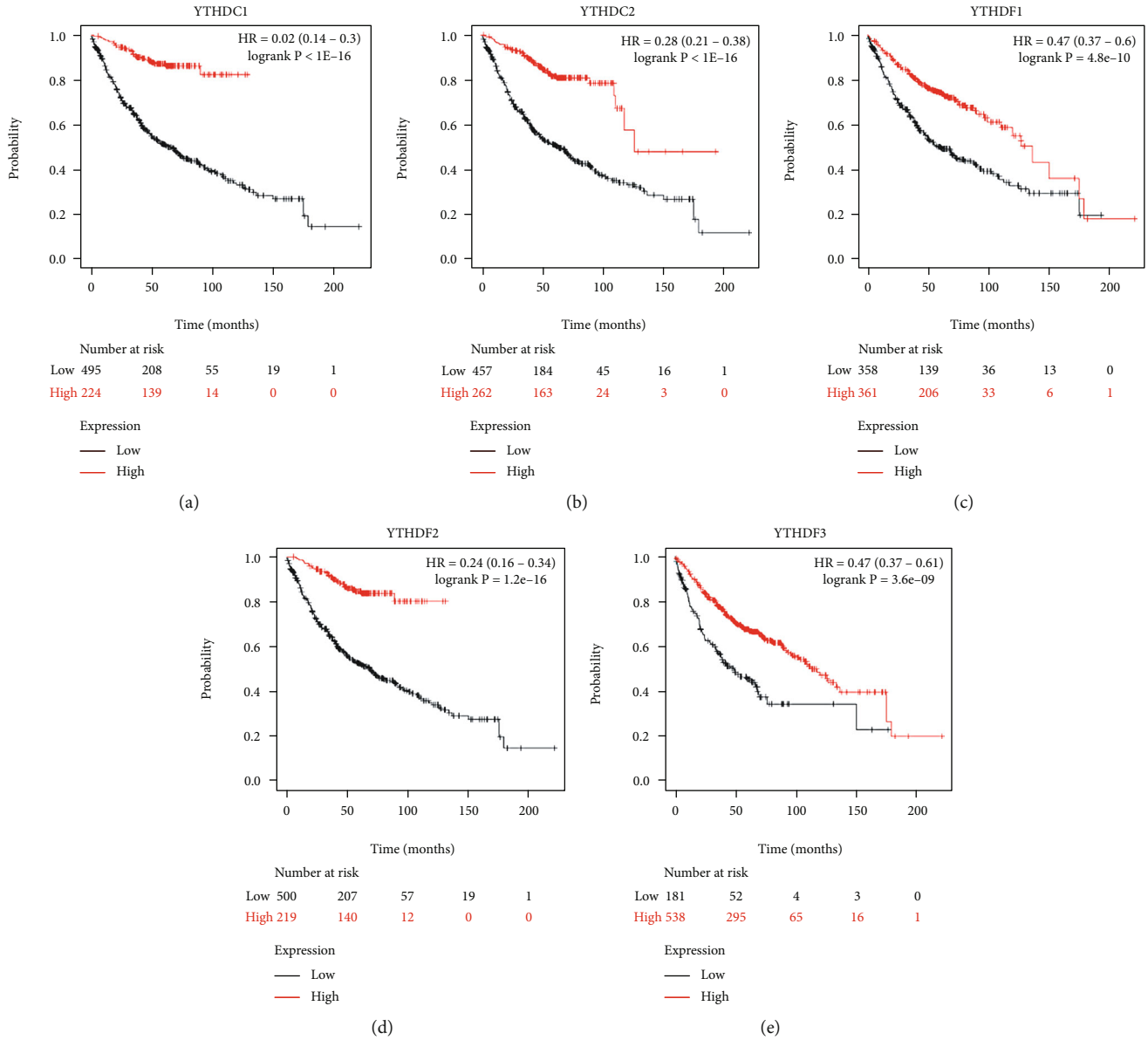


FIGURE 3: The correlations between the expression of YTH family members and overall survival (OS) of LUAD patients were analyzed through the Kaplan-Meier plotter website.

and YTHDF3 ($p < 0.05$) were associated with a better FP time (Figure 4). As for the PPS, it was YTHDC1 ($p < 0.001$), YTHDC2 ($p < 0.001$), and YTHDF2 ($p < 0.005$) that linked to a better prognosis (Figure 5).

3.3. Genetic Alterations of YTH Domain Family in LUAD. The frequency and types of genetic alterations in the YTH domain family in LUAD were obtained through the TCGA database and cBioPortal tool. As presented in Figure 6(a), the YTHDF1 genetic alteration rate was 26%, which was the highest in the YTH family. Genetic alteration rates of the other family members are 13% (for YTHDC1, YTHDC2, and YTHDF2) and 22% (YTHDF3). In the aspect of types of genetic alterations, gene amplification, missense mutation, truncating mutation, and mRNA high/low were the main genetic alterations in YTHDC1, YTHDF1, and YTHDF2.

Whereas barely missense mutation was observed in YTHDC2, and scarcely missense and truncating mutation were found in YTHDF3 (Figure 6(a)).

3.4. Interactive Network Analyses of YTH Domain Family and Associated Molecules. The interactive network of the YTH domain family generated by the STRING database demonstrated that YTHDF1, YTHDF2, and YTHDF3 were concordantly interacted with YTHDC1, revealing YTHDC1 acted as the hub node in this interactive network (Figure 6(b)). However, YTHDC2 was relatively dissociated from its family members in terms of the functional network of interactive molecules. Furthermore, 152 most frequent genes with changed expression level and concomitantly with close correlation to YTH domain family were identified from the cBioPortal database (Supplementary Table S2). Then, the

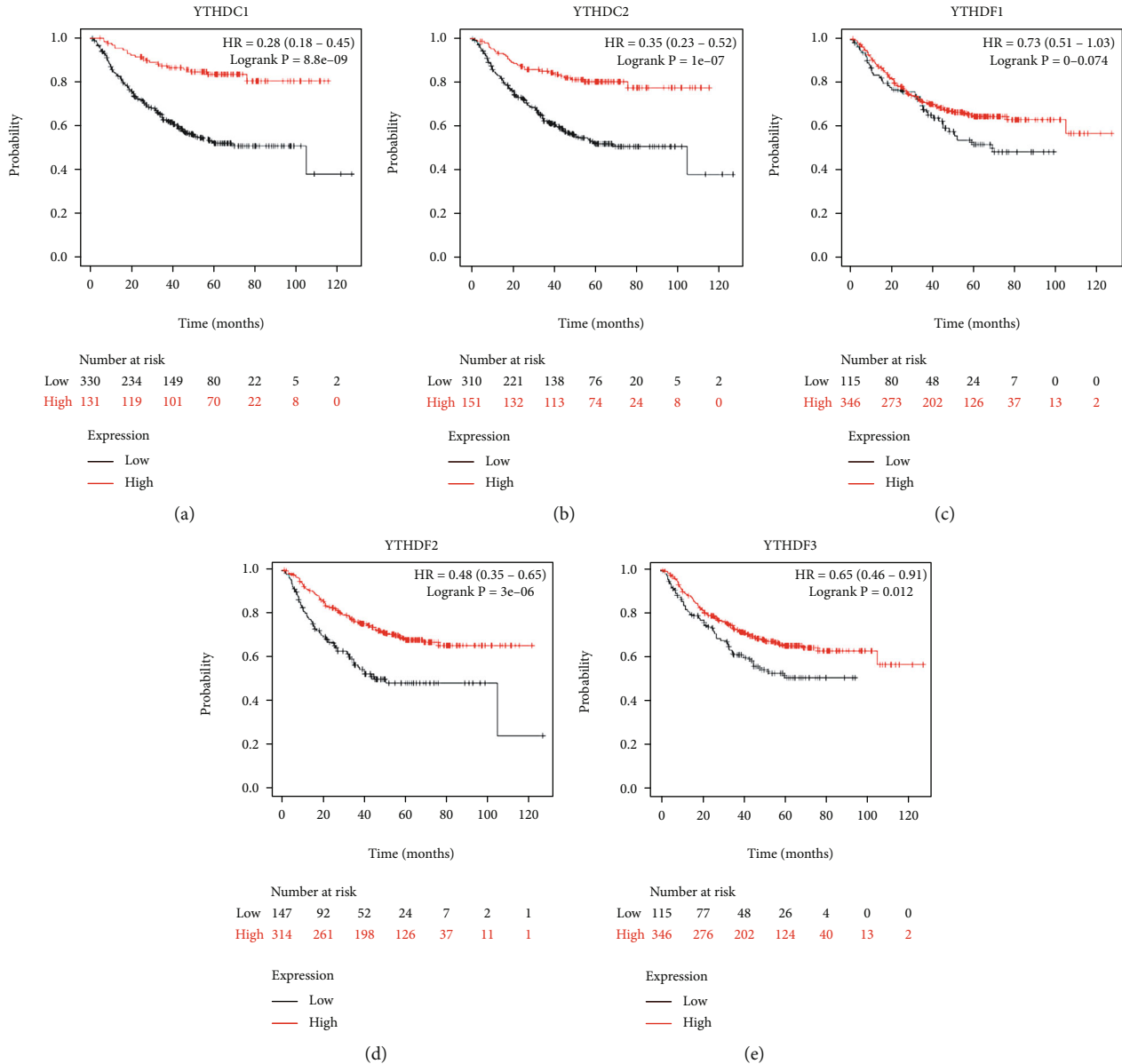


FIGURE 4: The correlations between the expression of YTH family members and first progression (FP) of LUAD patients were retrieved from the Kaplan-Meier plotter website.

interactive network of these 152 genes was established, which further suggested that molecules including MMP1, HP, MRC1, COL7A1, KRT14, ITGB4, CASR, and COL17A1 may serve as hub genes participating in the biological processes of the YTH domain family in LUAD (Figure 6(c)).

To further understand what kinds of functions are induced by the YTH domain family, the functional enrichment analysis was performed on the basis of the 152 YTH-associated genes by using the WebGestalt database. Consequently, YTH domain family members were mainly enriched in biological functions such as biological regulation, response to stimulus, metabolic process, multicellular organismal process, and developmental process. Moreover, YTH domain family members were also found to be highly enriched in the following cellular component, including membrane, vesicle, extracellular space, endomembrane

system, nucleus, protein-containing complex, and membrane-enclosed lumen, and so on. As for the enrichment of molecular function, protein binding, ion binding, hydrolase activity, nucleic acid binding, structural molecule activity, and molecular transducer activity are the top ones with the highest enrichment (Figure 7(a)). In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was used to exhibit the enrichment ratio of specific biological functions that contribute to the LUAD development. The top-ranked YTH-associated biological functions involved in the LUAD development were antigen processing and presentation, hemidesmosome assembly, formation of primary germ layer, and appendage development (Figure 7(b)).

3.5. Correlation between Immune Cell Infiltration and Each YTH Domain Family Member. Immune cell infiltration in

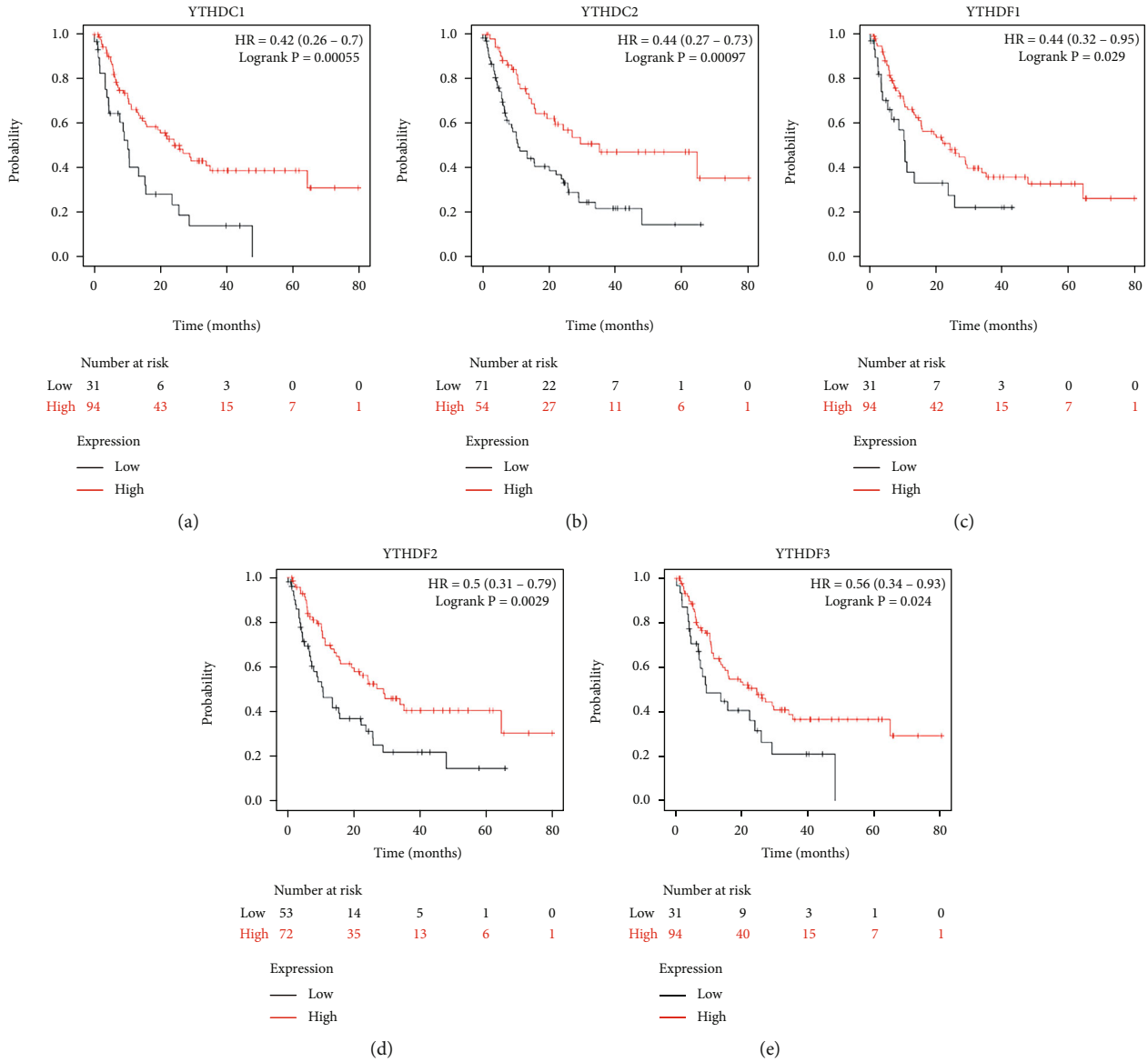


FIGURE 5: The correlations between the expression of YTH family members and postprogression survival (PPS) of LUAD patients were obtained from the Kaplan-Meier plotter website.

tumor is an indispensable component of tumor microenvironment and an independent index that reflects prognosis and lymphatic metastasis status [25–27]. Thus, we use the TIMER2.0 database to further illustrate the correlation between immune cell infiltration and each YTH domain family members. YTHDC1 expression was significantly correlated with infiltration of several types of immune cells ranging from macrophage ($Rho = 0.146, p = 1.14 \times 10^{-3}$) to neutrophil ($Rho = 0.224, p = 5.31 \times 10^{-7}$), CD4+ T cell ($Rho = 0.233, p = 1.77 \times 10^{-7}$), and CD8+ T cell ($Rho = 0.224, p = 5.32 \times 10^{-7}$) (Figure 8(a)). YTHDC2 expression was significantly associated with infiltration of macrophage ($Rho = 0.145, p = 1.25 \times 10^{-3}$), neutrophil ($Rho = 0.313, p = 1.09 \times 10^{-12}$), CD4+ T cell ($Rho = 0.235, p = 1.32 \times 10^{-7}$), CD8+ T cell ($Rho = 0.185, p = 3.69 \times 10^{-5}$), and dendritic cell ($Rho = 0.146, p = 5.22 \times 10^{-4}$)

(Figure 8(b)). In addition, in the cases of YTHDF1, YTHDF2, and YTHDF3, various immune infiltration signatures were observed to have a significant correlation (Figures 8(c)–8(e)). These data indicated the deep involvement of the YTH domain family in the immune cell infiltration in LUAD.

4. Discussion

RNA methylation is a vital posttranscriptional modification that participates in various human biological processes [28]. Recent researches on m6A RNA methylation have revealed its facilitating role in the initiation and development of various types of cancer, thus, has gradually become a new direction in oncology research and targeted drug development [29, 30]. All of the five YTH domain family members are m6A reader proteins—the key enzyme that

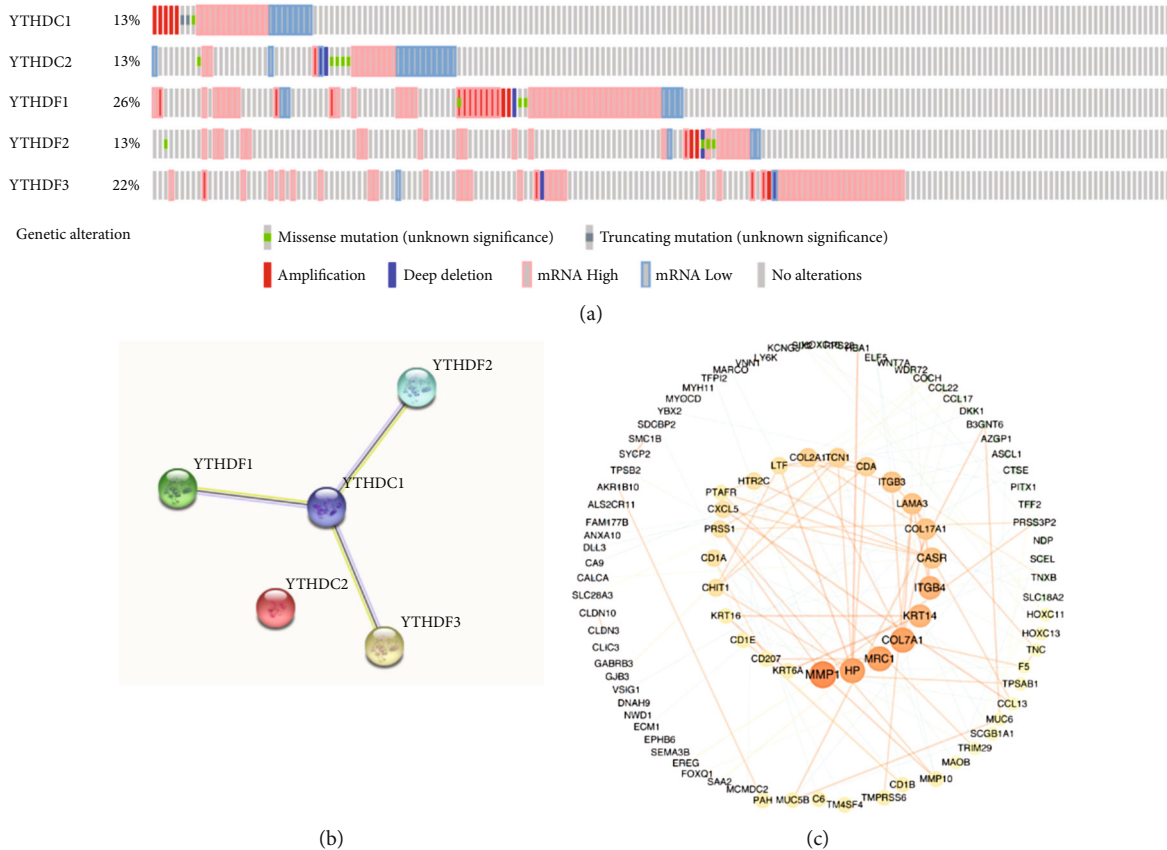


FIGURE 6: Genetic alterations analysis and molecular interaction analysis of the YTH family in LUAD. (a) Genetic alteration profiles of the YTH family in LUAD by using cBioPortal. (b) The interaction analysis within the YTH family from STRING. (c) The identification of 152 YTH-associated genes which was most frequently altered in LUAD through cBioPortal and Cytoscape.

regulates the methylation of target RNA by specific combination with m⁶A-containing mRNA. With huge potential value, there are multiple studies reporting the role of YTH family proteins in various cancer types. For instance, YTHDF1 was proved to be an oncogene in hepatocellular carcinoma (HCC) owing to its overexpression in HCC patients and association with poor prognosis [10, 31]. The oncogenic role of YTHDF1 may be achieved by the mechanisms of Snail-induced EMT [32] and m⁶A-dependent activation of the WNT/ β -catenin pathway [33]. However, the role of YTHDF2 in HCC was found to be contradictory, which acted as either oncogene in a m⁶A-dependent manner or tumor suppressor gene through EGFR and ERK/MAPK pathway [34, 35]. In the case of ovarian cancer, YTHDF1 was also found to be overexpressed and associated with poor clinical outcome. YTHDF1 accelerated the growth and metastasis of ovarian cancer in vivo and in vitro by promoting the m⁶A-modified translation of EIF3C [36]. In the field of lung cancer, the expression of YTHDF2 was aberrantly higher and facilitated the proliferation and growth of cancer cells, ribose-5-phosphate, and NADPH induced by pentose phosphate pathway might be the mechanism underlies [37]. YTHDF1 was demonstrated to promote proliferation of cancer cells in nonsmall cell lung cancer (NSCLC), whereas paradoxically, better prognosis and chemotherapy sensitivity were correlated with high YTHDF1 expression

and implied the complicated and multiple mechanisms lurking beneath the phenomena observed above [38].

According to our knowledge, a study that systematically focused on the expression, prognostic value, and pathophysiological function of the YTH domain family in LUAD is still lacking. Hence, we preliminarily explore the expression profiles of the YTH domain family and found that only the expression of YTHDC2 is significantly lower in all of the LUAD datasets included in this study, while YTHDF1, YTHDF2, and YTHDF3 are proved to be significantly upregulated only in one dataset. Undoubtedly, YTHDC2 expression is more convincing because data retrieved from multiple datasets showed high concordance. These indicated that YTHDC2 might be the potential tumor suppressor gene. YTHDF1, YTHDF2, and YTHDF3 might as well act as the potential oncogenes with a lower level of evidence.

Then, we explored whether the prognostic value of YTH proteins was in line with their expression trend. Only YTHDC1 passed this round of screening cause its lower expression was correlated with poorer prognosis with statistical significance when using OS, FP, and PPS as the endpoints of survival. While higher expression of YTHDF1, YTHDF2, and YTHDF3 was associated with better clinical outcome, which is in contradiction with their expression profiles in LUAD compared with normal lung tissue. These results pushed YTHDC2 to the foreground as a novel

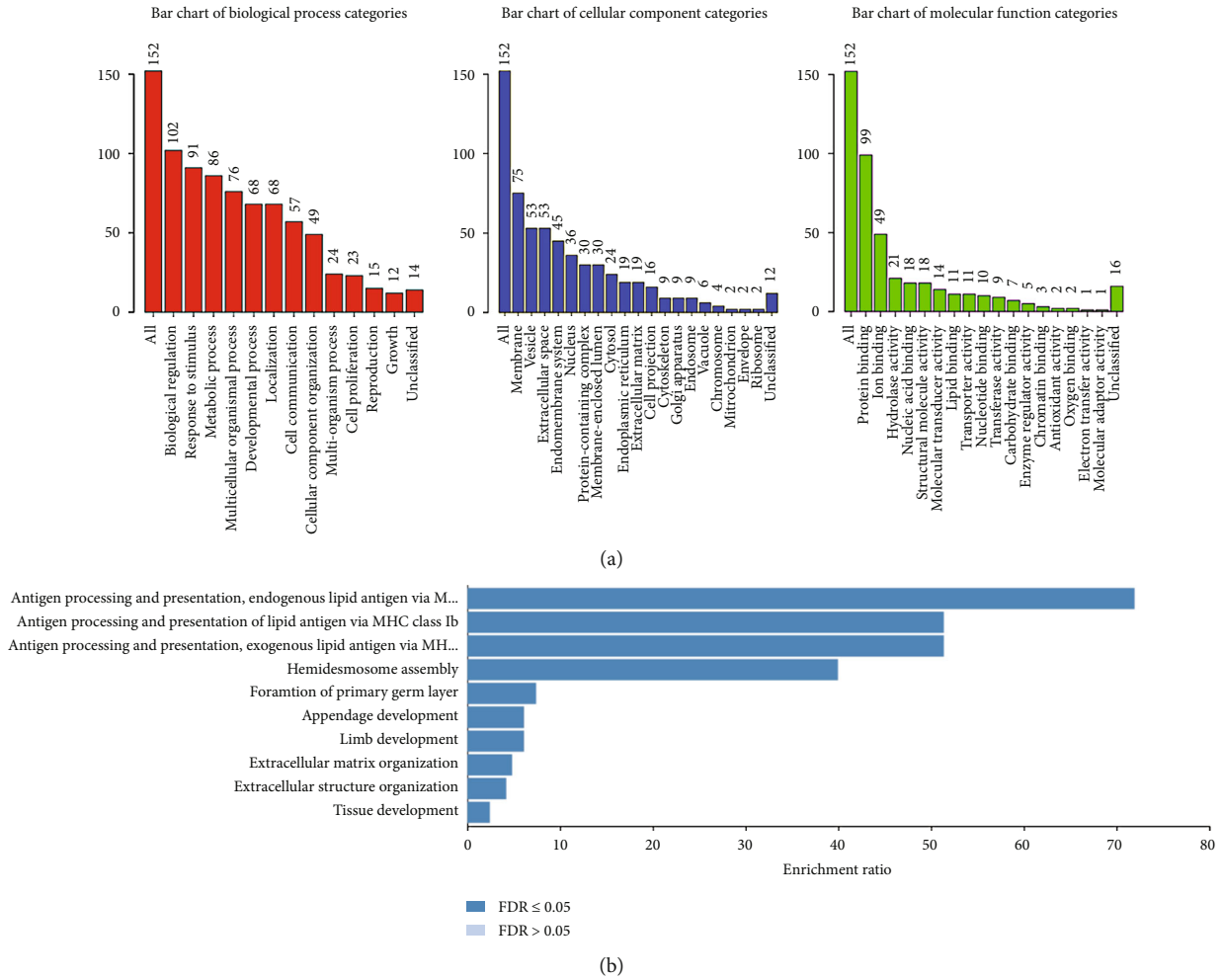


FIGURE 7: The biological pathway analysis of YTH family from WebGestalt database. (a) The biological process, cellular component, and molecular function which were associated with the YTH family were shown on the bar charts through Gene Ontology (GO) enrichment analysis. (b) Kyoto Encyclopedia of Genes and Genome (KEGG) enrichment analysis of YTH family.

potential target gene against LUAD. Of course, more solid evidence from experiments is needed.

To date, the role of YTHDF2 in lung cancer (especially in LUAD) remains to be further explored. YTHDC2 can promote 6PGD mRNA translation in lung cancer cells by means of m6A modification. We made a horizontal comparison of YTHDC2 in different types of cancer. Unlike the low expression of YTHDC2 in LUAD, YTHDC2 expression was increased in liver cancer and positively related to tumor malignancy [39]. Whereas opposite evidence of YTHDC2 as a tumor suppressor gene is also reported in liver cancer [35], covering the underneath mechanisms a heterogeneous veil. In addition, YTHDC2 exerts a promoting role in the colorectal cancer metastasis through the hypoxia/HIF-1 α /Twist1 signaling pathway [40]. Our study also for the first time suggested that smoking habits may negatively reflect the expression of YTHDC2 level in LAUD patients. Further investigations based on it may have the opportunities to make breakthrough in targeted drug development and precision medicine of LAUD with smoking habit.

The functional enrichment analysis [41] in this study screens out the biological process and functional interpretation of genes around the YTH family, which further broaden our understanding of the role of the YTH family in the pathophysiological process of LUAD. The major functions of the YTH family seem to point in the direction of immunomodulation, especially antigen processing and presentation. This was in accordance with the finding of a published paper, showing that gene signature of antigen processing and presentation machinery may predict the therapeutic effect of immune checkpoint inhibitors such as anti-PD-1/PD-L1 [42]. Another study gave a similar conclusion that impaired antigen processing and presentation may account for the acquired resistance of immune checkpoint inhibitors and thus led to treatment failure [43] in lung cancer. Therefore, the relationship between the YTH family and antigen processing and presentation in LUAD is worthy of further exploration.

m6A methylation has been reported to play essential roles in tumor immunity. Furthermore, profiles of immune cells infiltration in tumor could act as novel biomarkers that effectively improved the diagnosis and prognosis of many

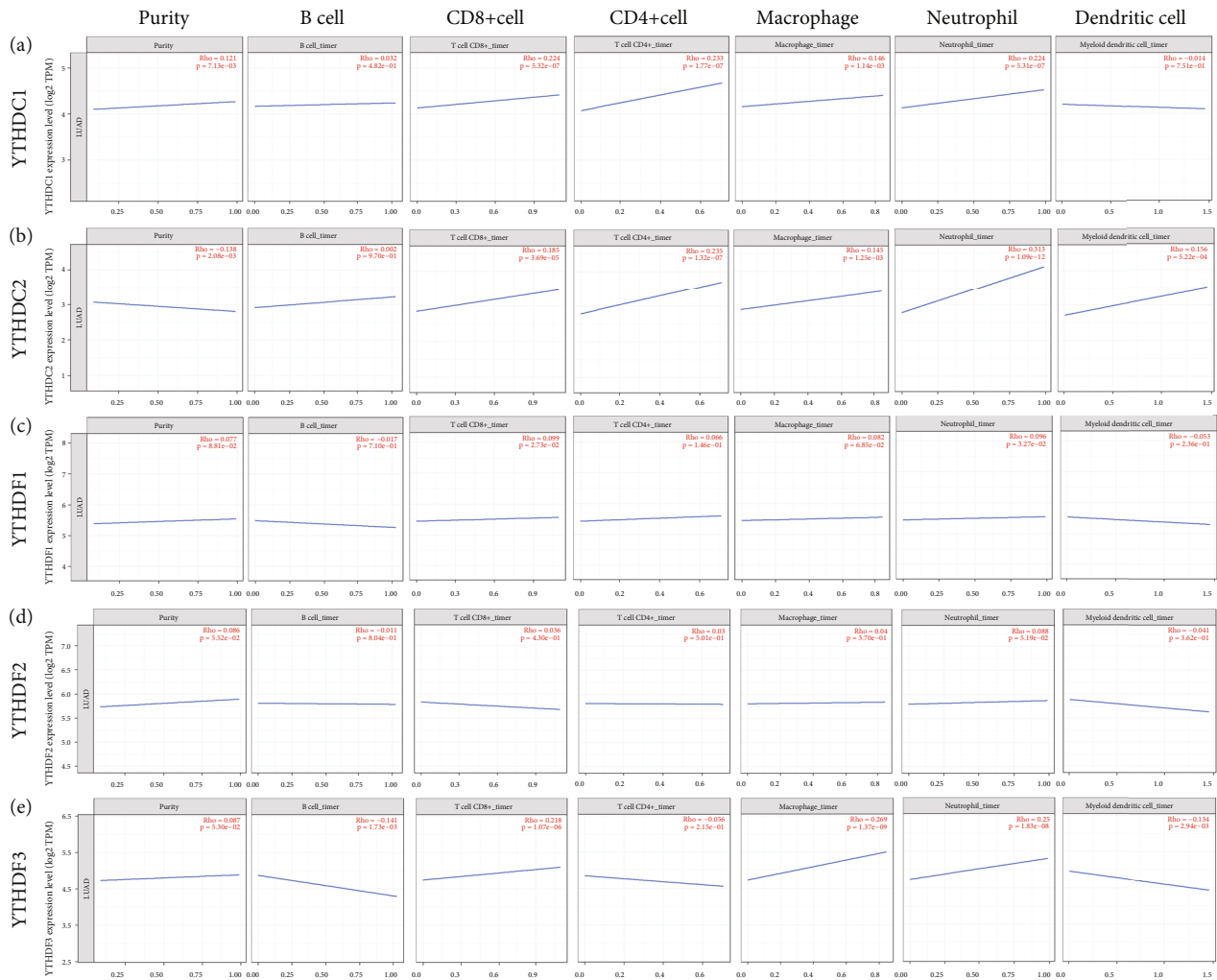


FIGURE 8: The correlation between immune cell infiltration and each YTH domain family member. The TIMER2.0 database was applied to explore the role of (a) YTHDC1, (b) YTHDC2, (c) YTHDF1, (d) YTHDF2, and (e) YTHDF3 in different immune cell infiltration (CD4+ T cells, CD8+ T cells, B cells, dendritic cells, macrophages, and neutrophils) around LUAD.

types of cancer [44, 45]. Our study found that in LUAD, most of the YTH family members were significantly correlated with the infiltration of CD4+ T cells, CD8+ T cells, macrophages, and neutrophils. A previous study presented that CD4+ T cells and CD8+ T cells were generally believed to control cancer outcome, while macrophages and neutrophils produce various factors that induce inflammation and stimulus tumor progression [46]. Taken together, the abnormal expression of YTH family members (especially YTHDC2) may alter the profiles of immune cell infiltration in LUAD through some kinds of specific mechanisms, thus influence the clinical outcome and the therapeutic effect of immunotherapy.

However, there were several limitations in our work. Our analysis in this study was mostly based on the data from the online databases, and further laboratory experiments were needed to verify these conclusions. Moreover, further research into the specific molecular mechanisms and molecules interactions would be needed in the future.

5. Conclusions

In conclusion, the molecular and expression profiles of the YTH family were dysregulated in LUAD. YTH family members (especially YTHDC2) are promising biomarkers and potential therapeutic targets that may bring benefit for patients with LUAD.

Data Availability

The original contributions presented in the study are included in the article/Supplementary Material, and further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Conception and design were done by J Li and K Hu. Writing, review, and/or revision of the manuscript were done by K Hu, J Li, and L Yao. Administrative, technical, or material support were done by Y Yan and L Zhou. All authors approved the final version of the manuscript.

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Supplementary Materials

Supplementary 1. Supplementary Figure S1: the prognostic effect of YTHDC2 on smoking and nonsmoking patients with LUAD using Kaplan-Meier plotter.

Supplementary 2. Supplementary Table S1: summary of bioinformatics databases used in this study for analyzing the role of the YTH domain family in LUAD.

Supplementary 3. Supplementary Table S2: the YTH domain family associated coexpressed genes in LUAD.

Supplementary 4. Supplementary Table S3: the association between the expression of individual YTH family members and clinical characters in LUAD patients.

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