Comprehensive Analysis of the Expression, Prognosis and Immune Infiltration of KPNA Family in Hepatocellular Carcinoma

Xiangyi Chen
Lanzhou University Second Hospital
Dechen Yu
Lanzhou University Second Hospital
Hai-Yu Zhou (zhouhy@lzu.edu.cn)
Lanzhou University Second Hospital
XiaoBo Zhang
Lanzhou University Second Hospital
Yicun Hu
Lanzhou University Second Hospital
Ruihao Zhang
Lanzhou University Second Hospital
Xidan Gao
Lanzhou University Second Hospital
Maoqiang Lin
Lanzhou University Second Hospital
Taowen Guo
Lanzhou University Second Hospital
Kun Zhang
Lanzhou University Second Hospital

Research Article

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Abstract

Background: The primary function of the Karyophorin alpha family (KPNAs) is to assist the transport of proteins from the cytoplasm to the nucleus. Studies have found that KPNAs are involved in the occurrence and development of a variety of tumors. However, the expression level of KPNAs family members in HCC, its influence on prognosis, its relationship with immune infiltration, and its clinical significance are still unclear.

Methods: We used UALCAN, GEPIA, HPA, TIMER, Kaplan-Meier Plotter, CBioPortal, String and Metascape databases to analyze the expression and mutation of KPNAs in Hepatocellular carcinoma (HCC), the expression level of KPNAs and the prognosis of patients, tumor immune cell infiltration, HCC clinicopathological characteristics Relationship. Finally, the biological functions of KPNAs related genes are analyzed.

Results: The protein and mRNA of KPNAs were significantly up-regulated in HCC, and their expression levels were closely related to the prognosis of patients and the clinical characteristics of the tumor (tumor grade, stage, etc.). In addition, KPNAs in HCC are prone to mutations, which are not conducive to the prognosis of patients. Moreover, the expression of HCC is positively correlated with the infiltration of immune cells. Enrichment analysis found that KPNAs-related genes are mainly related to biological processes such as nuclear and cytoplasmic signaling, protein chromosome localization, and their pathways mainly include cell cycle and MAPK signaling pathways.

Conclusion: KPNAs are significantly related to the occurrence, development and patient prognosis of HCC and may become the target of HCC immunotherapy in the future.

1 Introduction

Hepatocellular carcinoma (HCC) is a kind of cancer that seriously threatens the survival of human beings and ranks second in the global cause of cancer deaths. Statistics show that there are about 850000 new cases every year [1]. By 2030, the number of deaths from HCC will exceed one million [2]. And its incidence is still rising [3]. HCC mainly develops from liver cirrhosis. Hepatitis virus and alcohol are the leading causes of liver cirrhosis. Aflatoxin is also considered to be one of the factors that lead to HCC [4]. Current treatments for HCC include surgical resection, radiofrequency ablation, drug chemotherapy, liver transplantation and so on. However, about 70% of patients lose the opportunity for surgery because only early stage, no metastasis, and liver function can be compensated for the opportunity to have surgery [5]. And there is a risk of recurrence after surgery. When the tumor is deep and small and cannot be removed surgically, it can be treated by radiofrequency ablation [6]. For patients with large tumor volume and metastasis, chemotherapy and targeted therapy will be the first choice [5]. Although the treatment methods are improving, the prognosis of patients has not made substantial progress, so it is necessary to find a suitable drug treatment target and diagnostic marker.

The karyophorin alpha family (KPNAs) has seven members, KPNA1-7[7]. The primary function of the KPNA family is to actively transport proteins into the nucleus by recognizing nuclear localization signals (NLS) and is a crucial signal mediator for nuclear and cytoplasmic signal transduction. In addition, the KPNA family can also regulate the differentiation of oligodendrocytes [8, 9]. The KPNAs family plays an essential role in the occurrence and progression of tumors. Data analysis found that KPNA1 is associated with the prognosis of colorectal cancer [10]. KPNA2 promotes the occurrence and development of ovarian cancer, bladder cancer, and colon cancer. It also participates in the occurrence of pancreatic ductal cancer and facilitates immune evasion of tumor cells [11–14]. KPNA3 promotes the progression of colon cancer and HCC. It is also involved in the epithelial-mesenchymal transition (EMT) of HCC and mediates drug resistance [15, 16]. KPNA4 is associated with the occurrence and development of osteosarcoma, non-small cell lung cancer and thyroid cancer [17–19]. KPNA5 is involved in the activation of estrogen receptors in breast cancer [20]. KPNA6 is up-regulated in chronic myeloid leukemia [21]. KPNA7 is involved in tumor occurrence and cell growth in pancreatic cancer and breast cancer, and is also related to autophagy of pancreatic cancer cells [22, 23]. KPNA2 and KPNA3 participate in the occurrence and progression of HCC cancer, and promote the growth and invasion of HCC cells [24, 25]. In addition, KPNA2 can regulate the immune process of HCC [26]. KPNA3 is also associated with sorafenib resistance in HCC [16]. The study also found that the expression of KPNA2 and KPNA4 is related to the prognosis of HCC patients [26, 27]. However, other members of the KPNAs family have relatively limited research on HCC. Whether KPNAs can be used as indicators for the diagnosis and prognosis of HCC, and the specific mechanism of its involvement in HCC is still unclear.

Therefore, this article analyzes the expression level of KPNAs family members in HCC and the relationship between expression and prognosis and immune infiltration. The co-expressed genes and possible mechanisms of action were also predicted. It provides directions for the diagnosis and prognosis of HCC and future treatment.

2 Materials And Methods

UALCAN

UALCAN (http://ualcan.path.uab.edu/analysis.html) is an online analysis and data mining website whose data mainly comes from the TCGA database [28]. We used it to explore the expression level of KPNAs in HCC and its relationship with the clinical characteristics of HCC, such as grade and stage.

TIMER

TIMER (http://timer.cistrome.org/) is a database that can analyze gene expression levels in tumors and the relationship between the gene and immune infiltration [29]. We first used it to analyze the expression of KPNAs in HCC, and then analyzed the relationship between the expression of KPNAs in HCC and the infiltration of immune cells (CD4+, CD8+, B cell, myeloid dendritic cell, macrophage, neutrophil).

GEPIA

GEPIA (http://gepia2.cancer-pku.cn/) is a free database whose data mainly comes from the TCGA and Genotype-Tissue Expression (GTEx) project [30]. The difference in gene expression between normal tissues and tumors, the relationship between gene expression levels and tumor stages, and the impact of genes on patient prognosis can all be analyzed through this database. We used it to explore the expression of KPNAs in HCC. Filter conditions: p-value Cutoff: 0.01, fold change: 1.

HPA

The Human Protein Atlas (http://timer.cistrome.org/) database is a database that can analyze protein expression in different tissues and cells. And the database contains the immunohistochemical results of a variety of tumor tissues [31]. We used this database to analyze the expression of KPNAs in HCC and normal tissues.

Kaplan-Meier Plotter

The Kaplan-Meier Plotter database is a database that can analyze the prognosis of 21 tumors [32]. It can be used to explore the relationship between different expression levels of genes and patient prognosis. It was used to analyze the impact of KPNAs on OS (overall survival) and RFS (Relapse Free Survival) in HCC patients. If $p \leq 0.05$, it is considered that the difference in expression of this gene has a significant impact on the prognosis of patients.

CBioPortal

The CBioPortal (http://cbiop ortal.org) website contains various cancer gene data in the TCGA database [33, 34]. This website can be used to analyze gene mutations in tumors. We selected the data of 360 HCC patients (TCGA, Firehose Legacy) from the TCGA database to analyze the mutations of KPNAs. The selected Genomic profiles include putative copy-number alterations from GISTIC, mutations and mRNA expression z-score (RNA Sep V2 RSEM) with a z-score threshold ± 2.0. The top 20 genes that are most closely related to each KPNAs member are sorted out and used as co-expressed genes.

String

String (http://string-db.org) is a database that can predict protein-protein interaction (PPI)[35]. Up to now, the String database has collected 24,584,628 proteins from 5090 different organisms. We use the String tool to draw a PPI network of KPNAs related genes.

Metascape

Matescape (https://metascape.org/) is a gene function analysis tool [36]. We introduced KPNAs related genes into Metascape, set the species as human, and analyzed the GO and KEGG pathways, respectively.

3 Results

mRNA and protein expression levels of KPNAs in HCC

Analyzing the transcription levels of KPNAs in HCC and normal tissues in the ULCAN database, the results showed that the transcription levels of all members of the KPNAs family were significantly up-regulated in HCC tissues (Figure.1). Analyzing the data in the TIMER database got the same result (Figure.2a). In the GEPIA database, the expression levels of KPNAs family members in HCC are all increased, but compared with normal tissues, except for the statistically significant increase in KPNA2, the up-regulation of other members is not statistically significant (Figure.2b-h). Next, we used the HPA database to analyze the protein expression levels of KPNAs family members in HCC. As shown in Fig. 3, the protein expression level of KPNA2-6 in HCC tissues is significantly higher than that in normal liver tissues. KPNA1 is moderately expressed or not expressed in normal hepatocytes and cholangiocytes but is moderately expressed in cholangiocytes of cancer tissues. In conclusion, KPNAs family members are up-regulated in HCC.

The relationship between the expression level of KPNAs in HCC and clinicopathological characteristics

We used the UALCAN database to analyze the relationship between KPNAs and tumor stage, tumor grade and TP53 mutation status in HCC. As shown in Fig. 4, compared with normal tissues, KPNAs were significantly up-regulated in stage 1, 2, and 3 tumors. Compared with normal tissues and stage 4 tumors, except for the statistical significance of KPNA2 expression, the expression of other KPNAs family members is not statistically significant, which may be related to the limited number of stage 4 cases (n = 6). There are significant differences in the expression of KPNA2 in tumor stages 1 and 2, stage 1 and 3, KPNA4 in stage 1 and 2, stage 2 and 3, and KPNA6 expression between stage 1 and stage 3. As shown in Fig. 5, there is a close relationship between KPNAs and tumor grade. Compared with normal tissues, the higher the expression of KPNAs, the higher the tumor grade. In addition, the expression levels of some KPNAs members are also related to different grades of tumors. For example, the expression of KPNA2, KPNA4, KPNA5 and KPNA7 is significantly different between grade 1 and grade 3, grade 2 and grade 3. Moreover, the expression level of KPNA7 between grade 1 and grade 2, grade 2 and grade 4 of tumors is also statistically significant. We also analyzed whether the expression of KPNAs is related to the mutation of TP53. Compared with normal tissues and tumor tissues without mutations, the expression level of all members except KPNA3 in TP53 mutant tissues was significantly up-regulated (Fig. 6). In

short, the expression of KPNAs family members is significantly related to the staging and grade of HCC tumors and whether TP53 is mutated.

The relationship between KPNAs and the prognosis of HCC patients

Kaplan-Meier Plotter was used to evaluate the relationship between the expression level of KPNAs and the OS and RFS of patients. In general, the expression level of KPNAs is negatively correlated with the OS and RFS of HCC patients. The expression levels of KPNA2, KPNA4, KPNA5, and KPNA6 are statistically significant with the patient's OS, while the expression levels of KPNA2, KPNA5, and KPNA6 are significantly related to the patient's RFS (Fig. 7, Fig. 8). Therefore, KPNAs may be associated with the prognosis of HCC patients, have the potential to evaluate the prognosis of patients, and may be used as a biomarker to predict the prognosis of HCC patients in the future.

The relationship between the mutation of KPNAs and the prognosis of HCC patients

We used the cBioPortal tool to analyze the alteration in KPNAs in HCC patients and their relationship with the patient's prognosis. We found that 159 (44%) of 360 cases had changed. The alteration types mainly included Mutation, Amplification, Deep Deletion, mRNA High, mRNA Low and Multiple Alterations (Fig. 9b). The mutation rates of KPNA1-7 were 4%, 13%, 6%, 11%, 7%, 13%, and 8%, respectively (Figure.9a). In addition, as shown in Fig. 9c and d, the OS of patients with KPNAs mutations was significantly shortened ($p = 0.0166$), while the alteration of KPNAs had no significant effect on disease-free survival (DFS) ($P = 0.0571$)

The relationship between the expression of KPNAs in HCC and tumor immunity

Is the expression of KPNAs related to tumor immunity in HCC? We used the TIMER database to explore. As shown in Fig. 10, there is no significant correlation between the expression of a few KPNAs and tumor immune infiltration (e.g. KPNA1 and CD4 + T, KPNA2 and CD8 + T, KPNA6 and CD4 + T, KPNA7 and CD8 + T, KPNA7 and neutral Granulocytes). But overall the expression level of KPNAs is positively correlated with the immune infiltration of B cells, CD4 + T, CD8 + T, dendritic cells, macrophages and neutrophils. And this correlation is statistically significant. It proves that KPNAs participate in the immune infiltration process in HCC.

Co-expression and enrichment analysis of KPNAs related genes

The related genes of KPNAs are obtained from the cBioPortal database. We first sort out the top 20 genes that are most closely related to each member of the KPNAs family, and then summarize the above genes and remove duplicate genes. Use STRING online software to obtain the PPI of the above genes (Fig. 11). Metascape is used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The results are shown in Fig. 12a. The biological processes involved in KPNAs mainly include "sister chromatid segregation, NLS-bearing protein import into nucleus, peptidyl-serine phosphorylation, protein localization to chromosome, centromeric region, organelle localization," and so on. Cellular components such as "spindle, condensed chromosome, centromeric region, SWI/SNF complex, apicolateral plasma membrane, PML body" are related to KPNAs (Fig. 12b). KPNAs also regulate molecular functions, such as "nuclear import signal receptor activity, protein kinase activity, kinase binding, microtubule-binding, insulin receptor binding," and so on (Fig. 12c). The KEGG enrichment found that KPNAs-related pathways mainly include "Cell cycle, Tight junction, MAPK signaling pathway, Autophagy-animal, Hippo signaling pathway, etc." (Fig. 12d).

4 Discussion

Recent studies have found that the expression levels of specific genes in HCC have changed, proving that gene changes are involved in the occurrence and progression of HCC. For example, KPNA2 and KPNA3 can promote the growth and invasion of HCC cells [24, 25]. However, there is still a lack of systematic research on the specific mechanism of KPNAs family genes in HCC.

Our analysis found that in HCC, the mRNA and protein levels of KPNAs increased significantly. So we further analyzed the relationship between KPNAs expression level and HCC grade, stage and TP53 mutation. The results showed that the stage and grade of HCC and the mutation status of TP53 were significantly related to the expression of KPNAs. However, the expression levels of some KPNA family members are not correlated with high-stage (4) and high-grade (4) tumors. This may be related to the low number of high-stage (4) and high-grade (4) tumor cases in the database. This requires further verification. Studies have found that the expression of KPNA2 is increased in HCC, and MicroRNA-139 inhibits the growth, proliferation and invasion of HCC cells by down-regulating the expression of KPNA2, and promotes their apoptosis [24]. KPNA3 is up-regulated in HCC and participates in the growth and invasion of tumor cells [16]. Although there are relatively few studies on KPNAs in HCC staging and TP53 mutations, there are related studies in other tumors. For example, D'Antonio, A. et al. [37] analyzed the expression of KPNA2 in 65 cases of prostate cancer by immunohistochemistry and found that the expression level of KPNA2 was significantly related to the TNM staging of prostate cancer. These studies are consistent with our analysis. The KPNAs family is involved in the occurrence and development of HCC as proto-oncogenes, and is closely related to its clinical characteristics.

The expression level of KPNAs is an important factor affecting the prognosis of HCC patients. KPNA2 and KPNA3 are negatively correlated with the OS of HCC patients, and patients with higher KPNA4 expression have a worse prognosis [16, 24, 27]. Although the experimental results of KPNA3 have the same trend as our analysis results, our results show that KPNA3 has no statistical significance for the prognosis of patients ($p > 0.05$). This may be because the experimental results of Hu et al. [16] are derived from the analysis of small samples (78), while our results are derived from the systematic study of 370 cases. This requires a large amount of multi-center clinical data for further verification. The results of our analysis also showed that KPNAs gene changes in HCC patients. Xu et al. [38] also found KPNA2 expression abnormalities and mutations in HCC through bioinformatics analysis, which is consistent with our research results. In short, the KPNAs gene has changed in HCC, and its expression level is significantly related to the prognosis of HCC patients.

Tumor cell immune evasion is one of the mechanisms of tumor occurrence and progression [39]. Our analysis results show that KPNAs are positively correlated with the infiltration of immune cells (CD4+, CD8+, B cell, myeloid dendritic cell, macrophage, neutrophil). Wang et al.[40] found that compared with the control group, the content of CD4 + cells in the blood of HCC patients was higher, which was consistent with our analysis. The infiltration of CD8 + cells in HCC can improve the survival rate of patients and reduce the recurrence rate after surgery [41]. HCC patients with inflammatory cell infiltration (CD4 + and CD8+) have a better prognosis than patients without inflammatory cell infiltration, and the humoral immunity produced by B cells in lymphoid follicles is likely to be related to the prognosis of HCC patients [42]. The study also found that the functional defects of mouse dendritic cells promote the progress of HCC, which may be one of the reasons for the immune evasion of HCC [43]. These findings suggest that KPNAs can influence the progression of HCC and improve the prognosis of patients by regulating immune infiltration.

Our enrichment found that KPNAs and related genes are mainly related to biological processes such as nuclear and cytoplasmic signaling and protein chromosome localization. The enriched pathways mainly include cell cycle, MAPK signaling pathway, Hippo signaling pathway, etc. Previous studies have shown that KPNA is a protein receptor that mediates the entry of signal proteins into the nucleus. It can bind to proteins carrying NLS to achieve protein transport and signal transmission [44]. Chk2 is a protein involved in DNA damage repair, and its function requires KPNA2 to recognize NLS-3 on Chk2 [45]. This is consistent with the conclusion we reached through enrichment. Enriched signaling pathways include MAPK signaling pathways and so on. Studies have shown that MAPK signaling can enhance the transport activity of KPNA4. The enhancement of this activity depends on the phosphorylation of KPNA4, and the enhancement of this activity in head and neck squamous cell carcinoma (HNSCC) is related to the differentiation and malignancy of tumor cells. [46]. This will provide a certain direction for the study of the mechanism of KPNAs.

This study has some shortcomings. For example, our conclusion is based on a comprehensive analysis of multiple databases, and there may be deviations due to inconsistent data. And although some of the

conclusions in the article are consistent with the findings of the previously published articles, more conclusions still lack the support of experimental data, which will be the direction of our future efforts.

5 Conclusion

In summary, our research results found that KPNAs are up-regulated in HCC, participate in the occurrence and development of HCC, and are significantly related to clinical characteristics such as tumor staging, grade, and mutation of TP53. The higher the expression level of KPNAs, the worse the prognosis of HCC patients. Moreover, the KPNAs genes in HCC are prone to alteration such as mutation, amplification, and deep deletion. The OS of HCC patients with the above changes is significantly shorter than that of patients without genetic changes. In addition, the infiltration of immune cells (CD4+, CD8+, B cell, myeloid dendritic cell, macrophage, neutrophil) in HCC is also closely related to the expression of KPNAs. In short, we have systematically analyzed the role of KPNAs in HCC, and conducted enrichment analysis on the mechanism of action, which will provide a theoretical basis and research direction for related research.

Declarations

Data availability:

All data in the article comes from public databases, which have been clarified in the materials and methods section.

Author contributions:

X.Y.C., D.C.Y. and H.Y.Z. conceived and designed the idea to this paper; X.B.Z., Y.C.H. and R.H.Z. participated in its design and coordination and supervised the study. X.D.G., M.Q.L, T.W.G. and K.Z. analyzed the data and revised the final paper. All authors read and approved the final version of the manuscript. X.Y. C. was the first author of this article, X.Y. C. and D.C.Y. contributed equally to this work

Competing Interests:

The author(s) declare no competing interests.

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Figures

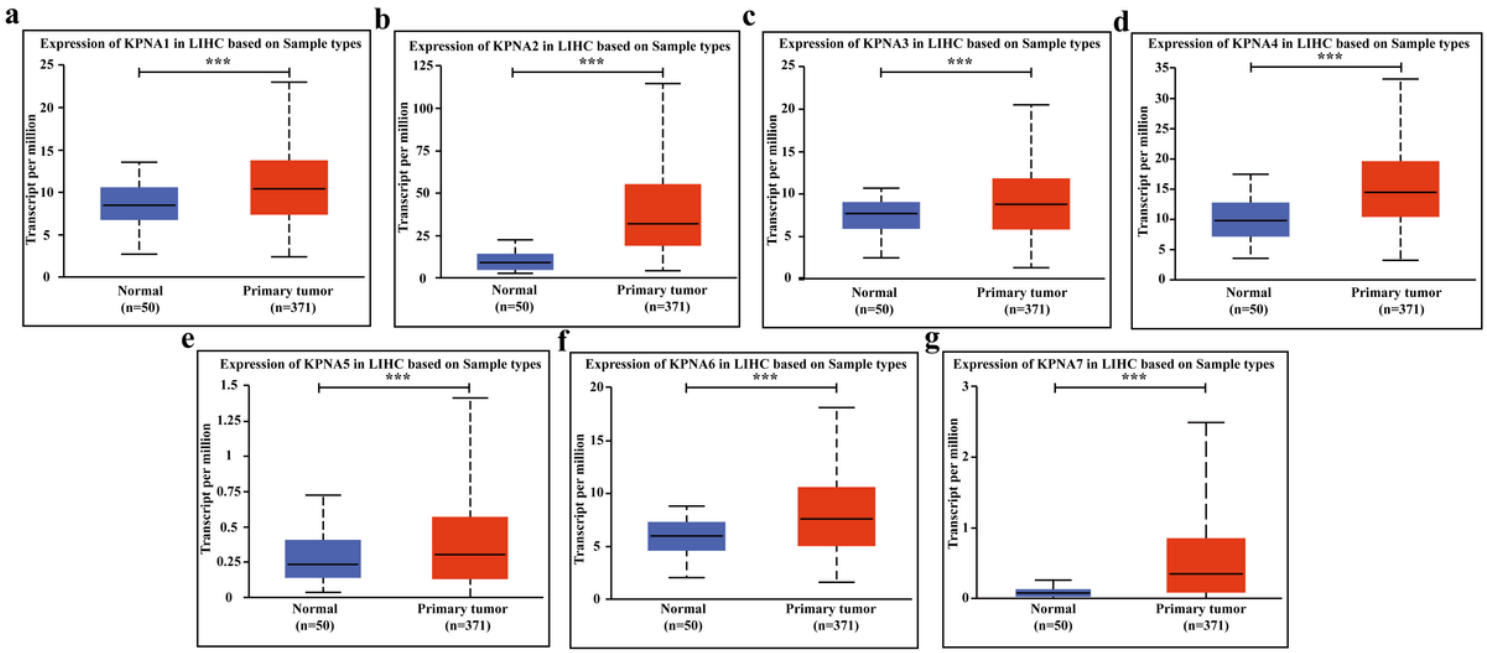

Figure 1

The mRNA expression level of KPNAs in HCC (UALCAN). LIHC: liver hepatocellular carcinoma; HCC: hepatocellular carcinoma. ***p□0.001.

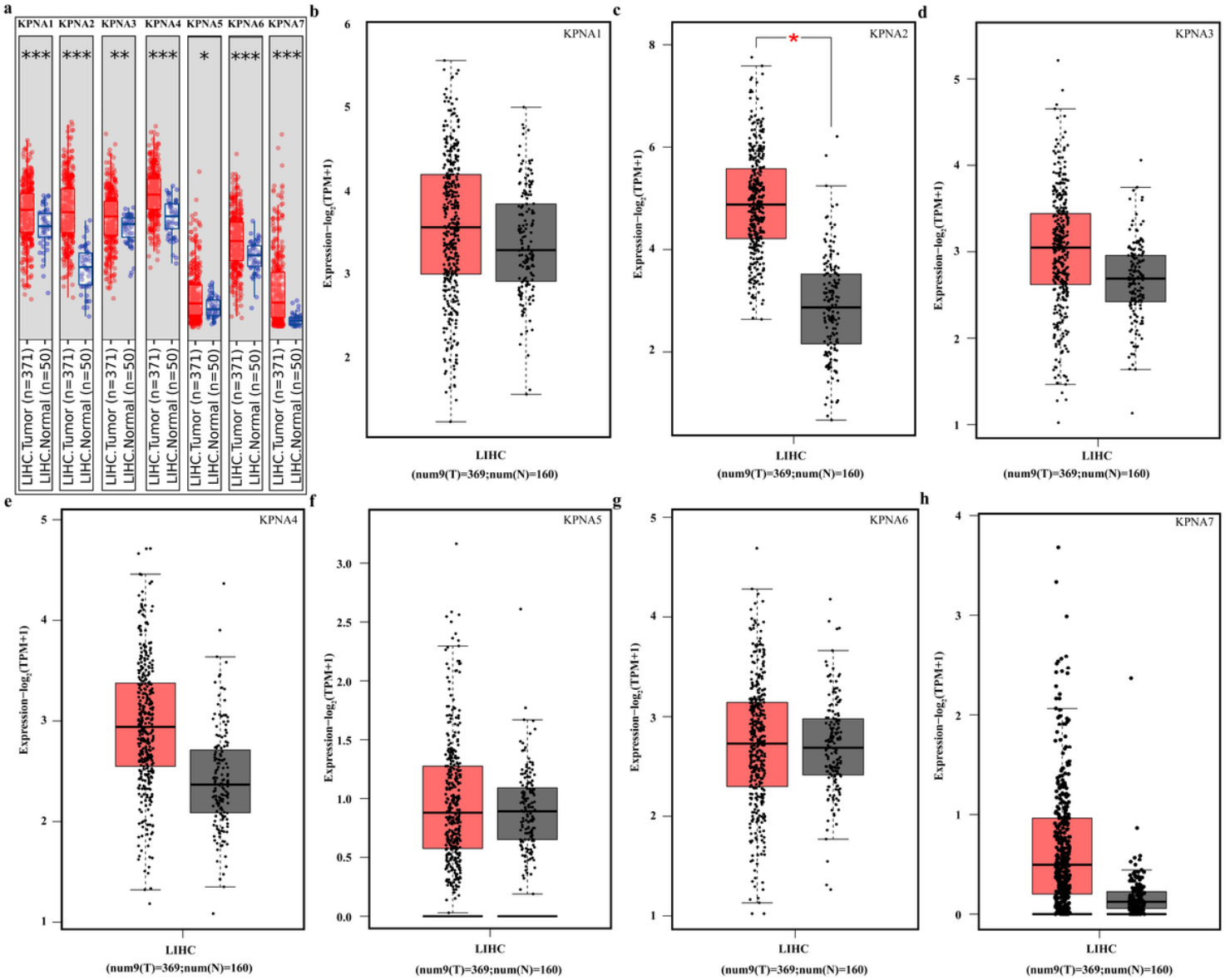

Figure 2

The mRNA expression level of KPNAs in HCC (A: TIMER, B-E: GEPIA). LIHC: liver hepatocellular carcinoma; HCC: hepatocellular carcinoma. *p□0.05,**p□0.01□***p□0.001.

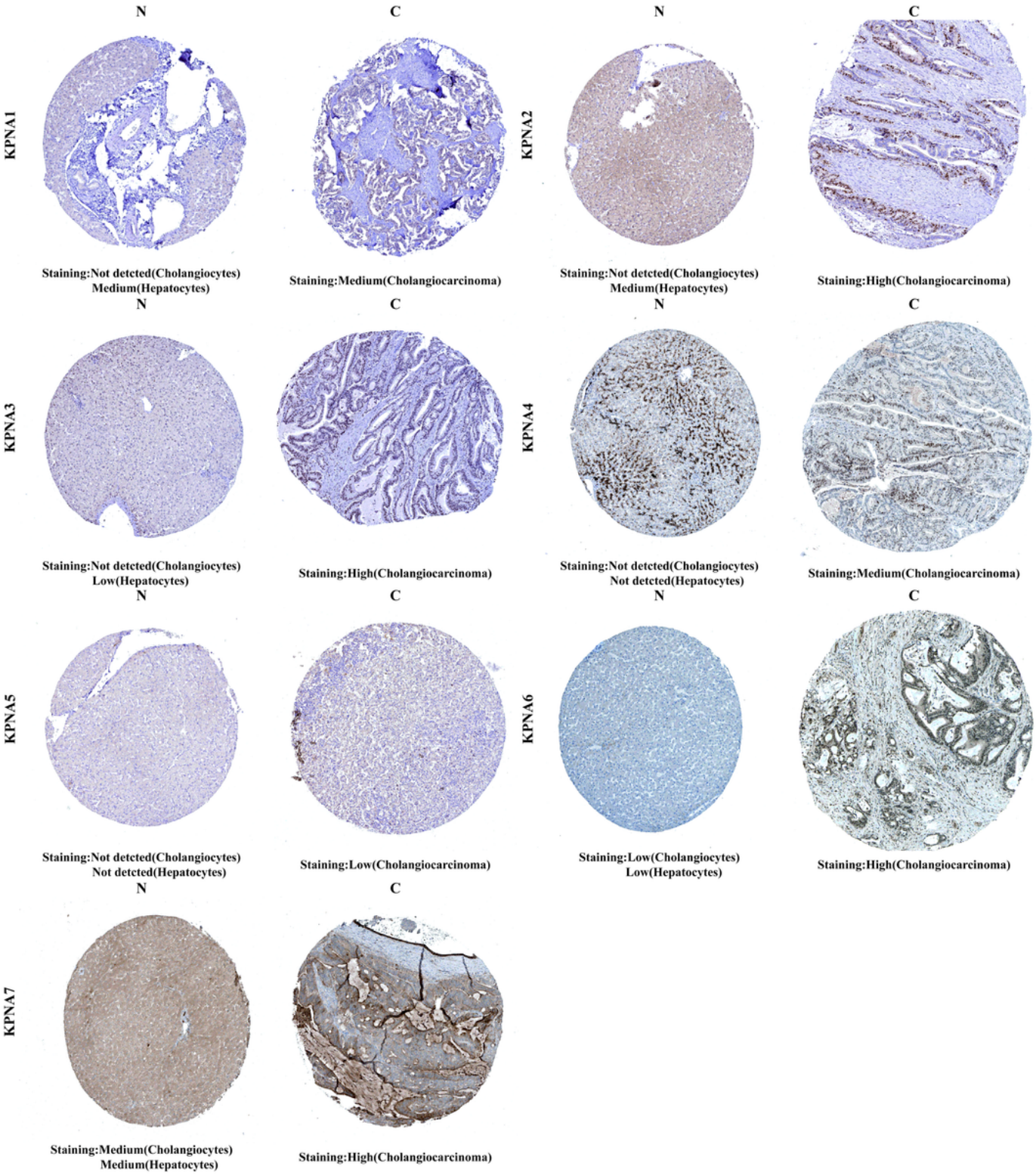

Figure 3

The protein expression level of KPNAs in HCC (HPA). HCC: hepatocellular carcinoma.

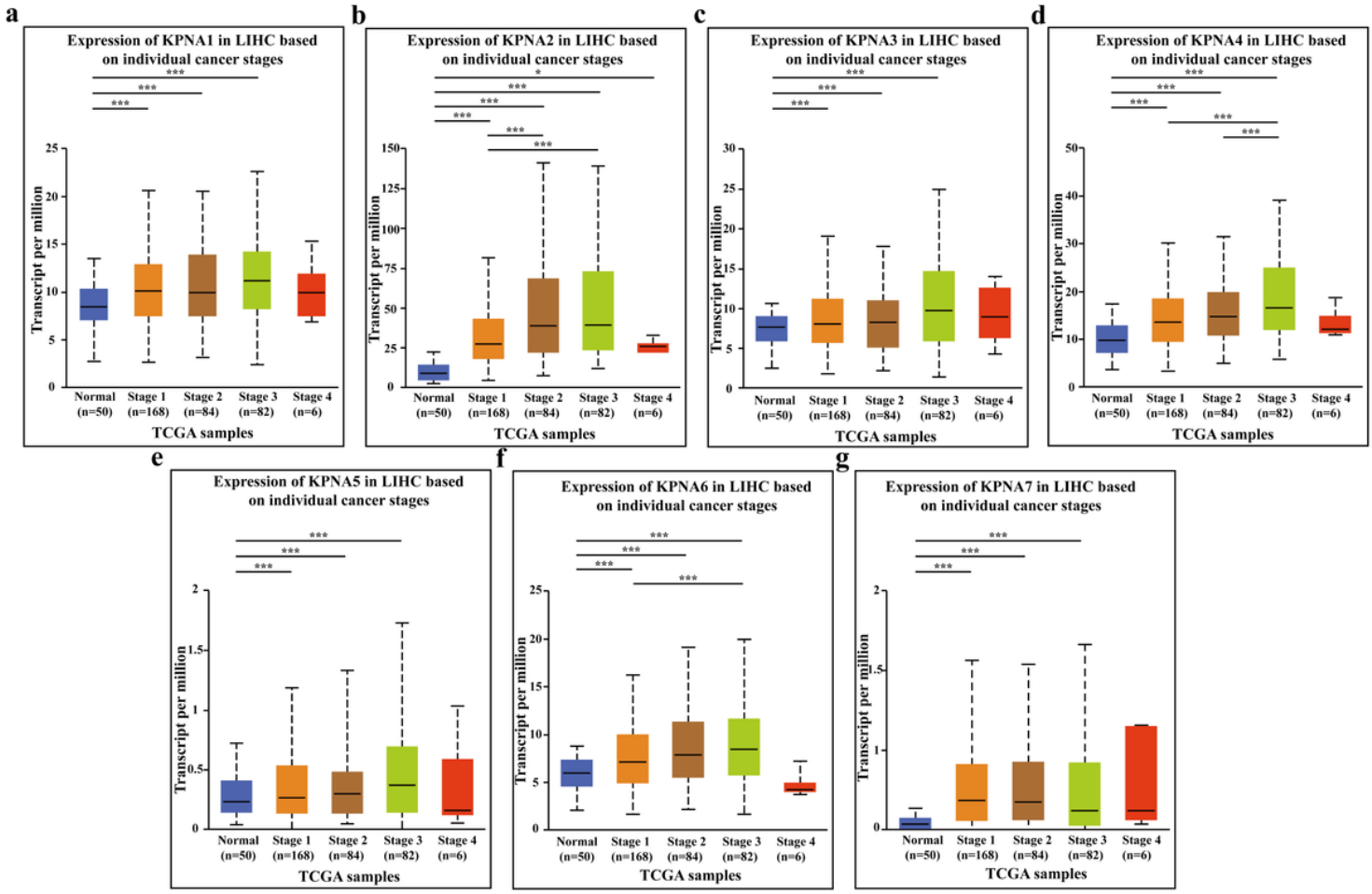

Figure 4

The relationship between the expression level of KPNAs and the stages of HCC (UALCAN). LIHC: liver hepatocellular carcinoma; HCC: hepatocellular carcinoma. *p□0.05,***p□0.001.

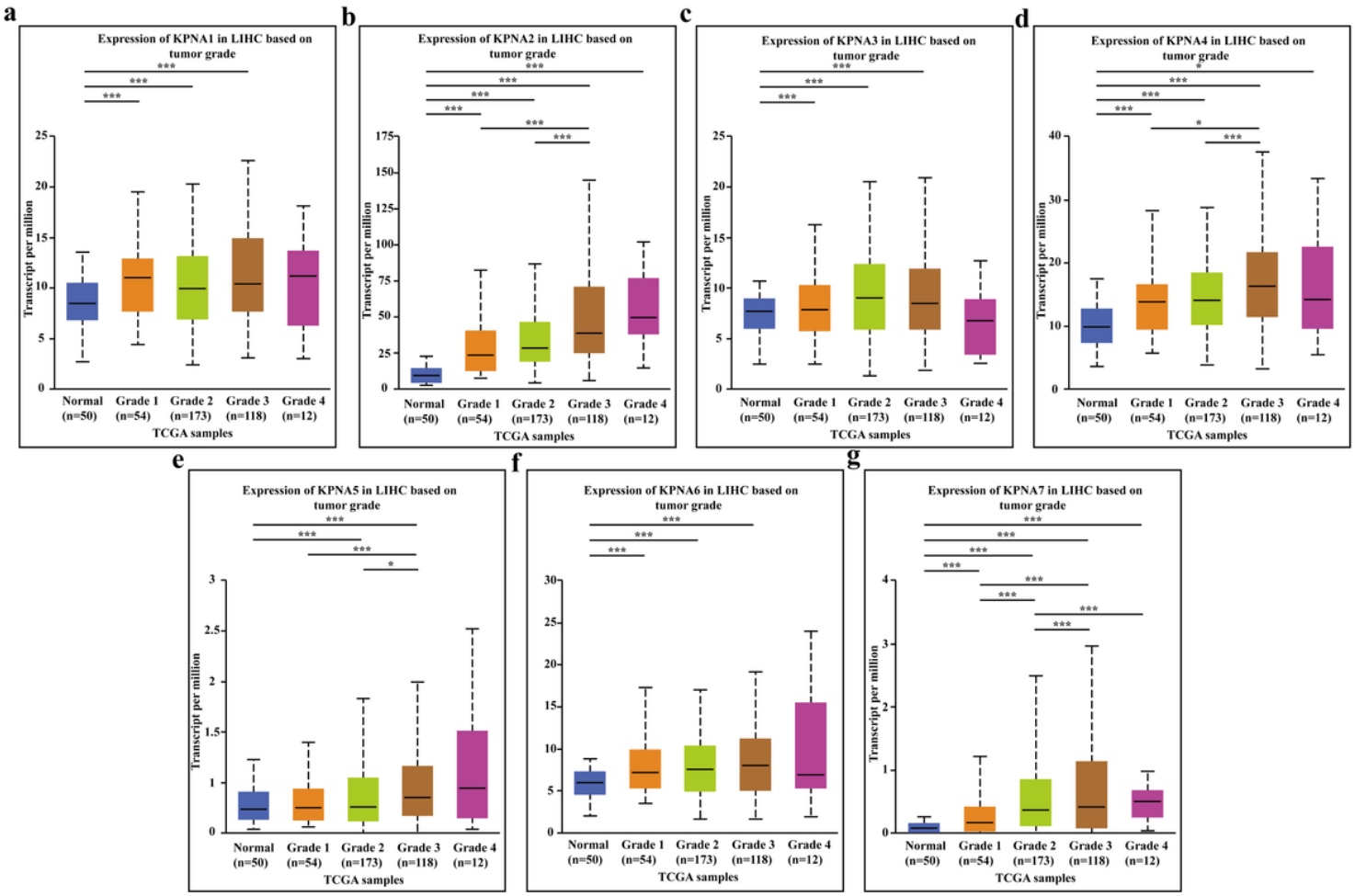

Figure 5

The relationship between the expression level of KPNAs and the grades of HCC (UALCAN). LIHC: liver hepatocellular carcinoma; HCC: hepatocellular carcinoma. *p□0.05, ***p□0.001.

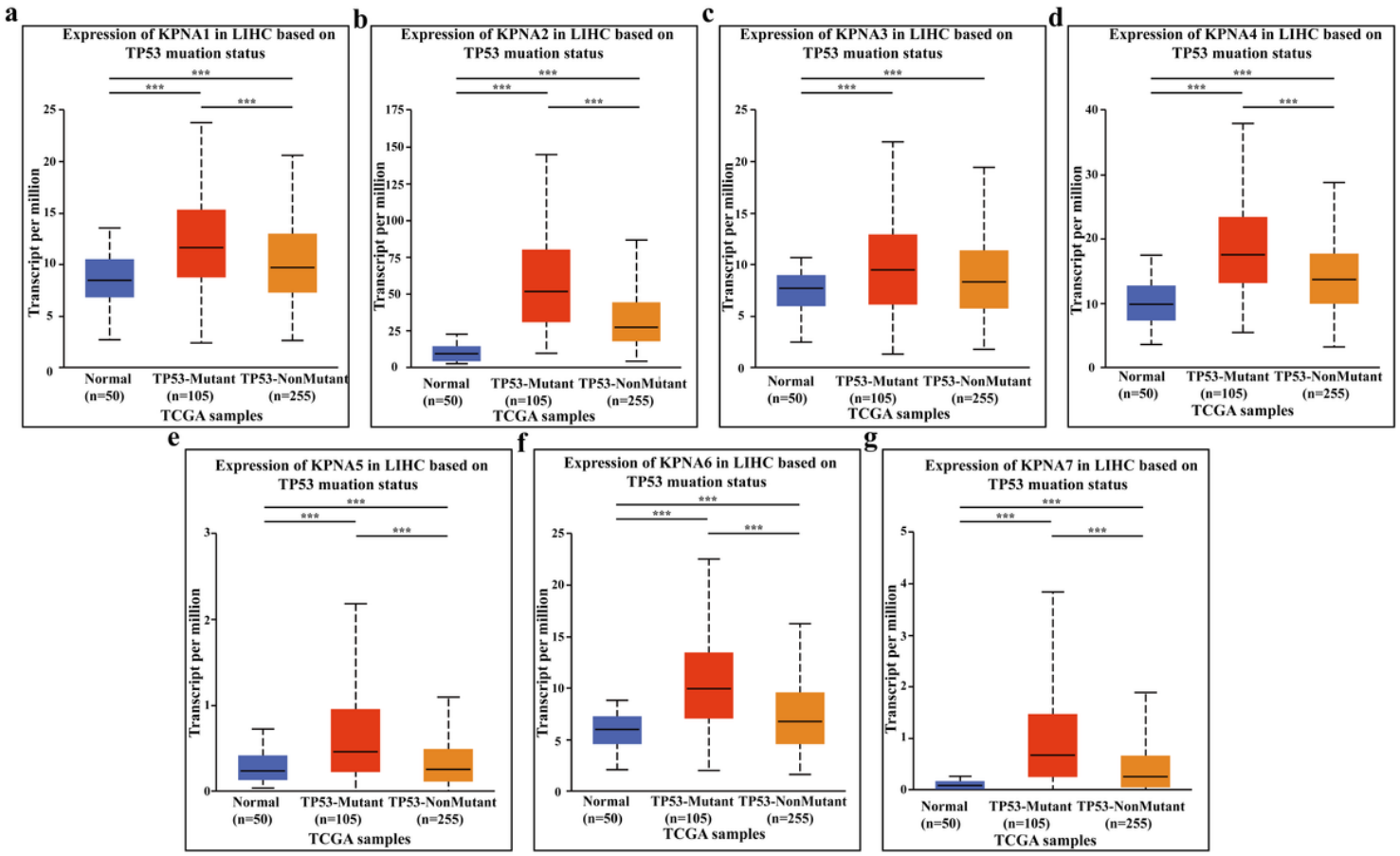

Figure 6

The relationship between KPNAs expression level and TP53 mutation in HCC (UALCAN). LIHC: liver hepatocellular carcinoma; HCC: hepatocellular carcinoma. ***p□0.001.

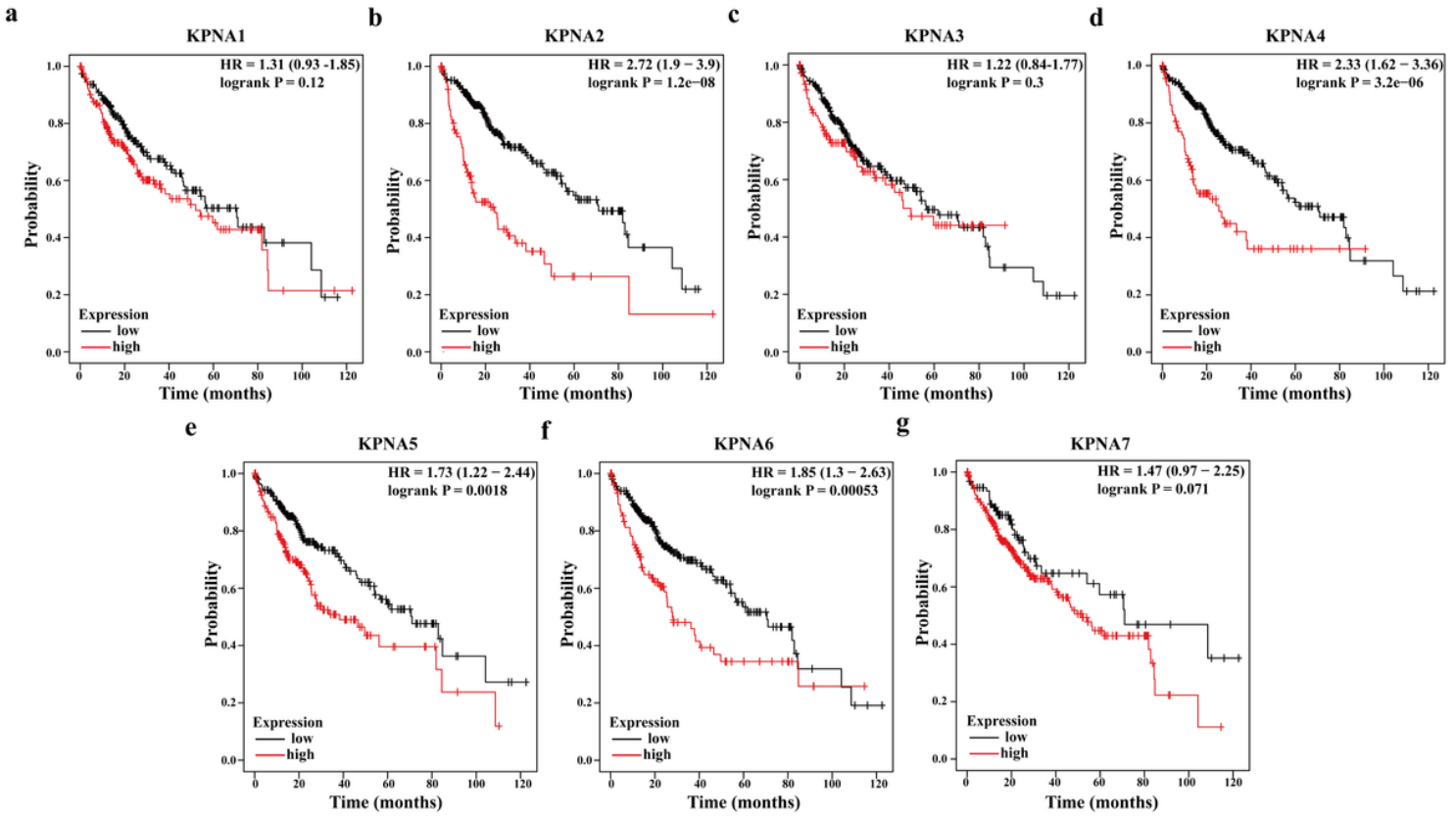

Figure 7

The relationship between mRNA expression level of KPNAs and OS in HCC patients (Kaplan-Meier Plotter). HCC: hepatocellular carcinoma; OS: overall survival

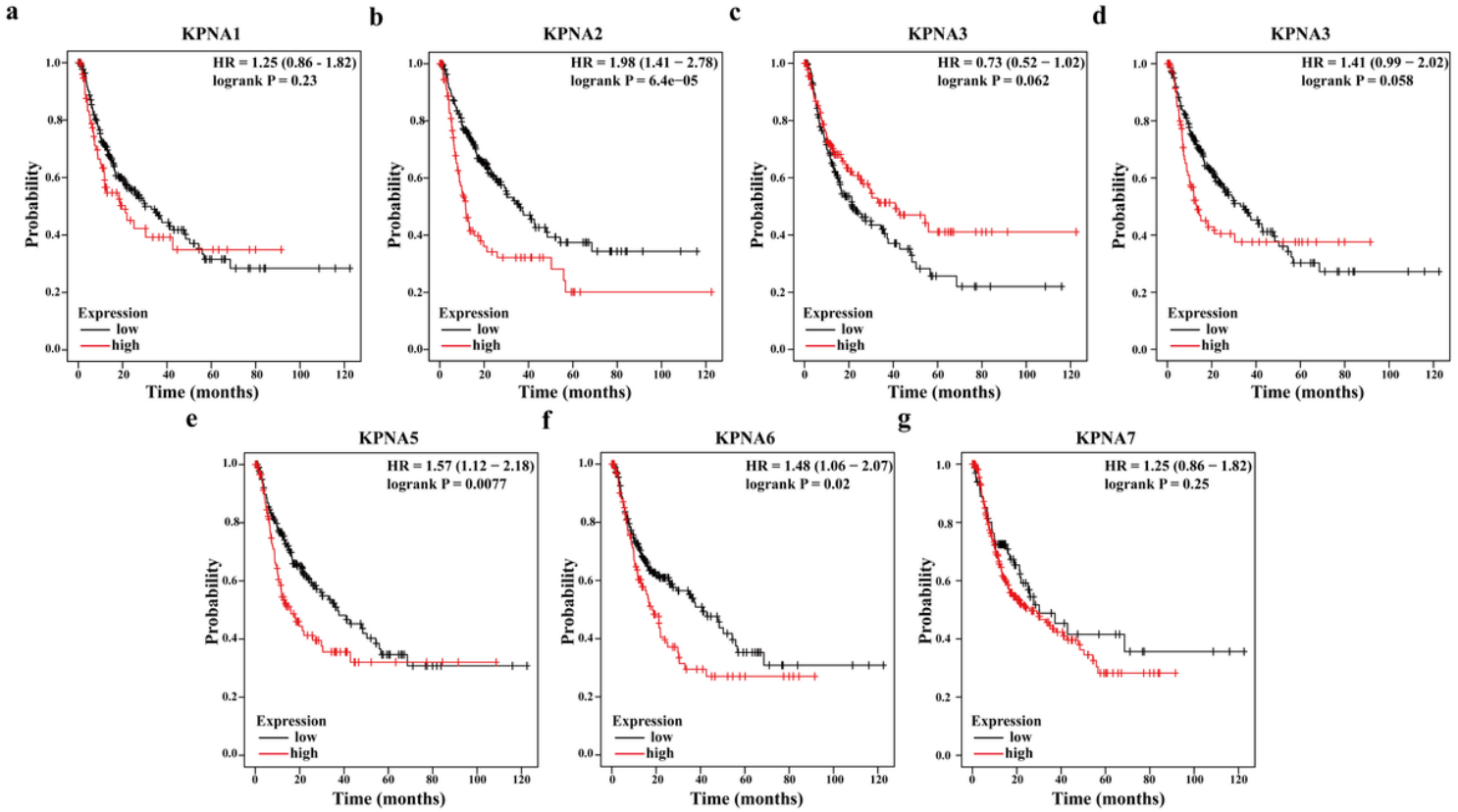

Figure 8

The relationship between mRNA expression level of KPNAs and RFS in HCC patients. (Kaplan-Meier Plotter). HCC: hepatocellular carcinoma; RFS: Relapse Free Survival.

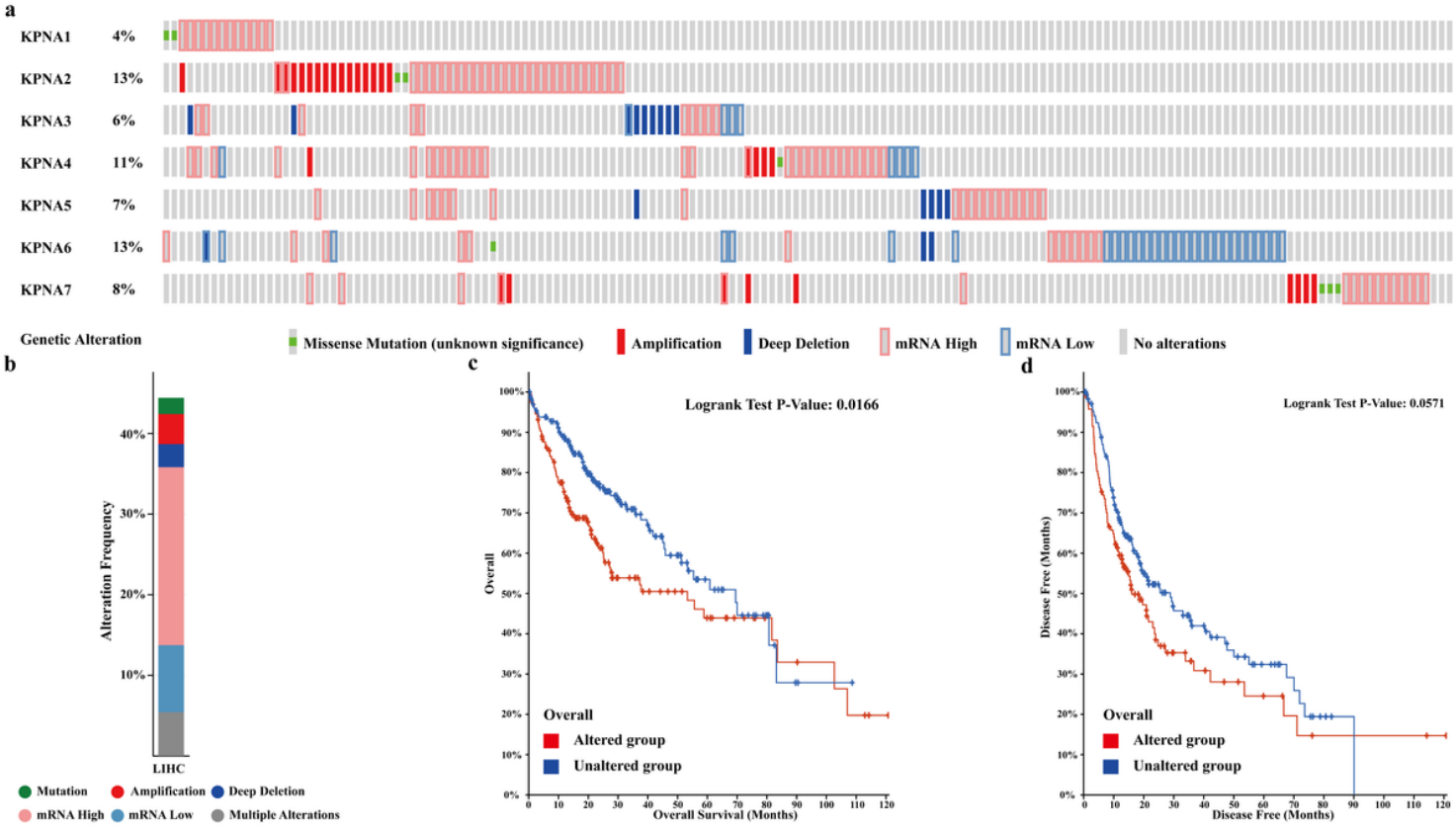

Figure 9

The genetic alteration of KPNAs in HCC and its relationship with prognosis (cBioPortal). (A–B) Summary of KPNAs gene alteration in HCC patients. (C–D) The effect of KPNAs gene alteration on OS and DFS in HCC patients. HCC: hepatocellular carcinoma; OS: overall survival; DFS: disease-free survival.

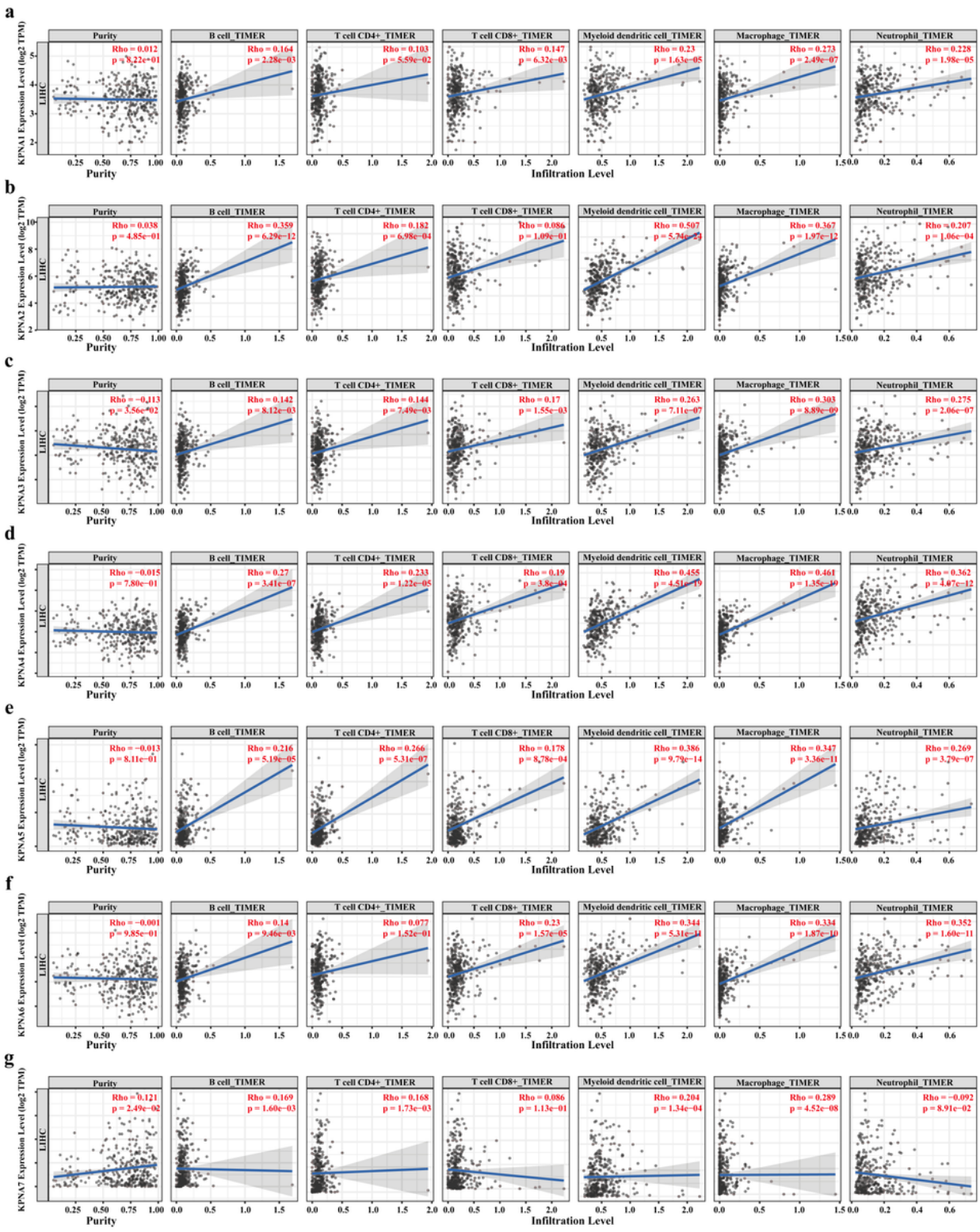

Figure 10

The relationship between the expression level of KPNAs and immune cell infiltration in HCC patients(TIMER). LIHC: liver hepatocellular carcinoma; HCC: hepatocellular carcinoma. p<0.05 is considered statistically significant.

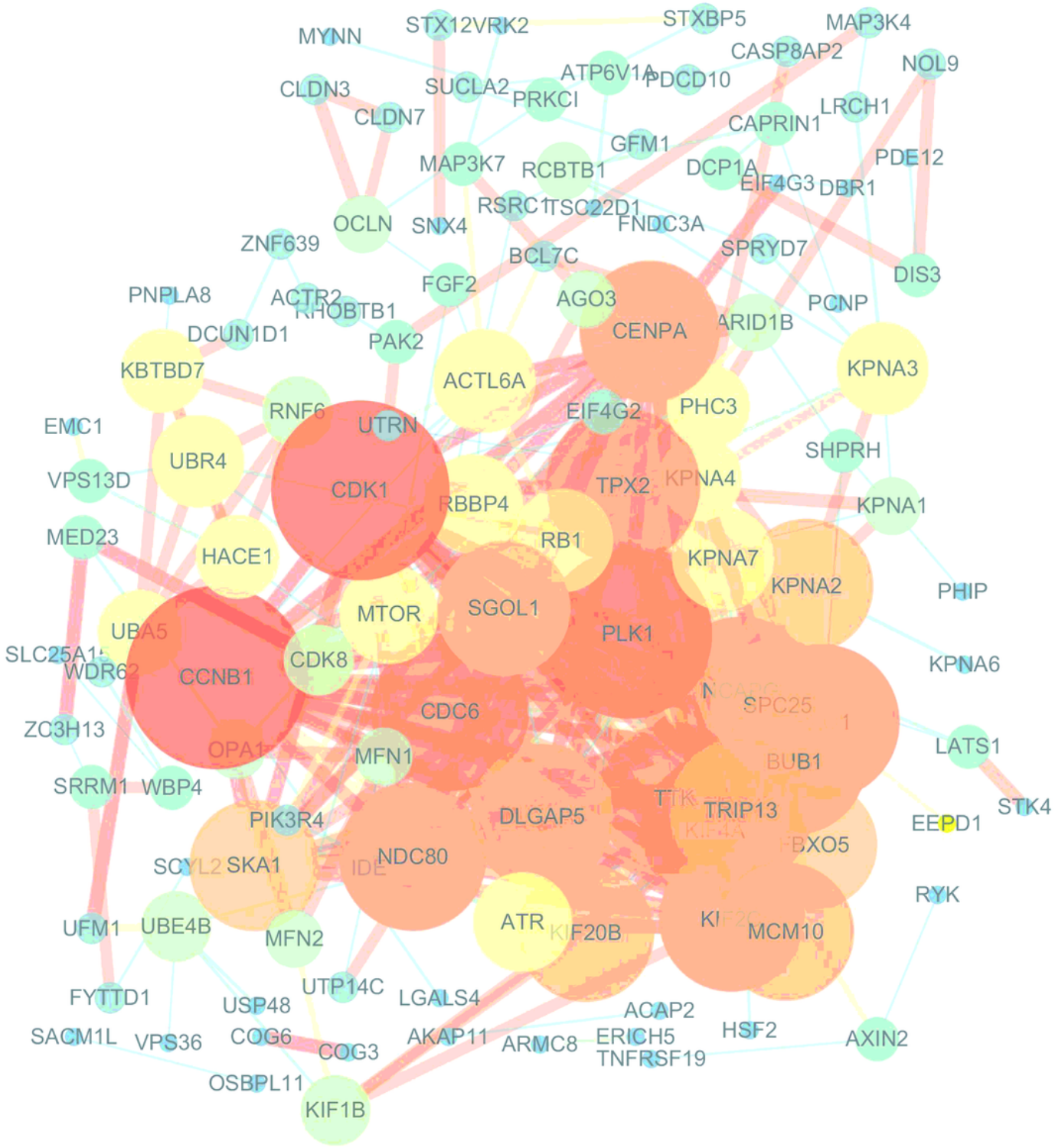

Figure 11

PPI network diagram of KPNAs related genes. PPI: protein-protein interaction.

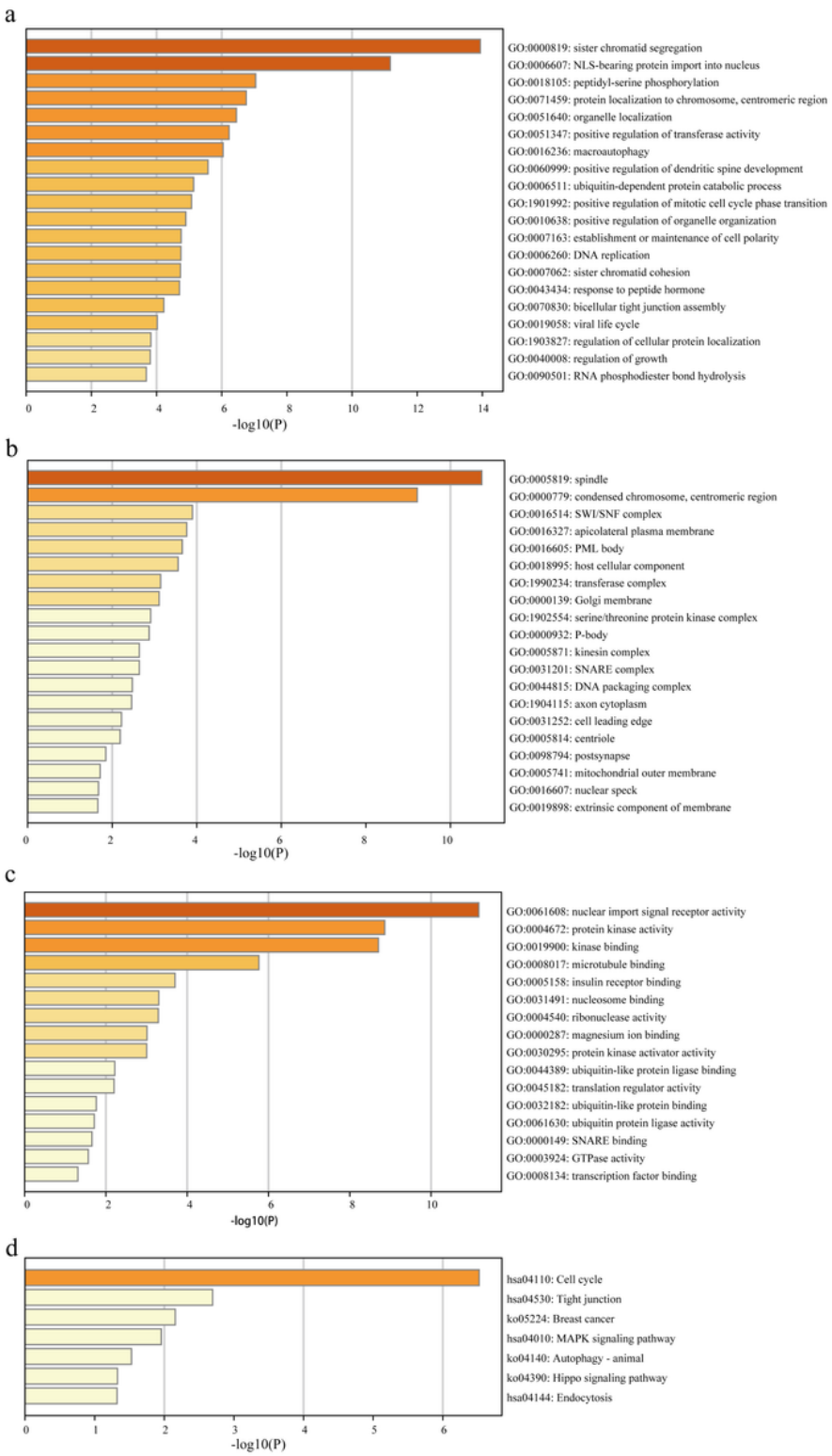

Figure 12

Enrichment analysis of GO and KEGG pathways of KPNAs-related genes in HCC patients. (A-C): Analysis of the biological process, cell composition and molecular function of KPNAs related genes, (D): Analysis of Signal Pathway of KPNAs Related Genes. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes