Prognostic value of members of the HSP70 family in Hepatocellular Carcinoma

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Abstract

BACKGROUND: Despite multiple functions in the disease, the prognosis of the heat shock protein (HSP) 70 family in Hepatocellular Carcinoma (HCC) remains unclear.

METHODS: The UALCAN database has provided information about the expression level of HSP70 family members in both HCC and in normal tissues. Overall survival (OS) was conducted by Kaplan-Meier plotter (KM plotter). Gene ontology (GO) and KEGG pathway enrichment analyses were accomplished by DAVID database. GeneMANIA and STRING were applied to construct gene-gene and protein-protein interaction (PPI) networks.

RESULTS: From the UALCAN database, we found that the expression levels of eight members of the HSP70 family in HCC patients were higher than in normal liver tissues. These eight members were, namely, HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA5, HSPA6, HSPA8 and HSPA9. From KM plotter database, high expression of HSPA1A, HSPA1B, HSPA6 and HSPA8 has been observed to be associated with worse OS in patients with HCC (hazard ratio [HR] =1.49, 95% confidence interval [CI]: 1.03-2.15, P=0.031; HR=1.49, 95%CI: 1.05-2.12, P=0.026; HR=1.53, 95%CI: 1.06-2.2, P=0.021 and HR=1.81, 95%CI: 1.21-2.71, P=0.0036, respectively). We also found that the high expressions of HSPA1A, HSPA1B, HSPA6 and HSPA8 were related to unfavorable OS of HCC patients with other factors such as clinical stage, gender, race and hepatitis virus. From GO and KEEG analysis, we detected a close relationship between these eight genes and Biological process, Cellular component, Molecular function and cancer related signaling pathways. The PPI network and GeneMANIA results showed that there was a strong co-expression relationship between the protein homology and the genes.

CONCLUSIONS: According to previous studies and analysis, when HSPA1A, HSPA1B, HSPA6 and HSPA8 were highly expressed in HCC patients, they generally demonstrated lower OS. Therefore, the members of HSP70 family are likely to be used as drug targets and prognostic biomarkers when treating HCC patients.

Introduction

Amongst one of the top six cancers prevalent across the world, hepatocellular carcinoma (HCC) is predicted to cause 782,000 deaths worldwide. The number of newly diagnosed HCC cases keeps growing at a rate of 841,000 as per 2018 [1].The data from Cancer Statistics (2015), have shown that HCC is ranked as the fifth most aggressive and common cancer types across China, with about 466 thousand new cases and 422 thousand deaths annually. However, the prognosis of HCC patients has remained to be unsatisfactory [2]. Even though during the past few years, the cancer diagnosis and treatment techniques have advanced considerably, the five-year OS performance of HCC patients still remains to be improved. Hence, there is an urgent need to conduct further studies on the initiation and developmental mechanisms of HCC, and to identify more effective drug targets and prognostic biomarkers to enhance the prognosis in patients using more specific therapeutic approaches.

The HSP70 family is composed of about 70-kDa molecular chaperones and has a highly conserved domain structure: including 15-kDa substrate-binding domain, 45-kDa N-terminal ATPase domain, C-terminal domain and 10-kDa helical lid domain [3, 4]. The HSP70 family is activated by stress, heat, apoptosis, and co-chaperones, which play an important role in protein folding, maintaining protein homeostasis, and increasing the survival of cells under various pressures [5, 6].The main eight members of HSP70 have been studied to be closely related to tumors, including HSPA1A (also known as HSPA1), HSPA1B (also known as HSP70-2), HSPA1L, HSPA2, HSPA5, HSPA6 (also known as HSP70B), HSPA8 and HSPA9[7]. Previous studies suggested that HSP70 was highly expressed in numerous cancer types, including liver, colorectal, breast, prostate and bladder carcinomas [8-12]. A prognostic effect has been detected in highly expressed HSP70 proteins [13, 14]. It is evident that protein of the HSP70 family is closely associated with tumors, but whether HSP70 has a prognostic impact on HCC is still unclear.

In this study, analysis data from the KM plotter database (http://kmplot.com/analysis/index.php?p=service&cancer=liver accessed February 16, 2020) was collected to evaluate the OS performance in HCC patients in correlation with expression of HSP70. This database currently includes the clinical outcome and expression levels of several types of cancers [15-18]. The information collected from this database might as well be applied to the analysis of specific genes which may have an influence on the overall survival (OS) of the HCC patients. In the past, a large number of researchers have identified and validated numerous genes which affect the mechanism of HCC, with the usage of the KM plotter database. Therefore, the KM plotter database is applied in our study for the evaluation of HSP70's potential to be used as drug targets and prognostic biomarkers.

UALCAN (http://ualcan.path.uab.edu accessed February 16, 2020) is a new data portal which has been established on the level 3 RNA-sequencing as well as the clinical information of thirty-one types of cancer provided by the Cancer Genome Atlas (TCGA). With the information provided by UALCAN, researchers are now able to conduct analysis on the expression of a target gene based on the samples of both normal and cancerous tissues, to evaluate how the gene expression level and clinicopathological features might impact the OS of cancer patients, and to discover new valuable genes for various types of cancers [19].

Materials And Methods

It is the Gene Expression Omnibus (GEO) [20-22], the Cancer Biomedical Informatics Grid (CBIG) and The Cancer Genome Atlas (TCGA) databases that provided the data for HCC patients which are included in the KM plotter database. It was the GEO database that has provided the datasets of HCC cases below: GSE9843 and GSE20017(http://kmplot.com/analysis/index.php?p=service&cancer=liver accessed February 16,2020)[23].

The UALCAN database (http://ualcan.path.uab.edu/analysis.html accessed February 16,2020) provided the information about individual expression levels of members of HSP70 in both HCC and in adjacent normal tissues.

The OS of patients with HCC was performed by the KM plotter database. Outcomes with P-values less than 0.05 were considered to have statistical significance.

The Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (https://david.ncifcrf.gov/ accessed February 20,2020) [24, 25] was used to perform gene ontology (GO) functional annotation analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) [26, 27] pathway analysis for various members of HSP70. Gene-gene interaction networks were generated with GeneMANIA [28, 29](http://genemania.org/ accessed February 23,2020) and protein-protein interaction networks were constructed with the Search Tool for the Retrieval if Interacting Genes and Proteins (STRING; https://string-db.org/ accessed February 23,2020) [30].

Results

High expression of eight members of the HSP70 family in HCC patient.

The UALCAN web portal was applied to detect the expression levels of the eight members of the HSP70 family in normal and in HCC tissues, respectively. Through the UALCAN, we observed the high expression of HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA5, HSPA6, HSPA8 and HSPA9 mRNA in 371 patients with liver cancer, as compared to their lower expression in 50 normal liver tissues, which was statistically significant (P<0.05, Fig. 1A-H).

Prognostic value of members of HSP70 in HCC from KM plotter database.

Firstly, the prognostic effect of HSPA1A was assessed using the information collected from the dataset, with the valid gene ID 3303(HSPA1A). Curves that described the survival performance were drawn as demonstrated in Fig. 2A (n=364). It turned out that highly expressed HSPA1A would significantly lead to a worse OS performance in patients with HCC ([hazard ratio (HR) =1.49; 95% confidence interval (CI): 1.03-2.15; P=0.031]).

Then, the prognostic effect of HSPA1B was examined using the information collected in this database, with the valid gene ID 3304(HSPA1B). It turned out that highly expressed HSPA1B would significantly lead to a worse OS performance in patients with HCC (HR=1.49; 95%CI: 1.05-2.12; P=0.026; Fig. 2B).

Subsequently, the prognostic effect of HSPA6 was also examined using the information collected from the database, with the valid gene ID 3310 (HSPA6). It turned out that highly expressed HSPA6 would significantly lead to a worse OS performance in patients with HCC (HR=1.53; 95%CI: 1.06-2.2; P=0.021; Fig. 2F).

Furthermore, the prognostic effect of HSPA8's was examined using the information collected from the database, with the valid gene ID 3312(HSPA8). It turned out that highly expressed HSPA8 would significantly lead to a worse OS performance in patients with HCC (HR=1.81; 95%CI: 1.21-2.71; P=0.0036; Fig. 2G).

In contrast, no significant correlation has been detected between the high expression of HSPA1L, HSPA2, HSPA5 and HSPA9 and OS performance in patients with HCC. Their ID, HR, CI and *P*- values are as follows: The HSPA1L gene ID was 3305, HR =1.2 (95% CI: 0.84–1.72), *P*=0.32 (Figure. 2C). The HSPA2 gene ID was 3306, HR =1.2 (95% CI: 0.81–1.76), *P*=0.36 (Figure. 2D). The HSPA5 gene ID was 3309, HR =1.25 (95% CI: 0.86–1.81), *P*=0.24(Figure. 2E). The HSPA9 gene ID was 3313, HR =0.72 (95% CI: 0.49-1.04), *P*=0.078(Figure. 2H).

In summary, a significant correlation was detected between the members of the HSP70 family, showing high expressions of HSPA1A, HSPA1B, HSPA6 and HSPA8 were associated with OS of HCC patients, while this correlation was not significant between the OS performance of HCC patients and the high expression of HSPA1L, HSPA2, HSPA5 and HSPA9.

Prognostic value of members of HSP70 in HCC from clinicopathological characteristics .

Besides, we further analyzed the related factors influencing the prognosis of HCC from clinicopathological characteristics. Including clinical stage (Table 1), cancer grade (Table 2), AJCC-T (Table 3), vascular invasion (Table 4), gender (Table 5), race (Table 6), alcohol consumption (Table 7), hepatitis virus (Table 8). From table 1, we found that high expression of HSPA1A、HSPA2、HSPA6、HSPA8 and HSPA9 was connected with poor OS in stage I HCC patients; High expression of HSPA1A and HSPA2 with Stage II had a similar outcome. In table 2, high HSPA1A, HSPA1L and HSPA8 mRNA expression was correlated with a poor prognosis for HCC patients with Grade 1; High expression of HSPA6 was associated with a worse OS for HCC patients with Grade 2; but the high expressions of HSPA5 and HSPA6 showed correlations with better OS for HCC patients with Grade 1. The results in table 3 illuminated that high expression of HSPA6 and HSPA8 was related to unfavorable OS of HCC patients with AJCC-T 1; High expression of HSPA1A with AJCC-T 1 showed a similar outcome. As shown in Table 4, High expression of HSPA8 was relevant to poor OS of HCC patients with no vascular invasion; On the contrary, High expression of HSPA8 showed a good OS from HCC patients with micro vascular invasion. As presented in Table 5, High expression of HSPA1A, HSPA1B, HSPA1L and HSPA6 had poor prognosis in female HCC patients; High expression of HSPA8 was associated with a poor prognosis for HCC male patients; In contrast, high expression of HSPA8 was associated with a better prognosis for HCC female patients. In table 6 we further investigated that high expression of HSPA1A, HSPA1B, HSPA1L, HSPA5 and HSPA8 was correlated with poor OS of white HCC patients; High expression of HSPA6 was relevant to worse OS in both white and Asian patients of HCC. In table 7, High expression of HSPA1L was relevant to worse OS for patients of HCC with alcohol consumption; Whether drinking or not, high expression of HSPA6 was associated with poor OS in HCC patients; High expression of HSPA9 showed a worse OS in HCC patients without alcohol consumption. Lastly table 8 demonstrated that high expression of HSPA1B was connected with worse OS in HCC patients, Whether or not infected with hepatitis virus; High expression of HSPA6 and HSPA8 was related to poor OS in HCC patients infected with hepatitis virus; High expression of HSPA1A, HSPA1L and HSPA9 had a connection with poor OS in HCC patients not infected with hepatitis virus.

GO functional annotation analysis and KEGG pathway analysis for members of HSP70.

Functional enrichment analysis together with the corresponding genes was investigated for the members of HSP70, with the usage of KEGG and GO analyses, conducted with DAVID, in which the biological processes (BPs), cellular components (CCs) and molecular functions (MFs) were predicted via GO analysis. According to the analysis, BPs included such as GO:1903265 positive regulation of tumor necrosis factor-mediated signaling pathway, GO:1902236 negative regulation of endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway, GO:2001240 negative regulation of extrinsic apoptotic signaling pathway in absence of ligand, GO:0030512 negative regulation of transforming growth factor beta receptor signaling pathway, and so on (Figure. 3A). CPs, such as GO:0005913 cell-cell adherens junction, GO:0000151 ubiquitin ligase complex, GO:0005739 mitochondrion,GO:0005829 cytosol,GO:0031012 extracellular matrix,GO:0016234 inclusion body,GO:0070062 extracellular exosome (Figure. 3B) were observed. MFs, such as GO:0031625 ubiquitin protein ligase binding,GO:0005524 ATP binding, GO:0019899 enzyme binding, GO:0016887 ATPase activity, GO:0098641 cadherin binding involved in cell-cell adhesion,GO:0001664 G-protein coupled receptor binding, GO:0044183 protein binding involved in protein folding (Figure. 3C) were detected. KEGG analysis such as, hsa04141:Protein processing in endoplasmic reticulum,hsa04612:Antigen processing and presentation, hsa04010:MAPK signaling pathway (Figure. 3D) were detected.

The PPI network was established with 13 nodes and 59 edges. Moreover, there was a strong co-expression relationship between the genes.

STRING (Figure. 4A) and GeneMANIA (Figure. 4B) were used in the construction of PPI and gene-gene interaction networks, respectively. The findings suggested that members of the HSP70 family interacted with each other in a complex manner. Besides, the co-expression of the HSP70 sub-members was also implied.

Discussion

As discussed previously, hepatocellular carcinoma (HCC) is a common cancer type, characterized with high incidence rates and severe aggressiveness. HCC patients generally have poor performance in five-year OS [1]. HSP70 was reported to be highly expressed in a number of cancer types, like esophagus, bladder, prostate, lung and colorectal cancers [9, 12, 31-33]. Chuma *et al* have found that HSP70 had the potential to be applied in the development of a sensitive marker to diagnose HCC in an early stage, from non-cancerous liver tissues to pre-cancerous lesion [34]. Gehrmann *et al* found that amongst 143 patients with serum HSP70, including 47 liver cancer patients, 46 cirrhosis patients and 50 chronic hepatitis patients, a significantly higher expression level of HSP70 was detected in the tissues of patients with liver diseases as compared to healthy individuals [35]. Our study also found the eight major sub-members of the HSP70 family (HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA5, HSPA6, HSPA8 and HSPA9) were highly expressed in 371 patients with HCC, which were consistent with the above reports. It is therefore

suggested that members of HSP70 family may be used as a diagnostic indicator for treating HCC in the future.

We previously found that eight members of the HSP70 family were highly expressed in HCC. Next, we will discuss the prognostic value of the high expression of HSP70 family members in HCC. First, HSPA1A has been shown to be a biomarker for tumor prediction. Jakobsson et al analyzed 53 patients with high-grade serous ovarian cancer using a protein mass spectrometer and found that unmethylated HSPA1 may be a prognostic marker for high-grade serous ovarian cancer patients[36]. Chen et al analyzed the expression of LLIM and SH3 protein 1 (LASP1) and HSPA1A in head and neck squamous cell carcinoma (HNSCC) by quantitative PCR and Western blot, and found that LASP1 and HSPA1A were highly expressed in HNSCC and were associated with poor OS of HNSCC patients[37]. HSPA1A has not been reported in the prognosis of HCC. In our present study, the high expression of HSPA1A in HCC patients tend to present a worse OS performance, and especially for HCC patients with Stage I+II, Grade 1, AJCC-T 2, white and female. Previous researches have reported the relationship between HSPA1B expression and prognosis in malignant tumor patients. Song's study indicated that Hepatitis B virus regulated HCC cell growth through microrna-340-5p activation of transcription factor 7 (ATF7)/HSPA1B axis, leading to increased cell proliferation and decreased apoptosis in HCC [38]. Another study also showed the relationship between lung cancer patients labeled with single nucleotide polymorphisms and the OS performance of 330 non-small cell lung cancer patients was evaluated, and the findings indicated the association between the variation of functional HSPA1B and the incidence risk and prognosis of lung cancer [39]. In our study, a high expression of HSPA1B was markedly related to an unfavorable OS in HCC patients and in particular for female and white patients, but was not associated with Stage I-IV, Grade 1-4 , AJCC-T 1-4 and hepatitis virus HCC patients. Some studies had found that HSPA6 mRNA expression was significantly related to the prognosis of malignant tumors. Yang *et al* showed the high expression of HSPA6 with earlier recurrence of HBV-related HCC [40]. In our present study, high expression of HSPA6 mRNA was associated with worse OS for HCC patients, especially for HCC patients with Stage I, Grade 2, AJCC-T 1, female and hepatitis virus. So far, there have been few reports on the prognosis of HSPA8.In our study, a high expression of HSPA1B was connected with a poor OS in patients with HCC in particular for male, white, hepatitis virus, Stage I, Grade 3 and AJCC-T 1. Overall, it is fair to conclude that highly expressed HSPA1A, HSPA1B, HSPA6 and HSPA8 mRNA were significantly likely to lead to poor OS performance in patients with HCC.

After discussing the prognostic value of HSP70 family members, we next study the functional enrichment analysis and biological behavior of HSP70 family members. HSP70 is secreted from tumor cells and is of great importance in signaling, especially when it comes to immune and inflammatory responses [41]. HSP70 proteins have been shown to have regulating effects on apoptosis and proliferation of cancer cells, which means they can be applied to the progression and prognosis of HCC as well as other types of malignant tumors [34, 42]. According to the studies of Udono *et al*, tumor-specific antigenic polypeptides in the cells of malignant tumors are bound via HSP70, so that the immune system can recognize them more easily [43]. From GO enrichment analysis, we found that HSP70 was involved in cell composition, biological functions, molecular function and signaling pathway by regulating how cells respond to heat

and oxidative stress, the apoptosis of cells, the ATP metabolic process, antigen processing and presentation and so on. Our report is also consistent with the above research.

Next, we study the signaling pathways that are mainly involved in HSP70 family members. It has been reported previously that HSP70 is involved in several pathways, including MAPK signaling pathway, necrosis tumor factor-mediated signaling pathway, the apoptotic signal pathway, etc. Stankiewicz *et al* found that by primarily oppressing the activation of Bax, HSP70 inhibited cell apoptosis induced by extracellular heat, thus preventing mitochondrias from releasing pro-apoptotic factors [44]. Other study showed two major mechanisms through which the cell apoptosis in colon and pancreatic cancers was oppressed by HSP70: lysosomal stabilization and cytosolic calcium attenuation [45]. Another report showed that in the TNF-induced cell apoptosis of primary culture of human fibroblasts, IMR90, there was specific interference of HSP72 with the Bid-dependent apoptotic pathway by preliminarily inhibiting the stress kinase c-jun N-terminal kinase (JNK)[46]. Zhe *et al* had showed that by activating TLR4 and TLR2, and subsequently activating the intracellular JNK1/2/MAPK signaling pathway, the cell proliferation of HCC tumors can be promoted by extracellular HSP70/HSP70-peptide complexes (HSP70-PCs) [47]. Our study found that HSP70 was involved in the negative regulation of mitochondrial outer membrane permeabilization, involved in apoptotic signaling pathway, negative regulation of endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway, negative regulation of extrinsic apoptotic signaling pathway in absence of ligand, positive regulation of tumor necrosis factor-mediated signaling pathway and MAPK signaling pathway. It is basically consistent with the signaling pathways involved by HSP70 as reported above.

Finally, we further discuss the potential therapeutic value of the HSP70 family. It has been also reported that the vaccine constructed by HSP70 is used to treat liver cancer in mice model expressing alpha-fetoprotein(AFP). Such as, constructing a therapeutic peptide vaccine through connecting AFP epitope with HSP70 function peptide to get HSP70-P/AFP-P. Then, the HSP70-P/AFP-P was administered into BALB/c mice. The natural killer cells and CD8 + T cells in BALB/c mice reacted strongly and had a protective effect on the AFP-producing tumors. Therefore, it is safe to assume that HSP70/AFP-P has a huge potential in the application of the treatment of patients diagnosed with AFP-expressing malignant tumors [48].

Certainly, there are a number of limitations in the present study. Firstly, the number of patients included in liver cancer research is just over 300, which is a relatively small sample size. Secondly, the research only focused on the differences between tissues of HCC patients and normal tissues, and did not investigate further based on gender, age, etiology and clinical stage of patients with HCC. Therefore, a stratified survival analysis for HCC should be conducted with larger and separate samples in the future studies. Our result for this study is solely obtained by using bioinformatics tools, without any experimental verification, so it needs to be verified by large random multi-center sample studies.

Taken together, it can be concluded that HSP70 members, HSPA1A, HSPA1B, HSPA6 and HSPA8, have the potential to be used as effective biomarkers to enhance the OS in patients with HCC. Nevertheless, this

result will also require further confirmation and validation, as it has been obtained solely with the usage of bioinformatics approaches.

Declarations

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Authors' contributions

Haixing Jiang designed the study and performed bioinformatics analysis. Ziyu Liang analyzed the data and wrote the manuscript. Shanyu Qin and Kang Liu analyzed the data. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table1.The prognostic value of eight mRNA expressions in HCC patients with different clinical stage.

Genes	Stage	cases	HR(95%CI)	P-value
HSPA1A	I	170	1.83(1-3.36)	0.047
	II	83	2.93(1.09-7.83)	0.025
	III	83	0.76(0.41-1.42)	0.39
	IV	4	NA	NA
HSPA1B	I	170	1.57(0.77-3.21)	0.21
	II	83	1.71(0.75-3.89)	0.19
	III	83	1.75(0.95-3.23)	0.07
	IV	4	NA	NA
HSPA1L	I	170	0.79(0.41-1.52)	0.47
	II	83	0.59(0.24-1.48)	0.26
	III	83	0.76(0.36-1.59)	0.46
	IV	4	NA	NA
HSPA2	I	170	0.45(0.21-1.01)	0.047
	II	83	3.05(1.32-7.03)	0.006
	III	83	0.75(0.41-1.38)	0.35
	IV	4	NA	NA
HSPA5	I	170	1.78(0.96-3.31)	0.063
	II	83	1.62(0.74-3.54)	0.22
	III	83	0.62(0.31-1.24)	0.17
	IV	4	NA	NA
HSPA6	I	170	2.19(1.19-4.05)	0.01
	II	83	1.9(0.71-5.07)	0.19
	III	83	1.52(0.82-2.81)	0.18
	IV	4	NA	NA
HSPA8	I	170	2.55(1.28-5.09)	0.0059
	II	83	2.43(0.83-7.09)	0.093
	III	83	1.31(0.72-2.38)	0.37
	IV	4	NA	NA
HSPA9	I	170	2.38(1.28-4.44)	0.005
	II	83	0.45(0.15-1.32)	0.13
	III	83	0.24(0.13-0.47)	5.2e-6
	IV	4	NA	NA

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval; NA, not available.

Table 2.The prognostic value of eight mRNA expressions in HCC patients with different cancer grade.

Genes	Grade	cases	HR(95%CI)	P-value
HSPA1A	1	55	3.16(1.2-8.35)	0.015
	2	174	0.62(0.34-1.14)	0.12
	3	118	0.75(0.41-1.37)	0.35
	4	12	NA	NA
HSPA1B	1	55	1.86(0.68-5.09)	0.22
	2	174	0.57(0.3-1.08)	0.081
	3	118	1.49(0.76-2.91)	0.24
	4	12	NA	NA
HSPA1L	1	55	7.03(1.87-26.4)	0.00086
	2	174	1.32(0.79-2.23)	0.29
	3	118	0.53(0.28-0.98)	0.039
	4	12	NA	NA
HSPA2	1	55	0.58(0.18-1.81)	0.34
	2	174	1.55(0.91-2.65)	0.11
	3	118	1.53(0.8-2.95)	0.2
	4	12	NA	NA
HSPA5	1	55	0.22(0.08-0.66)	0.0029
	2	174	1.4(0.8-2.44)	0.23
	3	118	2.19(0.97-4.93)	0.053
	4	12	NA	NA
HSPA6	1	55	0.38(0.14-1.02)	0.045
	2	174	1.69(1.01-2.83)	0.045
	3	118	2.06(0.96-4.45)	0.059
	4	12	NA	NA
HSPA8	1	55	2.54(0.96-6.72)	0.052
	2	174	1.49(0.82-2.72)	0.19
	3	118	3.3(1.71-6.37)	0.00016
	4	12	NA	NA
HSPA9	1	55	0.4(0.15-1.06)	0.056
	2	174	1.37(0.82-2.28)	0.22
	3	118	0.57(0.31-1.07)	0.079
	4	12	NA	NA

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval; NA, not available.

Table 3.The prognostic value of eight mRNA expressions in HCC patients with different AJCC_T.

Genes	AJCC_T	cases	HR(95%CI)	P-value
HSPA1A	1	180	2.09(0.89-4.94)	0.085
	2	90	3.08(1.25-7.59)	0.01
	3	78	0.73(0.4-1.34)	0.31
	4	13	NA	NA
HSPA1B	1	180	1.57(0.79-3.1)	0.19
	2	90	1.93(0.9-4.16)	0.087
	3	78	1.77(0.95-3.31)	0.071
	4	13	NA	NA
HSPA1L	1	180	0.79(0.42-1.49)	0.47
	2	90	0.65(0.28-1.51)	0.31
	3	78	0.75(0.41-1.38)	0.35
	4	13	NA	NA
HSPA2	1	180	0.51(0.25-1.06)	0.066
	2	90	2.15(1.04-4.44)	0.035
	3	78	0.73(0.39-1.37)	0.33
	4	13	NA	NA
HSPA5	1	180	1.78(0.99-3.21)	0.052
	2	90	1.46(0.69-3.09)	0.32
	3	78	0.62(0.3-1.26)	0.18
	4	13	NA	NA
HSPA6	1	180	2.34(1.31-4.19)	0.0032
	2	90	1.72(0.81-3.65)	0.15
	3	78	1.7(0.9-3.21)	0.1
	4	13	NA	NA
HSPA8	1	180	2.69(1.36-5.31)	0.003
	2	90	1.66(0.71-3.88)	0.24
	3	78	0.77(0.42-1.4)	0.39
	4	13	NA	NA
HSPA9	1	180	2.4(1.32-4.34)	0.003
	2	90	0.41(0.14-1.18)	0.087
	3	78	0.27(0.14-0.53)	4.7e-5
	4	13	NA	NA

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval; NA, not available; AJCC, American Joint Committee on Cancer.

Table 4.The prognostic value of eight mRNA expressions in HCC patients with vascular invasion.

Genes	Vascular invasion	Cases	HR(95%CI)	P-value
HSPA1A	None	203	1.31(0.77-2.23)	0.32
	Micro	90	1.58(0.7-3.56)	0.26
HSPA1B	None	203	1.31(0.78-2.2)	0.31
	Micro	90	0.55(0.21-1.46)	0.22
HSPA1L	None	203	1.7(1.01-2.87)	0.043
	Micro	90	0.33(0.14-0.77)	0.0066
HSPA2	None	203	1.79(1-3.22)	0.049
	Micro	90	0.73(0.34-1.55)	0.41
HSPA5	None	203	1.59(0.95-2.67)	0.074
	Micro	90	1.83(0.69-4.87)	0.22
HSPA6	None	203	1.67(0.98-2.86)	0.058
	Micro	90	3.2(1.1-9.31)	0.024
HSPA8	None	203	2.84(1.51-5.36)	0.00075
	Micro	90	4.25(1-18.09)	0.033
HSPA9	None	203	1.62(0.96-2.73)	0.07
	Micro	90	1.45(0.67-3.13)	0.35

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

Table 5.The prognostic value of eight mRNA expressions in HCC patients with gender.

Genes	Gender	Cases	HR(95%CI)	P-value
HSPA1A	Male	246	0.72(0.43-1.23)	0.23
	Female	118	2.3(1.29-4.08)	0.0035
HSPA1B	Male	246	1.53(0.95-2.48)	0.081
	Female	118	2.01(1.14-3.56)	0.014
HSPA1L	Male	246	0.77(0.49-1.19)	0.23
	Female	118	2.05(1.03-4.11)	0.039
HSPA2	Male	246	1.23(0.79-1.92)	0.36
	Female	118	1.97(0.98-3.97)	0.052
HSPA5	Male	246	1.37(0.85-2.2)	0.19
	Female	118	0.57(0.28-1.15)	0.11
HSPA6	Male	246	1.65(0.96-2.82)	0.067
	Female	118	2.24(1.27-3.95)	0.004
HSPA8	Male	246	2.31(1.48-3.59)	0.00014
	Female	118	0.48(0.23-1)	0.046
HSPA9	Male	246	0.68(0.42-1.11)	0.13
	Female	118	1.61(0.93-2.8)	0.086

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

Table 6.The prognostic value of eight mRNA expressions in HCC patients with race.

Genes	Race	Cases	HR(95%CI)	P-value
HSPA1A	White	181	2.19(1.34-3.57)	0.0013
	Asian	155	0.7(0.38-1.28)	0.24
HSPA1B	White	181	1.77(1.1-2.83)	0.016
	Asian	155	1.81(0.93-3.52)	0.077
HSPA1L	White	181	1.91(1.17-3.12)	0.0086
	Asian	155	0.83(0.46-1.5)	0.54
HSPA2	White	181	0.77(0.46-1.29)	0.33
	Asian	155	1.52(0.84-2.76)	0.16
HSPA5	White	181	0.7(0.42-1.14)	0.15
	Asian	155	2.03(1.02-4.02)	0.038
HSPA6	White	181	1.99(1.23-3.22)	0.0041
	Asian	155	2.44(1.21-4.95)	0.01
HSPA8	White	181	2.06(1.11-3.83)	0.019
	Asian	155	2.41(1.32-4.37)	0.0029
HSPA9	White	181	1.63(1.03-2.56)	0.03
	Asian	155	0.43(0.24-0.8)	0.0056

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

Table 7.The prognostic value of eight mRNA expressions in HCC patients with alcohol consumption.

Genes	Alcohol consumption	Cases	HR(95%CI)	P-value
HSPA1A	Yes	115	1.81(0.9-3.64)	0.092
	None	202	1.6(0.99-2.61)	0.055
HSPA1B	Yes	115	1.82(0.9-3.67)	0.089
	None	202	1.71(1.08-2.72)	0.022
HSPA1L	Yes	115	2.02(1.01-4.01)	0.041
	None	202	0.74(0.44-1.24)	0.25
HSPA2	Yes	115	0.7(0.34-1.44)	0.33
	None	202	0.77(0.47-1.24)	0.27
HSPA5	Yes	115	0.76(0.39-1.49)	0.43
	None	202	1.64(0.93-2.9)	0.083
HSPA6	Yes	115	0.8(0.41-1.56)	0.50
	None	202	1.7(1.06-2.72)	0.027
HSPA8	Yes	115	2.53(1.35-4.76)	0.0028
	None	202	1.97(1.04-3.75)	0.034
HSPA9	Yes	115	0.66(0.33-1.32)	0.24
	None	202	1.87(1.16-3)	0.0089

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

Table 8. The prognostic value of eight mRNA expressions in HCC patients with hepatitis virus.

Genes	Hepatitis virus	Cases	HR(95%CI)	P-value
HSPA1A	Yes	150	1.7(0.84-3.44)	0.14
	None	167	1.77(1-3.12)	0.048
HSPA1B	Yes	150	2.16(1.06-4.39)	0.029
	None	167	1.59(1-2.55)	0.049
HSPA1L	Yes	150	0.51(0.26-0.97)	0.038
	None	167	1.7(1.04-2.76)	0.032
HSPA2	Yes	150	0.64(0.3-1.35)	0.24
	None	167	1.35(0.81-2.25)	0.25
HSPA5	Yes	150	2.39(0.93-6.15)	0.061
	None	167	1.31(0.76-2.25)	0.32
HSPA6	Yes	150	2.98(1.16-7.65)	0.017
	None	167	1.38(0.86-2.24)	0.018
HSPA8	Yes	150	3.49(1.23-9.88)	0.012
	None	167	1.46(0.89-2.4)	0.13
HSPA9	Yes	150	1.91(0.99-3.68)	0.05
	None	167	1.74(1.05-2.89)	0.03

Abbreviation: HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

Figures

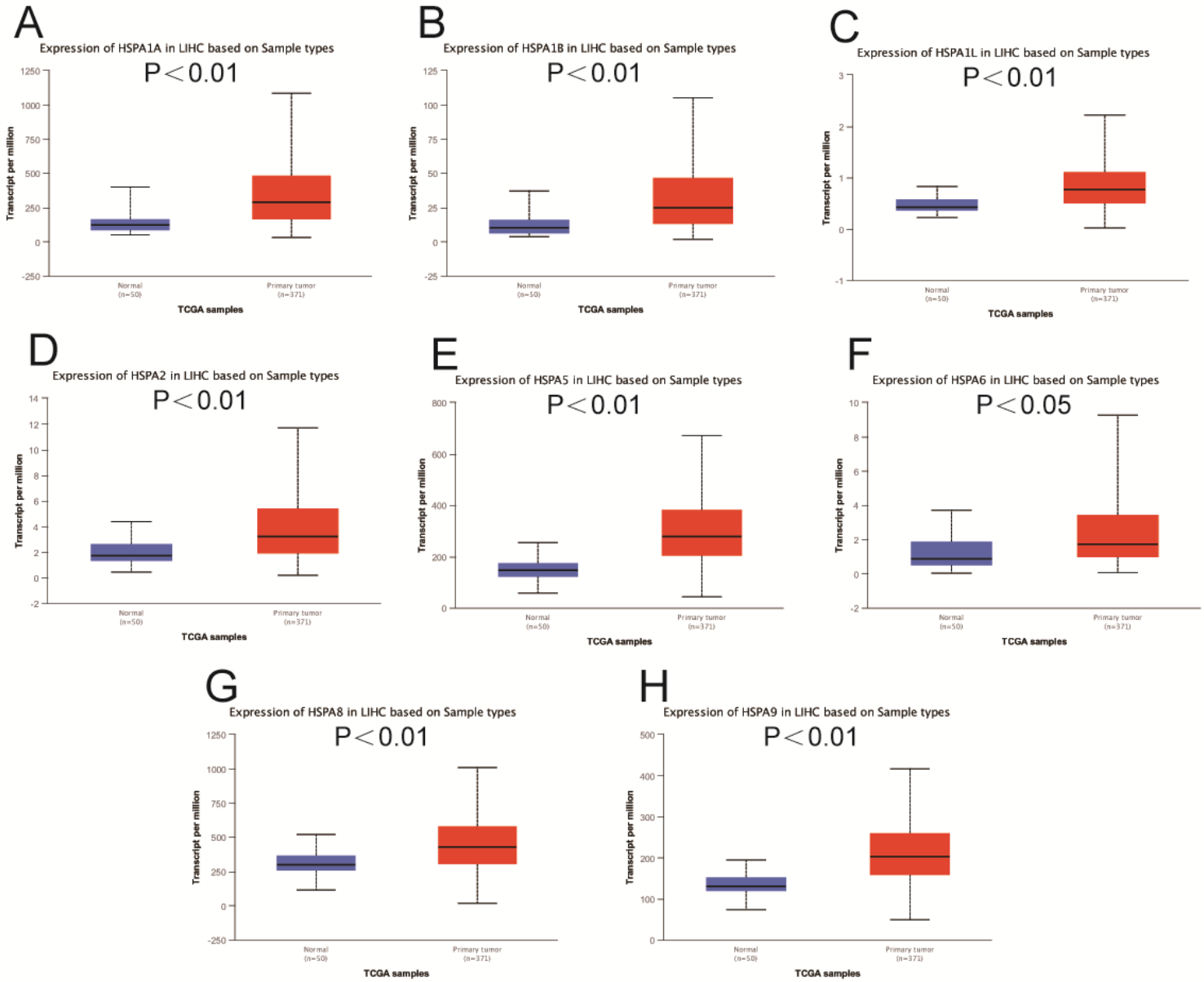

Figure 1

The eight mRNA expression levels of the HSP70 family members in HCC patients and normal liver tissues from UALCAN. High expression of eight members of the HSP70 family in 371 HCC patients compared to 50 normal liver tissues (A=HSPA1A, B=HSPA1B, C=HSPA1L, D=HSPA2, E=HSPA5, F=HSPA6, G=HSPA8, H=HSPA9H). *P<0.05, ** P<0.01.

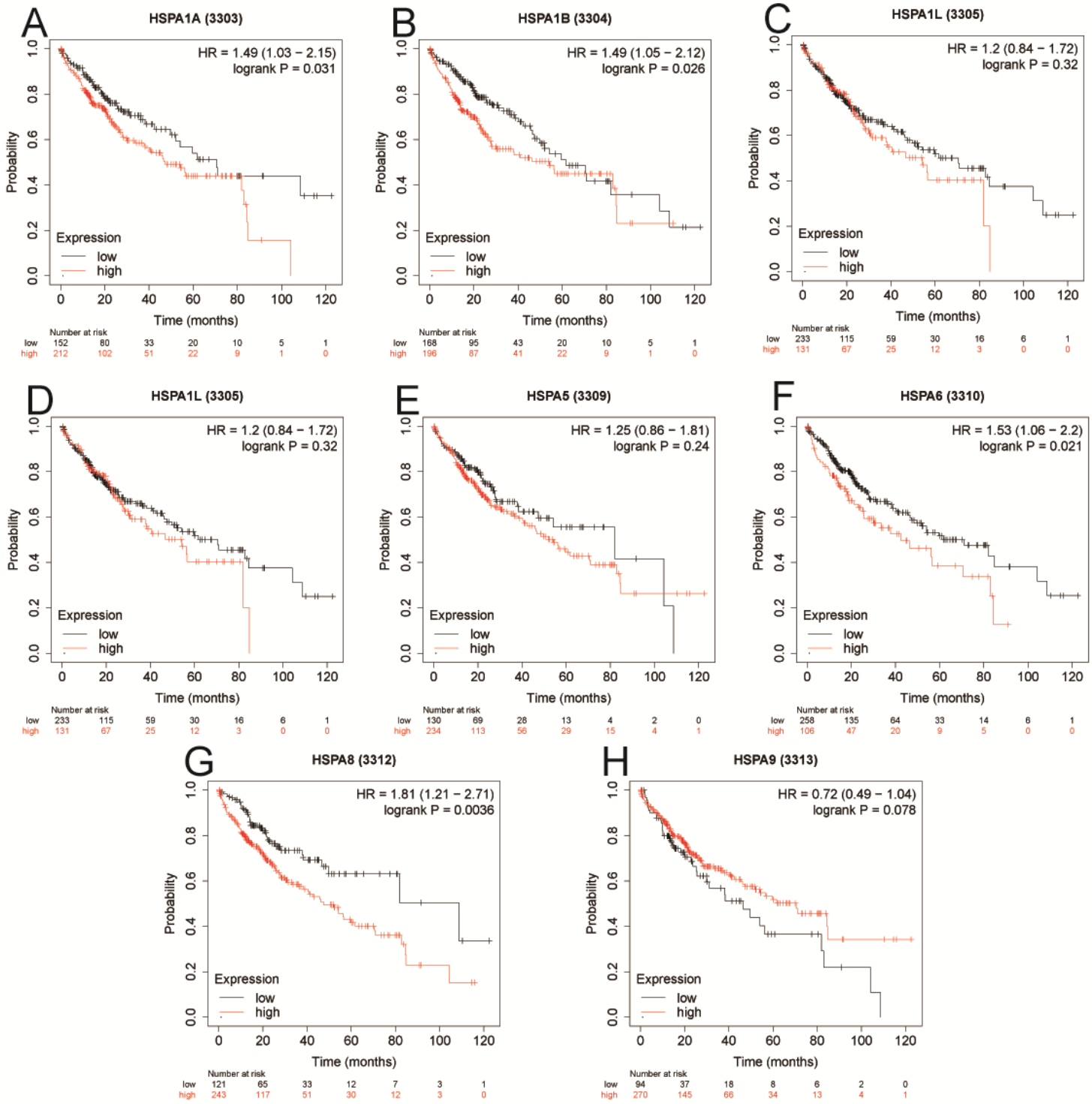

Figure 2

Kaplan-Meier analysis of the prognostic value of eight mRNA expression in HCC patients. High mRNA expressions of HSPA1A, HSPA1B, HSPA6 and HSPA8 were associated with shorter OS of HCC patients (A-B, F-G), while high expressions of HSPA1L, HSPA2, HSPA5 and HSPA9 showed no correlation with prognosis of HCC patients(C-E,H).

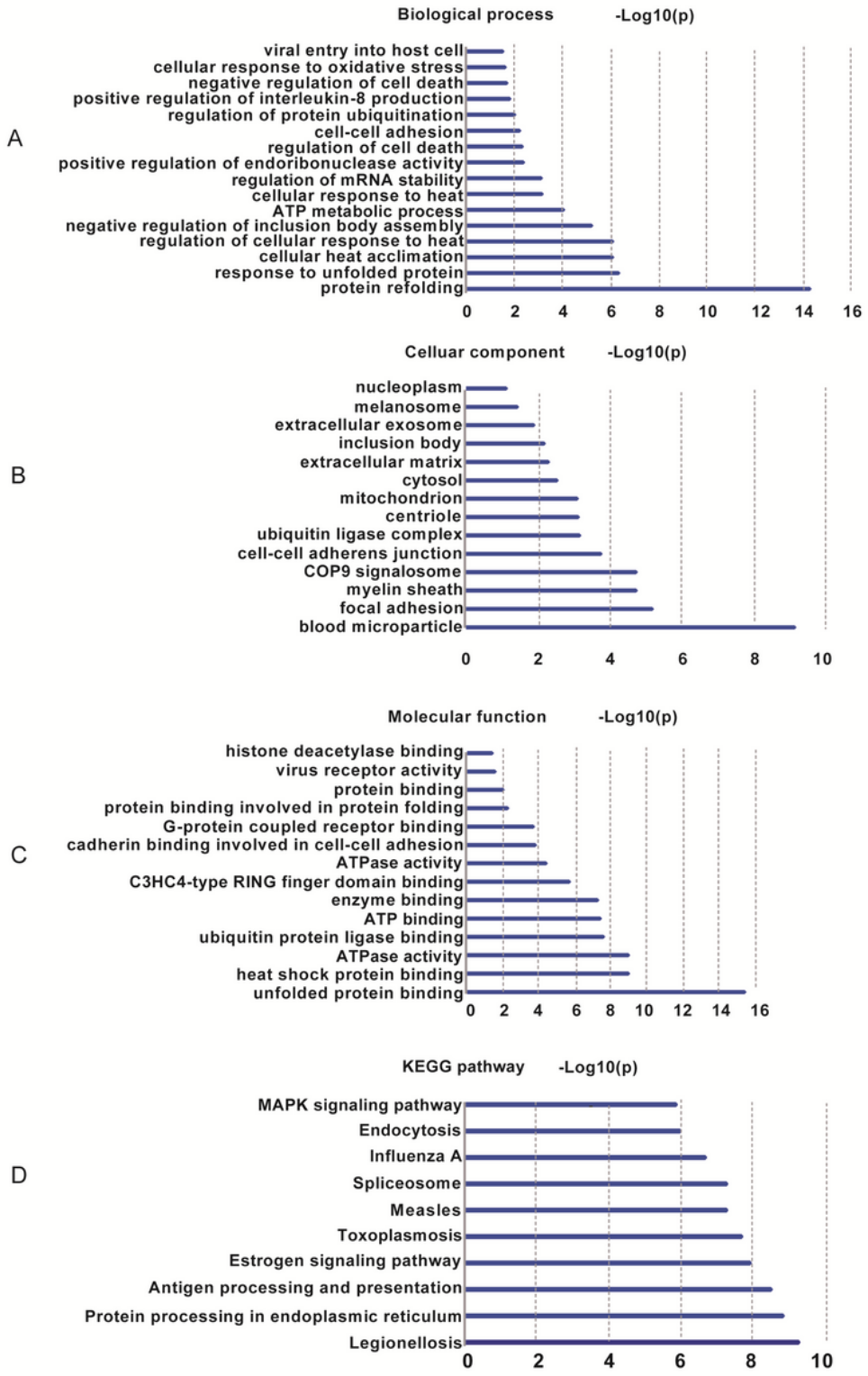

Figure 3

Analysis of GO terms and KEGG performed by DAVID. GO functional enrichment analysis predicted three main functions of eight HSP70 family members, including biological process, cellular components and molecular functions (A-C). KEGG pathway analysis on eight HSP70 family members (D).
mitogen-activated protein kinase (MAPK)

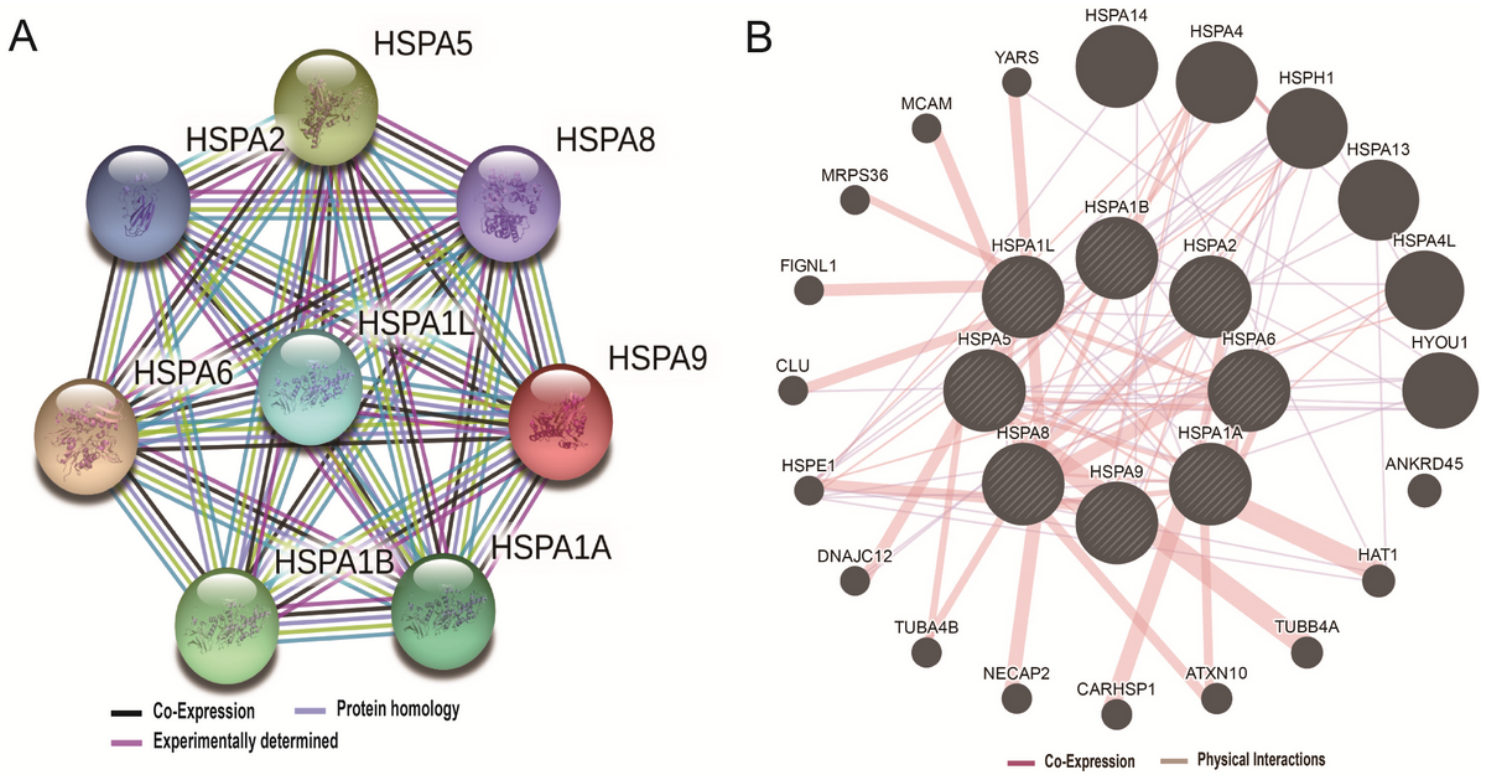

Figure 4

PPI and gene-gene interaction networks were constructed by STRING and GeneMANIA (A-B). It showed a strong co-expression relationship between the protein homology and the genes.