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DATABASE ANALYSIS

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Prognostic Significance of the Hsp70 Gene Family in Colorectal Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Colorectal cancer (CRC) is a deadly form of cancer worldwide. Heat shock protein 70 (*Hsp70*) belongs to the family of human HSPs and plays an essential role in multiple cellular developments and in responding to environmental changes. However, studies on the relationship between CRC and the *Hsp70* family are rare.

Material/Methods: Data pertaining to 438 patients with CRC was downloaded from The Cancer Genome Atlas database. To investigate the prognostic significance of the *Hsp70* genes, survival and joint-effect analyses were conducted. The correlation between prognosis-related *Hsp70* genes and clinical factors in CRC was analyzed using a nomogram. Gene set enrichment analysis (GSEA) was performed to explore the complex enrichment pathway in CRC with the prognosis-related *Hsp70* genes.

Results: According to multivariate Cox regression survival analysis, low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were correlated with improved overall survival (OS). According to the joint-effects survival analysis, the joint low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were related to improved OS. The 1-, 3-, 5-, and 10-year survival rates of patients with CRC were predicted by constructing a nomogram model based on *HSPA1A*, *HSPA1B*, *HSPA1L*, and tumor stage. The GSEA results indicated the biological roles of *HSPA1A*, *HSPA1B*, and *HSPA1L* in CRC.

Conclusions: Low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were strongly correlated with improved prognosis in CRC and might serve as latent prognostic biomarkers in CRC.

Keywords: Colorectal Neoplasms, Hereditary Nonpolyposis • HSP70 Heat-Shock Proteins • Prognosis • Survival Analysis

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Background

Colorectal cancer (CRC) causes many fatalities worldwide. However, timely diagnosis and therapy can slow the progression of CRC [1]. It has been found that during advanced stages of CRC, when metastasis has commenced, carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199) are elevated, and these antigen levels are being utilized in clinical practice with limited effectiveness [2].

Molecular chaperones aid in the dissolution of misfolded proteins. Consequently, they fulfill a crucial physiological role [3]. Heat shock protein 70 (*Hsp70*) is classified under the family of HSPs and has important functions in relation to different cellular processes that respond to environmental changes and survival [4]. Historically, *Hsp70* has been regarded as an essential anti-stress defense system that keeps tumor cells alive. *Hsp70* interacts at key points in cellular apoptotic pathways [5]. Elevated expression of extracellular *Hsp70* is an indicator of a worse prognosis in the cancer process. *Hsp70* inhibition leads to an anti-tumor system activation and apoptotic process in cancer [6]. The human *Hsp70* is a multigene family consisting of 17 genes and 30 pseudogenes [7], and *Hsp70* proteins are most likely related to the functional part of their differentiated C-terminal and N-terminal domains. The selection of the most effective and ideal molecule for anti-chaperone agents is based on the *Hsp70* gene family [8].

Studies have demonstrated that *Hsp70* is the worst independent prognostic factor in primary colon cancer [9], and the clinical value of *Hsp70* overexpression in patients diagnosed with colon cancer has been summarized [10]. However, studies to date have not summarized the prognostic significance of all *Hsp70* family genes in the context of CRC. Hence, this study is aimed at investigating the prognostic significance of *Hsp70* family expression by using data from 438 patients with CRC obtained from The Cancer Genome Atlas (TCGA) database.

Material and Methods

Data Preparation

The TCGA dataset is a substantial network database for researchers (*https://cancergenome.nih.gov/*), which stores information on different genomes of primary tumors and matched normal tissues [11]. In this study, we analyzed data from 438 patients with CRC, which included *Hsp70* gene family expression and clinical data. Scatter plots were generated for the *Hsp70* gene family in CRC and matched normal tissues.

Interaction and Function Analysis of the Hsp70 Gene Family

The Pearson correlation coefficient was used for the correlation analysis of *Hsp70* genes. The coexpression correlation of *Hsp70* genes was performed in GeneMANIA (*www.genemania.org*) [12]. The functional bioinformatics analysis of *Hsp70* genes was conducted using the online tool DAVID (*david.ncifcrf.gov/tools.jsp*) [13].

Survival and Joint-effect Analysis of the Hsp70 Gene Family

Univariate and multivariate Cox proportional hazard ratios (HRs) were used to determine the effects of all *Hsp70* gene expressions on overall survival (OS). Adjustments included patient

Table 1. The clinical data for 438 patients with colorectal cancer.

Variables	Patients (n=438)	No. of events (%)	MST (days)	HR (95% CI)	Log-rank *P*
Age (years)					
<60	122	81.1	3039	Ref.	0.398
≥60	316	76.3	2535	1.223 (0.766-1.952)	
Sex					
Female	204	78.4	2990	Ref.	0.545
Male	234	76.9	2320	1.131 (0.759-1.686)	
TNM stage					
I	73	94.5	3234	Ref.	<0.001
II	167	83.8	2838	2.24 (0.781-6.421)	
III	126	75.4	2856	4.068 (1.434-11.538)	
IV	61	49.2	1114	11.291 (3.980-32.026)	
Missing	11				

MST – median survival time; HR – hazard ratio; CI – confidence interval; TNM – tumor-node-metastasis.

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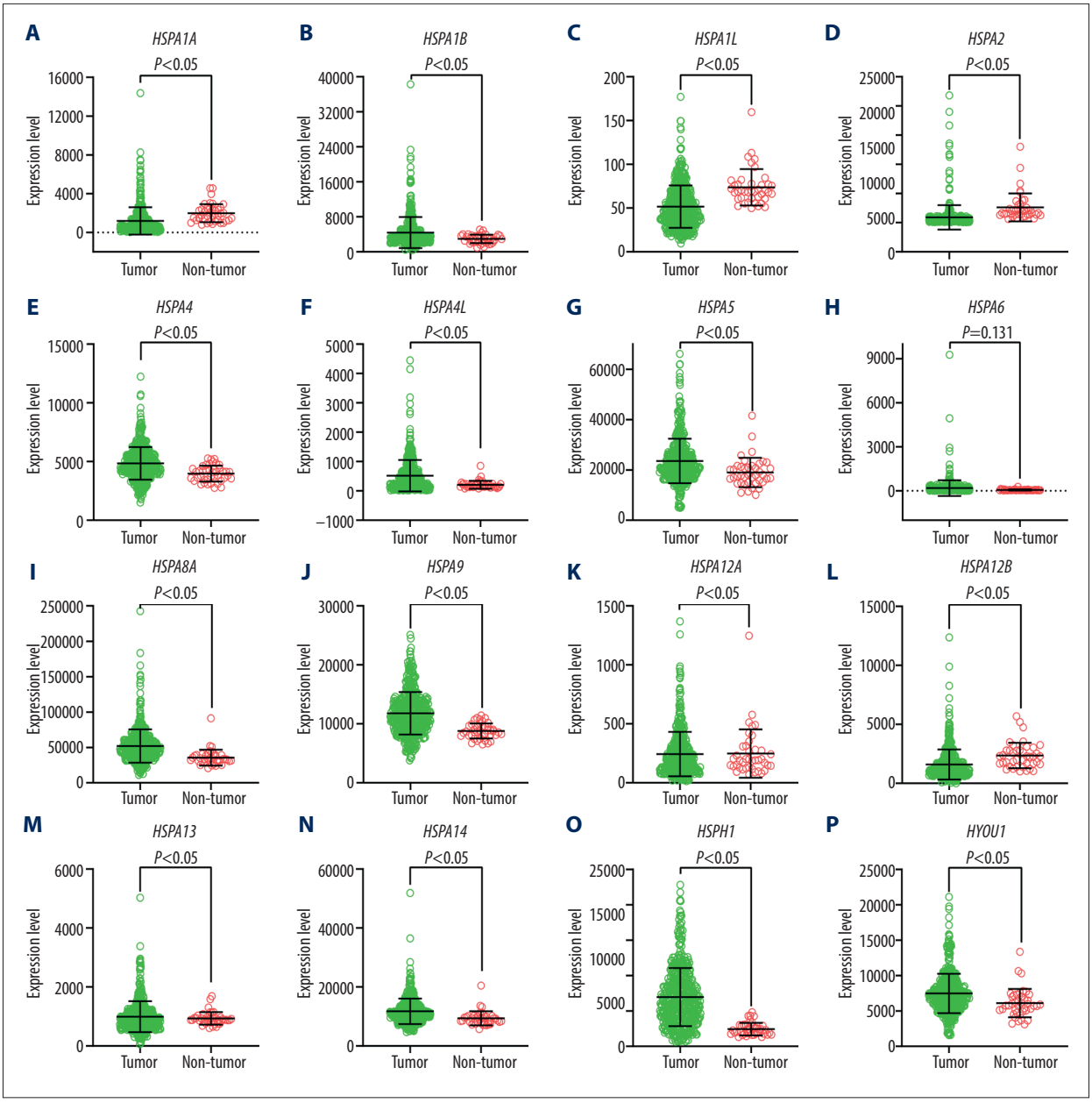

Figure 1. Expression levels of *Hsp70* genes in colorectal cancer and normal colon tissue. (**A**) *HSPA1A*; (**B**) *HSPA1B*; (**C**) *HSPA1L*; (**D**) *HSPA2*; (**E**) *HSPA4*; (**F**) *HSPA4L*; (**G**) *HSPA5*; (**H**) *HSPA6*; (**I**) *HSPA8*; (**J**) *HSPA9*; (**K**) *HSPA12A*; (**L**) *HSPA12B*; (**M**) *HSPA13*; (**N**) *HSPA14*; (**O**) *HSPH1*; (**P**) *HYOU1*.

tumor-node-metastasis (TNM) stage, age, and sex. Following this, joint-effect analysis was conducted with the significant *Hsp70* genes that exhibited prognostic value for CRC.

Nomogram

A nomogram was formulated for the prognosis-related *Hsp70* genes and clinical factors in CRC. The 1-year, 3-year, 5-year, and 10-year survival rates in CRC patients were predicted using the nomogram [14].

Gene set Enrichment Analysis

Gene set enrichment analysis (GSEA) v.3.0 (*http://software.broadinstitute.org/gsea/msigdb/index.jsp*) was used to analyze the enrichment pathway in CRC with the prognosis-related *Hsp70* genes [15]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) datasets were used for analysis. Statistical significance was indicated by $P<0.05$ and a false discovery rate <0.25.

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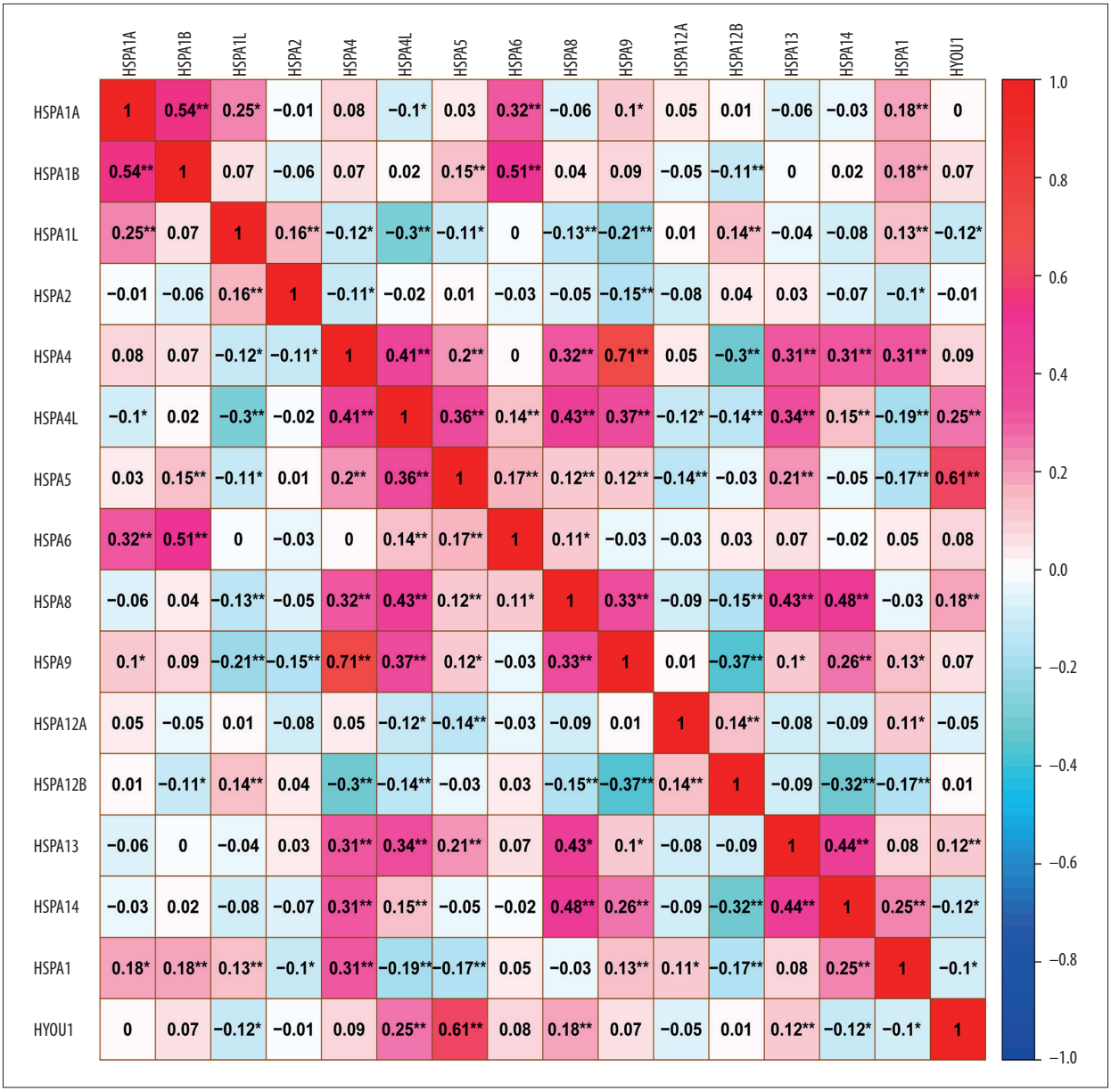

Figure 2. Pearson's correlation analysis for *HSPA1A*, *HSPA1B*, *HSPA1L*, *HSPA2*, *HSPA4*, *HSPA4L*, *HSPA5*, *HSPA6*, *HSPA8*, *HSPA9*, *HSPA12A*, *HSPA12B*, *HSPA13*, *HSPA14*, *HSPH1*, and *HYOU1*.

Statistical Analysis

SPSS version 25.0 (IBM, Chicago, IL, USA) was used for statistical analysis. The calculation of survival analysis was carried out with Cox proportional hazards regression and Kaplan-Meier analyses, which yielded log-rank *P* values, HRs, and 95% confidence intervals (CIs). Results were considered statistically significant when $P<0.05$.

Results

Clinical Characteristics

The clinical data from 438 patients with CRC was used in the analysis. **Table 1** lists the correlations between clinical characteristics and OS in the patients with CRC [16]. The results showed that TNM stage was related with OS. **Figure 1** illustrates each of the *Hsp70* family gene levels in samples of CRC and normal colon tissue. *HSPA1A*, *HSPA1B*, *HSPA1L*, *HSPA2*, *HSPA4*, *HSPA4L*, *HSPA5*, *HSPA8*, *HSPA9*, *HSPA12B*, *HSPA14*, *HSPH1*, and *HYOU1* gene levels were statistically significant.

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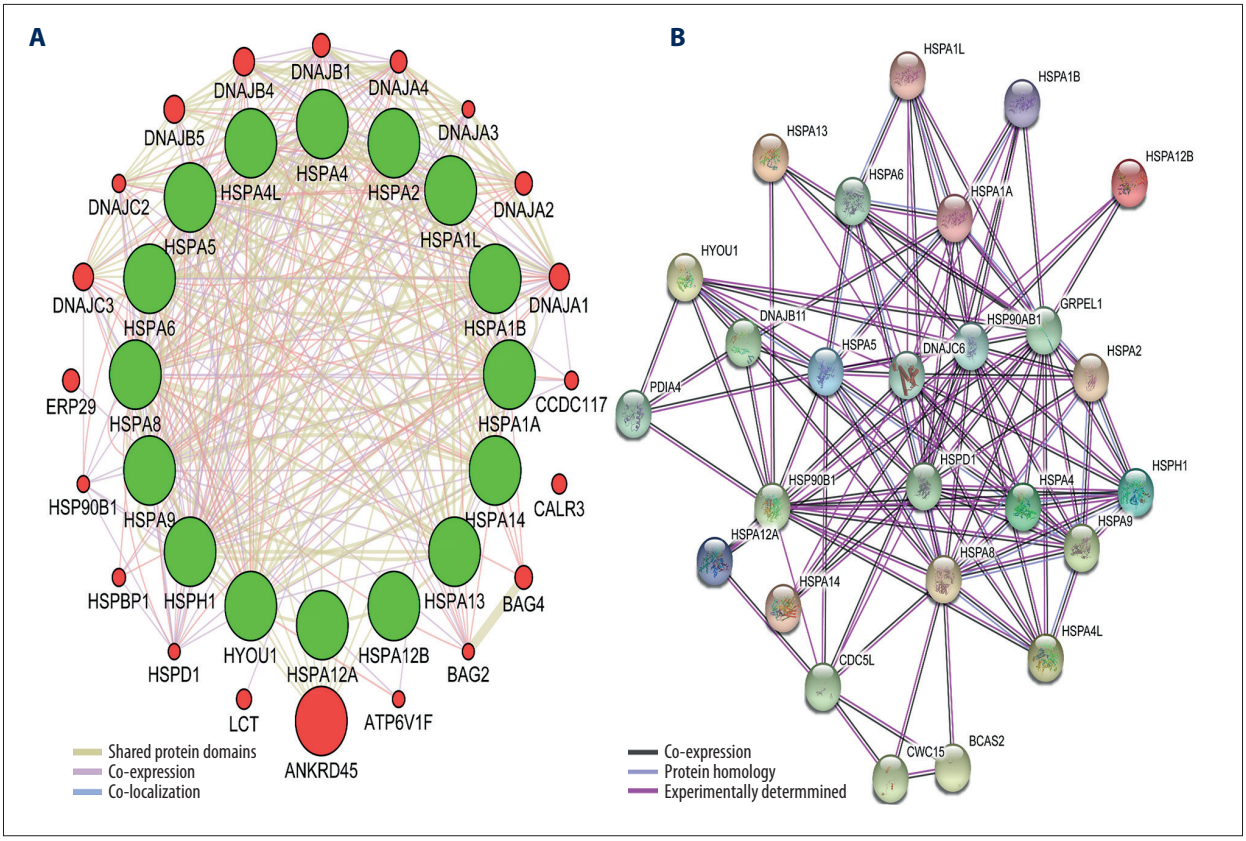

Figure 3. Gene-gene and protein–protein interaction network for *Hsp70* gene family. (**A**) Gene-gene interaction network; (**B**) Protein–protein interaction network.

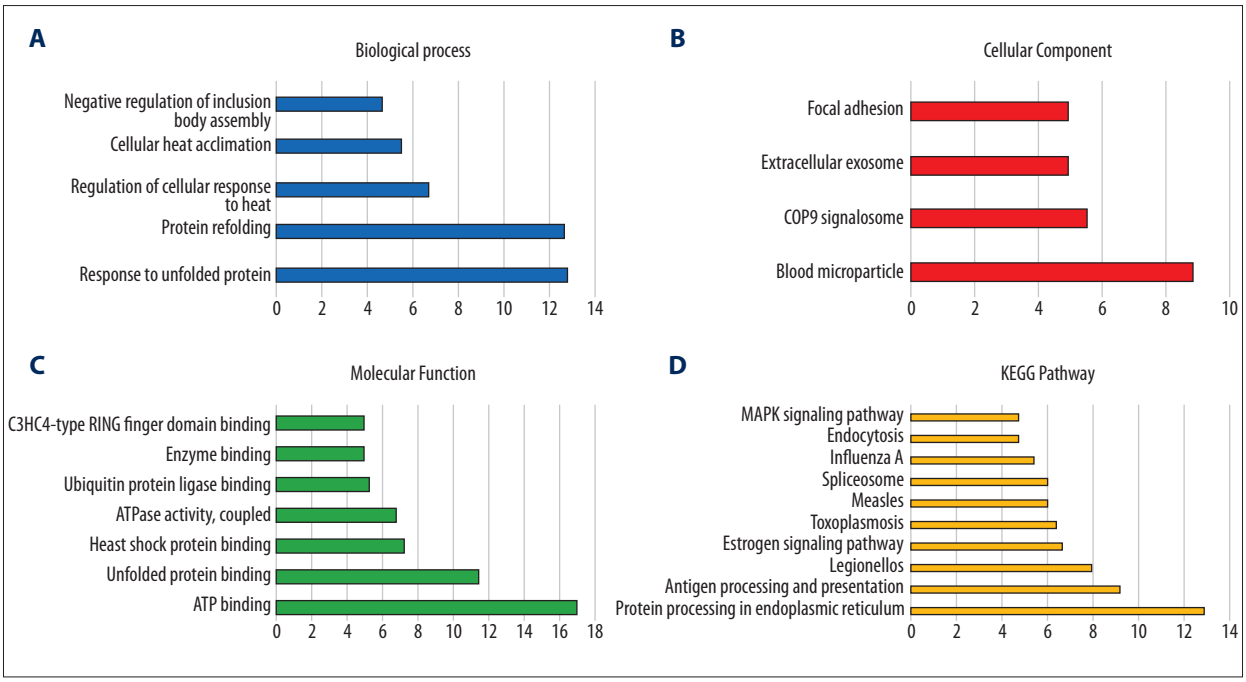

Figure 4. GO and KEGG pathway analysis of *Hsp70* gene family carried out by the online tool DAVID. (**A**) Biological process; (**B**) cellular component; (**C**) molecular function; (**D**) KEGG pathway.

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Table 2. Prognostic survival analysis of *Hsp70* family genes.

Gene expression	Patients (n=438)	No. of events (%)	MST (days)	Crude HR (95% CI)	Crude P	Adjusted HR (95% CI)	Adjusted P*
HSPA1A					<0.001		
Low	219	85.8	2969	Ref.		Ref.	0.004
High	219	69.4	2412	0.435 (0.284-0.666)		0.514 (0.327-0.808)	
HSPA1B					0.037		
Low	219	83.6	2966	Ref.		Ref.	0.044
High	219	71.7	2462	0.645 (0.427-0.974)		0.643 (0.419-0.988)	
HSPA1L					0.004		
Low	219	83.1	3046	Ref.		Ref.	0.046
High	219	72.1	2247	0.545 (0.362-0.821)		0.650 (0.425-0.993)	
HSPA2							
Low	219	76.7	2584	Ref.	0.776	Ref.	0.720
High	219	78.5	2810	0.944 (0.635-1.404)		1.077 (0.717-1.619)	
HSPA4							
Low	219	76.3	2222	Ref.	0.103	Ref.	0.180
High	219	79.0	2852	0.717 (0.481-1.069)		1.325 (0.879-1.998)	
HSPA4L							
Low	219	76.3	2054	Ref.	0.134	Ref.	0.622
High	219	79.0	2908	0.734 (0.490-1.100)		0.900 (0.591-1.370)	
HSPA5							
Low	219	79.0	2734	Ref.	0.656	Ref.	0.674
High	219	76.3	2594	1.095 (0.736-1.629)		1.092 (0.725-1.644)	
HSPA6							
Low	219	80.8	2732	Ref.	0.067	Ref.	0.284
High	219	74.4	2591	1.455 (0.975-2.171)		1.254 (0.829-1.899)	
HSPA8							
Low	219	73.5	2481	Ref.	0.050	Ref.	0.333
High	219	81.7	2790	0.668 (0.446-0.999)		0.815 (0.538-1.233)	
HSPA9							
Low	219	74.0	2335	Ref.	0.017	Ref.	0.059
High	219	81.3	2962	0.612 (0.409-0.915)		0.670 (0.442-1.016)	

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Table 2 continued. Prognostic survival analysis of *Hsp70* family genes.

Gene expression	Patients (n=438)	No. of events (%)	MST (days)	Crude HR (95% CI)	Crude P	Adjusted HR (95% CI)	Adjusted P*
HSPA12A							
Low	219	78.1	2634	Ref.	0.863	Ref.	0.605
High	219	77.2	2688	1.035 (0.697-1.539)		1.114 (0.741-1.674)	
HSPA12B							
Low	219	76.3	2644	Ref.	0.935	Ref.	0.697
High	219	79.0	2712	0.984 (0.661-1.464)		0.922 (0.612-1.388)	
HSPA13							
Low	219	74.0	2666	Ref.	0.189	Ref.	0.484
High	219	81.3	2570	0.763 (0.510-1.142)		0.863 (0.571-1.305)	
HSPA14							
Low	219	76.7	2646	Ref.	0.388	Ref.	0.645
High	219	78.5	2666	0.840 (0.565-1.249)		0.909 (0.604-1.366)	
HSPH1							
Low	219	78.5	2672	Ref.	0.836	Ref.	0.131
High	219	76.7	2661	1.043 (0.701-1.551)		0.721 (0.472-1.102)	
HYOU1							
Low	219	74.4	2617	Ref.	0.244	Ref.	0.185
High	219	80.8	2692	0.788 (0.528-1.177)		0.757 (0.501-1.143)	

* Adjusted for TNM stage. *Hsp70* – heat shock protein 70; MST – median survival time; HR – hazard ratio; CI – confidence interval.

Interaction and Function Analysis of the Hsp70 Gene Family

The Pearson correlation coefficient was used to analyze the correlation of *Hsp70* genes (**Figure 2**). **Figure 3** illustrates the gene-gene and protein–protein interaction network of the *Hsp70* gene family. **Figure 4** illustrates the GO pathway functional analysis and KEGG pathway functional analysis.

Survival and Joint-effect Analysis of the Hsp70 Gene Family

Table 2 summarizes the univariate and multivariate survival analyses of the *Hsp70* genes. Low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were associated with improved OS in univariate survival analysis. Meanwhile, the elevated expression level of *HSPA9* was related to improved OS (**Figure 5**). Moreover, multivariate survival analysis demonstrated that lower expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were significantly associated with improved OS.

Based on the multivariate survival analysis of *HSPA1A*, *HSPA1B*, and *HSPA1L*, a joint-effects framework was performed with different groups (**Table 3**). As illustrated in **Figure 6**, low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* in Groups 1, 4, 7, and 10 were significantly correlated with improved OS.

Nomogram

The nomogram was utilized for investigating the association between *HSPA1A*, *HSPA1B*, *HSPA1L*, and tumor stage in CRC. The points of each variable could be calculated. **Figure 7** shows the prediction of the 1-, 3-, 5-, and 10-year survival rates.

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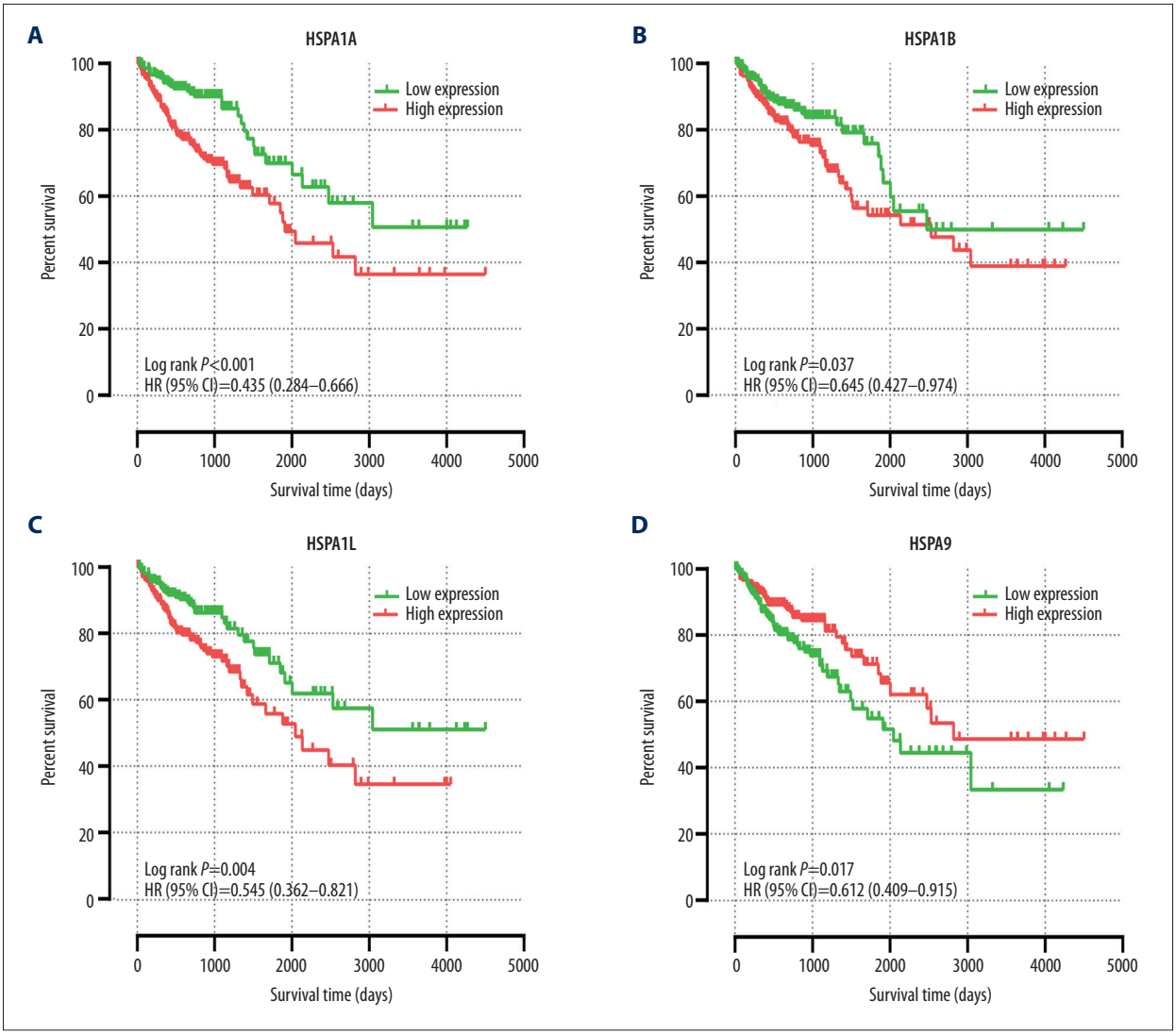

Figure 5. The univariate survival analysis of *HSPA1A*, *HSPA1B*, *HSPA1L*, and *HSPA9* ($P<0.05$). Kaplan-Meier survival curves concerning (**A**) *HSPA1A*; (**B**) *HSPA1B*; (**C**) *HSPA1L*; (**D**) *HSPA9* expression.

GSEA Analysis

To investigate the enrichment pathway with *HSPA1A*, *HSPA1B*, and *HSPA1L*, GSEA analysis was conducted. As illustrated in **Figure 8**, according to the GSEA, the low expression of *HSPA1A* was positively correlated with the cell cycle, DNA replication, RNA degradation, and P53 pathway. As illustrated in **Figure 9**, the GSEA indicated that the high expression of *HSPA1B* was positively correlated with the spliceosome, heat shock protein binding, RNA polymerase II promoter transcription elongation, DNA-templated transcription elongation, chaperone-mediated protein folding, and positive regulation of gene-expression epigenetics. The GSEA also indicated that a low expression of *HSPA1L* was positively correlated with the cell cycle, DNA replication, DNA helicase activity, and P53 pathway (**Figure 10**).

Discussion

The TCGA database was utilized to illustrate the importance of *Hsp70* genes in predicting the prognosis of patients with CRC. We found that gene levels were statistically significantly higher in CRC tissue samples than in normal colon tissue samples for *HSPA1A*, *HSPA1B*, *HSPA1L*, *HSPA2*, *HSPA4*, *HSPA4L*, *HSPA5*, *HSPA8*, *HSPA9*, *HSPA12B*, *HSPA14*, *HSPH1*, and *HYOU1*. Furthermore, interaction and functional analyses were established in the investigation of the *Hsp70* genes. According to the multivariate survival analysis and joint-effects analysis, low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* had a strong association with improved OS. Additionally, a nomogram based on *HSPA1A*, *HSPA1B*, *HSPA1L*, and tumor stage was formulated for predicting 1-, 3-, 5-, and 10-year survival rates in the patients with CRC. The investigation of potential molecular

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Table 3. Grouping according to *HSPA1A*, *HSPA1B*, and *HSPA1L*.

Group	Composition	Group	Composition
1	Low *HSPA1A* +low *HSPA1B*	10	Low HSPA1A +low HSPA1B+ low *HSPA1L*
2	Low *HSPA1A* +high *HSPA1B*	11	Low HSPA1A +low HSPA1B+ high *HSPA1L*
	High *HSPA1A* +low *HSPA1B*		Low HSPA1A +high HSPA1B+ low *HSPA1L*
3	High *HSPA1A* +high *HSPA1B*		High *HSPA1A* +low *HSPA1B*+ low *HSPA1L*
4	Low *HSPA1A* +low *HSPA1L*		High *HSPA1A* +high *HSPA1B*+ low *HSPA1L*
5	Low *HSPA1A* +high *HSPA1L*		High *HSPA1A* +low *HSPA1B*+ high *HSPA1L*
	High *HSPA1A* +low *HSPA1L*		Low *HSPA1A* +high *HSPA1B*+ high *HSPA1L*
6	High *HSPA1A* +high *HSPA1L*	12	High *HSPA1A* +high *HSPA1B*+ high *HSPA1L*
7	Low *HSPA1B* +low *HSPA1L*		
8	Low *HSPA1B* +high *HSPA1L*		
	High *HSPA1B* +low *HSPA1L*		
9	High *HSPA1B* +high *HSPA1L*		

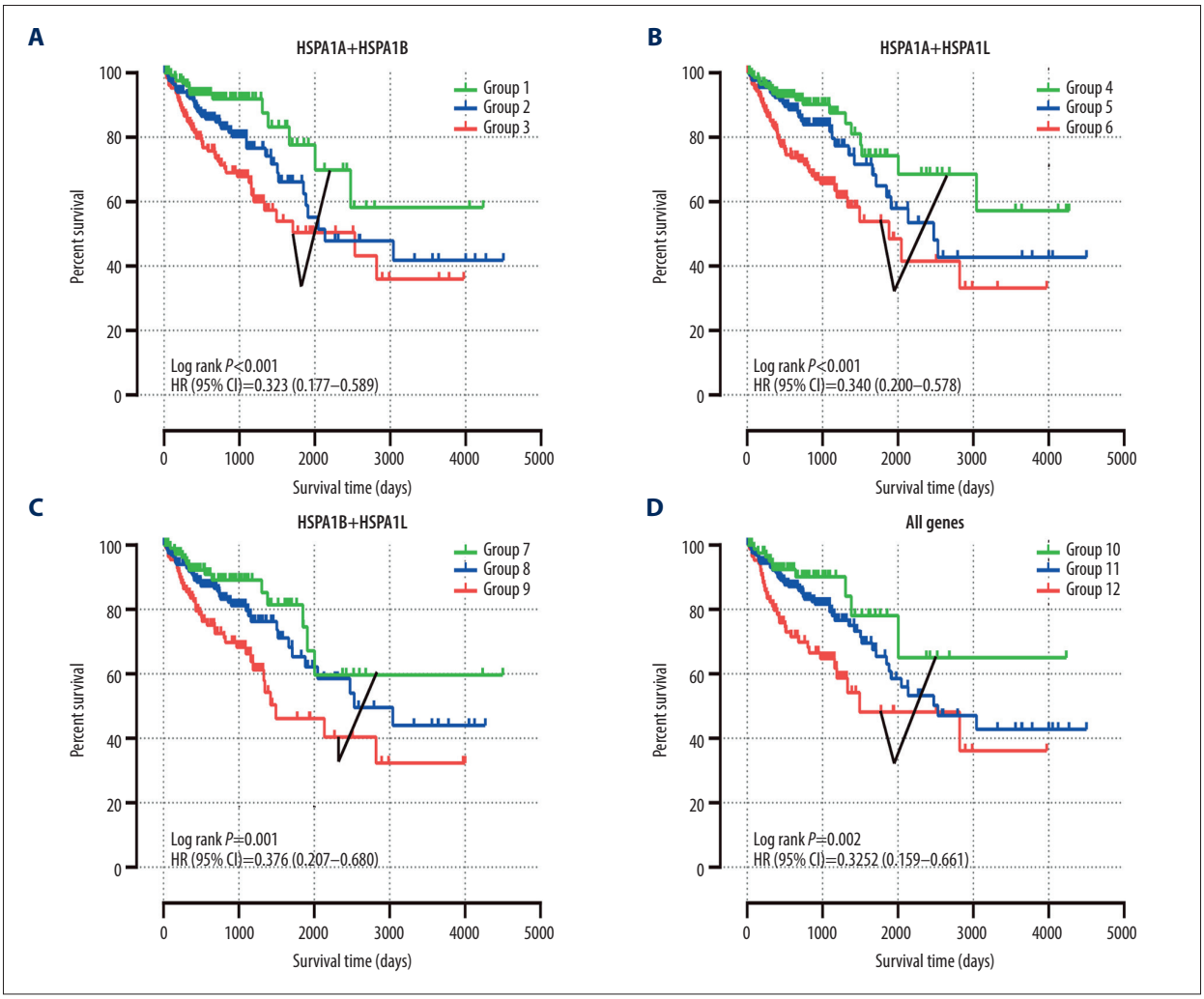

Figure 6. The joint-effects analysis of the influence of combined *HSPA1A*, *HSPA1B*, and *HSPA1L*. Kaplan-Meier survival curves concerning (**A**) *HSPA1A+HSPA1B*; (**B**) *HSPA1A+HSPA1L*; (**C**) *HSPA1B+HSPA1L*; (**D**) *HSPA1A+HSPA1B+HSPA1L*.

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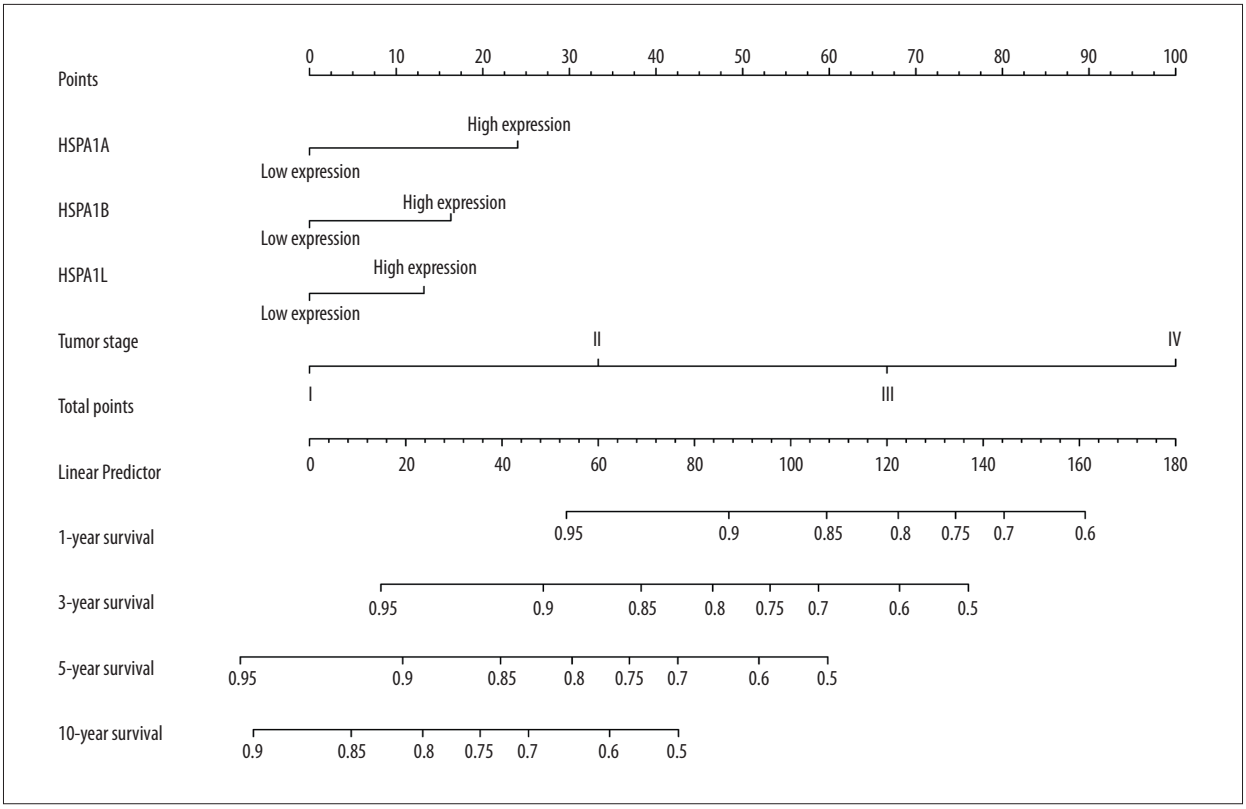

Figure 7. A nomogram model was performed to analyze the prognosis correlation of *HSPA1A*, *HSPA1B*, *HSPA1L* and tumor stage in CRC. The points of each variable were calculated at the top of the nomogram. A vertical line down to the 1-, 3-, 5-, and 10-year survival lines allowed for the determination of survival probabilities.

mechanisms with *HSPA1A*, *HSPA1B*, and *HSPA1L* was facilitated by GSEA analysis. Accordingly, low expression of *HSPA1A* exhibited a positive correlation with the cell cycle, DNA replication, RNA degradation, and P53 pathway. Furthermore, an elevated expression level in *HSPA1B* was positively correlated with the spliceosome, heat shock protein binding, RNA polymerase II promoter transcription elongation, DNA-templated transcription elongation, chaperone-mediated protein folding, and positive regulation of gene-expression epigenetics. In addition, the low expression of *HSPA1L* was positively correlated with the cell cycle, DNA replication, DNA helicase activity, and P53 pathway.

Since the *Hsp70* genes are important members of the HSPs family, they were assumed to be responsible for multiple cellular developments and for responding to environmental changes [4]. The human *Hsp70* is a multigene family which consists of 17 genes and 30 pseudogenes [7]. It also includes 1 putative gene, *HSPA7* [17]. In the present study, we selected data from 16 *Hsp70* genes to investigate the significance of *Hsp70* genes in the prediction of prognosis in patients with CRC. According to the multivariate survival analysis, low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were significantly related with improved OS. Previous studies have done detailed analysis of the evolutionary history of *HSPA1A*, *HSPA1B*, and *HSPA1L* [18], and these genes, respectively, encode 3 highly analogous *Hsp70* proteins, namely, *Hsp70-1*, *Hsp70-2*, and *Hsp70-hom*, which are located on chromosome 6p21.3 [19]. The genes *HSPA1A* and *HSPA1B* have been studied extensively, and their coded proteins are thought to be completely interchangeable because only 2 amino acids are different [20]. In a majority of human tissues, the expression levels of *HSPA1A* and *HSPA1B* are expressed much more than are other *Hsp70* family genes. Furthermore, *HSPA1L* is highly expressed in testis [7].

It has been demonstrated that *HSPA1A* plays an essential role in cancer development. Apparently, *HSPA1A* could be significant in the development of cancer cells, protecting them from oxidative stress, hypoxia, inflammatory cytokines, and the anti-apoptotic pathway [21]. It has been demonstrated that *HSPA1A* is essential to the survival of different cancer cells [22-24]. It has also been established that *HSPA1A* has a role on changes in the immune system [4]. Moreover, the *HSPA1A* and *HSPA1L* genes could be related to the prognosis in ovarian epithelial cancer [25].

Similar to *HSPA1A*, *HSPA1B* also assumes a vital role in cancer. It has been reported that *HSPA1B* variations are related to lung

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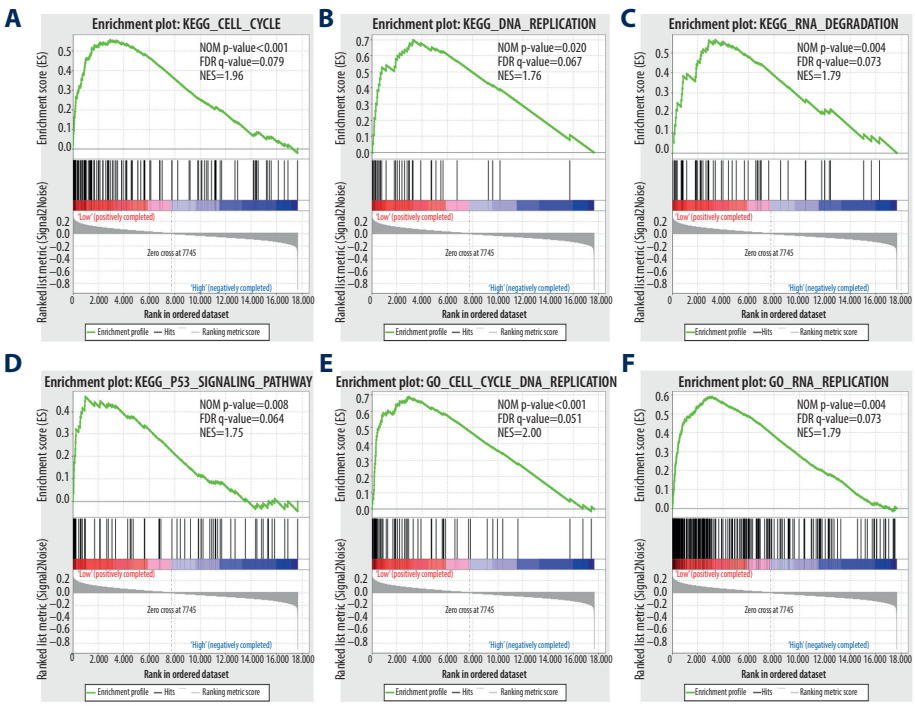

Figure 8. Gene set enrichment analysis shows the enrichment analysis of *HSPA1A*. (**A–F**) Statistical significance was implied by NOM *P*<0.05 and FDR<0.25. NOM – normalized; FDR – false discovery rate; NES – normalized enrichment score.

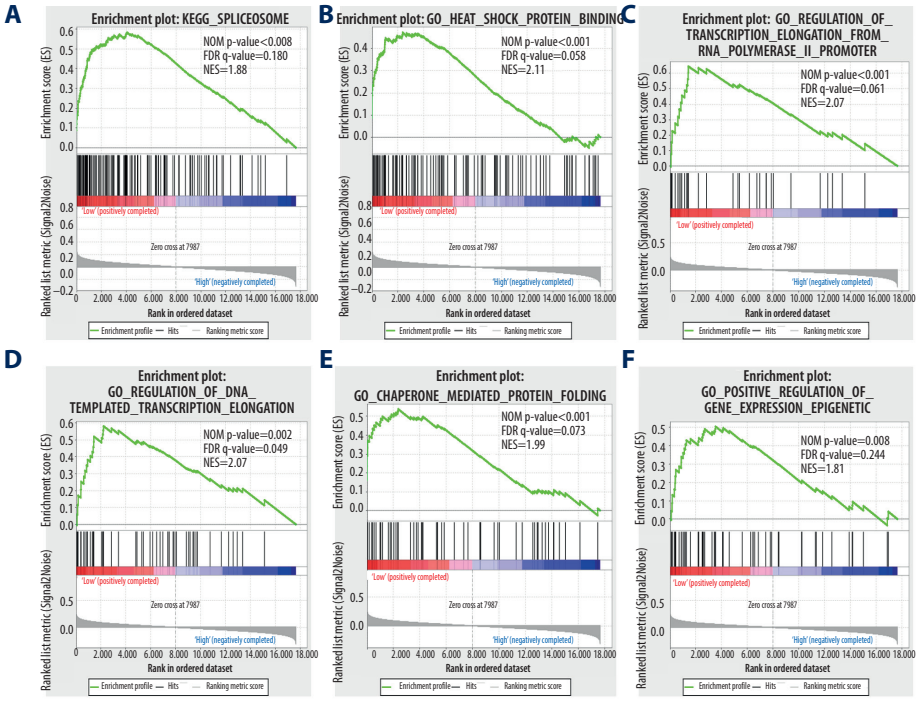

Figure 9. Gene set enrichment analysis shows the enrichment analysis of *HSPA1B*. (**A–F**) Statistical significance was implied by NOM *P*<0.05 and FDR<0.25. NOM – normalized; FDR – false discovery rate; NES – normalized enrichment score.

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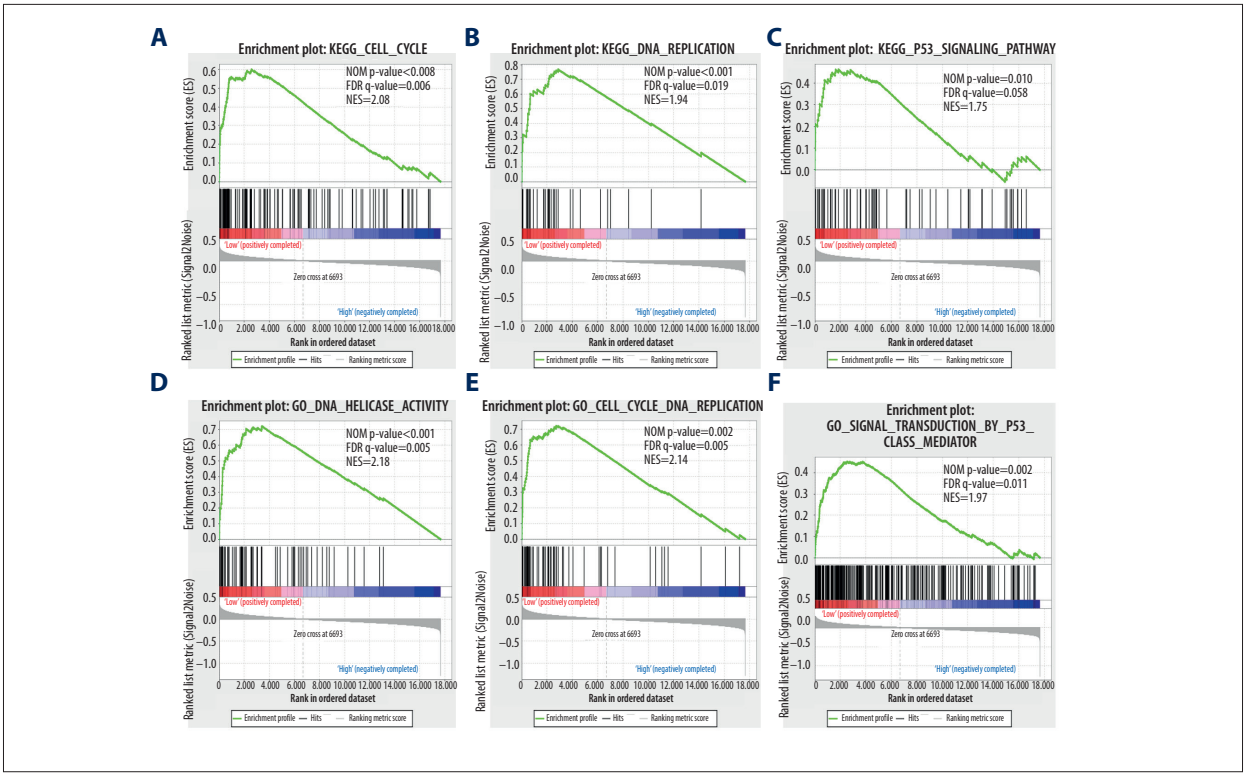

Figure 10. Gene set enrichment analysis shows the enrichment analysis of *HSPA1L*. (**A–F**) Statistical significance was implied by NOM $P<0.05$ and FDR<0.25. NOM – normalized; FDR – false discovery rate; NES – normalized enrichment score.

cancer risk and survival [19]. Numerous studies have shown that *HSPA1B* is related to the growth of tumors in colorectal and breast cancer [26,27]. Additionally, variant *HSPA1L* could be related to prostate cancer risk [28]. *Hsp70* exhibits various anticancer therapies, including playing the role of lifeguard and having anti-apoptotic effects in cancer cells [6,29,30]. It also plays a role in the regulation of the intrinsic, extrinsic, and caspase-independent pathways [31,32]. GSEA was used to discover the potential underlying molecular mechanisms of *HSPA1A*, *HSPA1B*, and *HSPA1L* in CRC. It is likely that these genes possess anticancer effects by affecting the cell cycle, DNA replication, and P53 pathway.

This study has a number of limitations. First, the public databases lack detailed clinical information. Second, the patient data were obtained from a single source. To generalize the results, it will be necessary to validate the conclusions through the analysis of independent data in future studies. Finally, since this study is mainly a bioinformatics study using data from a public database, it lacks empirical conclusiveness. The anticancer properties of *HSPA1A*, *HSPA1B*, and *HSPA1L* in CRC should be tested through various in vitro and in vivo experiments. Studies have demonstrated that *Hsp70* genes have prognostic significance in some common tumors [9,33,34]; however, the present study is the first to report on the significance of the *Hsp70* family of genes in estimating the prognosis of patients with CRC.

Conclusions

Through comprehensive analysis, we identified the potential molecular mechanisms of *HSPA1A*, *HSPA1B*, and *HSPA1L* in CRC. Additionally, we discovered that low expression levels of *HSPA1A*, *HSPA1B*, and *HSPA1L* were significantly correlated with an improved prognosis in CRC. Importantly, *HSPA1A*, *HSPA1B*, and *HSPA1L* have potential value as prognostic biological markers in CRC.

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Conflicts of Interest

None.

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