Prognostic Significance of the Hsp70 Gene Family in Colorectal Cancer

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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) | 0.545 |
| Sex           |                 |                   |            |             |            |
| Female        | 204             | 78.4              | 2990       | Ref.        | 0.545      |
| Male          | 234             | 76.9              | 2320       | 1.131 (0.759-1.686) | <0.001 |
| TNM stage     |                 |                   |            |             |            |
| I             | 73              | 94.5              | 3234       | Ref.        | <0.001     |
| II            | 167             | 83.8              | 2838       | 2.24 (0.781-6.421) | 0.031     |
| III           | 126             | 75.4              | 2856       | 4.068 (1.434-11.538) | 0.012     |
| IV            | 61              | 49.2              | 1114       | 11.291 (3.980-32.026) | 0.001     |
| Missing       | 11              |                   |            |             |            |

MST – median survival time; HR – hazard ratio; CI – confidence interval; TNM – tumor-node-metastasis.
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 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.
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, HSPA6, HSPA8, HSPA9, HSPA12A, HSPA12B, HSPA13, HSPA14, HSPH1, and HYOU1 gene levels were statistically significant.

### 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 $P < 0.05$.

### Figure 2

Pearson’s correlation analysis for HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA4, HSPA4L, HSPA5, HSPA6, HSPA8, HSPA9, HSPA12A, HSPA12B, HSPA13, HSPA14, HSPH1, and HYOU1.
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.
| Gene expression | Patients (n=438) | No. of events (%) | MST (days) | Crude HR (95% CI) | Crude P | Adjusted HR (95% CI) | Adjusted P* |
|-----------------|-----------------|------------------|------------|-------------------|---------|---------------------|-----------|
| **HSPA1A**      |                 |                  |            |                   |         |                     |           |
| Low             | 219             | 85.8             | 2969       | Ref.              | 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.    | 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.    | 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.              | Ref.    | 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.              | Ref.    | 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.              | Ref.    | 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.              | Ref.    | 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.              | Ref.    | 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.              | Ref.    | 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.              | Ref.    | Ref.                | 0.059     |
| High            | 219             | 81.3             | 2962       | 0.612 (0.409-0.915)| 0.670   | (0.442-1.016)       |           |
The Pearson correlation coefficient was used to analyze the correlation of \( \text{Hsp70} \) genes (Figure 2). Figure 3 illustrates the gene-gene and protein–protein interaction network of the \( \text{Hsp70} \) 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 \( \text{Hsp70} \) genes. Low expression levels of \( \text{HSPA1A}, \text{HSPA1B}, \) and \( \text{HSPA1L} \) were associated with improved OS in univariate survival analysis. Meanwhile, the elevated expression level of \( \text{HSPA9} \) was related to improved OS (Figure 5).

Moreover, multivariate survival analysis demonstrated that lower expression levels of \( \text{HSPA1A}, \text{HSPA1B}, \) and \( \text{HSPA1L} \) were significantly associated with improved OS.

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

**Nomogram**

The nomogram was utilized for investigating the association between \( \text{HSPA1A}, \text{HSPA1B}, \text{HSPA1L}, \) 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.
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
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 |
| 3     | High HSPA1A +low HSPA1B | 12    | High HSPA1A +high HSPA1B + low HSPA1L |
| 4     | Low HSPA1A +low HSPA1L  |       | High HSPA1A +low HSPA1B + low HSPA1L |
| 5     | High HSPA1A +low HSPA1L  |       | High HSPA1A +low HSPA1B + high HSPA1L |
| 6     | High HSPA1A +high HSPA1L |       | Low HSPA1A +high HSPA1B + high HSPA1L |
| 7     | Low HSPA1B +high HSPA1L  |       | High HSPA1A +high HSPA1B + high HSPA1L |
| 8     | Low HSPA1B +high HSPA1L  |       | High HSPA1A +low HSPA1B + high HSPA1L |
| 9     | High HSPA1B +high HSPA1L |       | Low HSPA1A +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.
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

![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.](image)
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.
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 PS3 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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