ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20210495

Tumorigenic and immunological roles of Heat shock protein A2 in pancreatic cancer: a bioinformatics analysis

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SUMMARY

OBJECTIVE: Heat shock protein A2 has been reported to be tightly associated with tumorigenesis and tumor progression. This study aimed to determine the oncogenic and immunological roles of Heat shock protein A2 in pancreatic cancer by bioinformatics.

METHODS: Expression of Heat shock protein A2 in tumorous and normal specimens of pancreatic cancer was analyzed using the Cancer Genome Atlas and the Cancer Genome Atlas + Genotype-Tissue Expression data sets, respectively. Relationships of Heat shock protein A2 expression with immune infiltrates in pancreatic cancer were assessed. Heat shock protein A2-associated coexpressed genes in pancreatic cancer were obtained, followed by the implementation of enrichment analysis.

RESULTS: The data demonstrated that Heat shock protein A2 was significantly overexpressed in tumorous samples compared with normal samples. Heat shock protein A2 expression was remarkably positively interrelated with CD8+ T cell, neutrophil, dendritic cell, and macrophage, but not with CD4+ T and B cells. Heat shock protein A2 expression was markedly positively relevant to both cancer-associated fibroblast and endothelial cell. Enrichment data revealed that Heat shock protein A2 was intimately involved in the tumorigenesis and progression of pancreatic cancer.

CONCLUSION: Heat shock protein A2 is upregulated in pancreatic cancer and is closely associated with tumor immunity and aggressive progression.

KEYWORDS: HSPA2. Pancreatic cancer. Carcinoma. Pancreas. Gene expression.

INTRODUCTION

Pancreatic cancer (PC) is a fatal solid malignancy that seriously endangers human health and is the seventh leading cause of malignancy-related death worldwide[1]. Due to the rapid progression of the disease, most patients first identified have reached an advanced stage, losing the best time for curative resection. Moreover, PC is insensitive to radiotherapy and chemotherapy. Targeted therapy and immunotherapy are currently the most promising adjuvant anticancer therapies. Therefore, the search for biomarkers associated with tumor immunity and aggressiveness is a significant insight for the development of new targeted therapies or immunotherapies for PC.

Heat shock-associated 70-kDa proteins (HSP70s) are a family of stress proteins with an approximate 70-kD molecular weight that have antioxidative, antiapoptosis, and immunoregulatory functions[2]. HSPA2, also known as HSP70-2, is one of the elements of the HSP70s group, initially identified in male germ cells and is associated with spermatogenesis[3]. Former publications have demonstrated that HSPA2 is highly expressed in many cancers, including malignancies of the cervix, bladder, esophagus, lung, liver, colorectum, breast, and pancreas[4-15], suggesting that HSPA2 is involved in cancer development. Moreover, immunoinformatics analysis has showed an intimate association of HSPA2 with immune responses in several human tumors, revealing the potential of HSPA2 as a molecular biomarker for cancer immunotherapy[16].

Although HSPA2 overexpression has been reported, the immunological role and biological function of HSPA2 have not been elucidated in PC. Therefore, this study evaluated the expression, clinical value, immunological effect, biological role, and potential mechanisms of HSPA2 in PC through multiple bioinformatics platforms. These data contribute to our understanding of the oncogenic and immunological roles of HSPA2 in PC from a cancer omics perspective.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: This study was supported by the Zhejiang Provincial Natural Science Foundation of China (no. LGF19H290004).
Received on January 09, 2022. Accepted on February 08, 2022.

METHODS

Data acquisition and gene expression analysis

The mRNA expression data of 178 tumor specimens and 77 normal specimens were downloaded from the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). These specimens were obtained from 234 cases, including 177 TCGA-PAAD, 49 ORGANOID-PANCREATIC, and 8 HCMI-CMDC. Some cases provided multiple specimens. The tumor specimens were all from TCGA-PAAD cases. Among the 77 normal specimens, 4 were from TCGA-PAAD cases, 55 were from ORGANOID-PANCREATIC cases, and 18 were from HCMI-CMDC cases. The HSPA2 mRNA data were then extracted using Perl software, and the difference in expression between tumor and normal tissues was analyzed using R software.

The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn/), an open platform matching TCGA and Genotype-Tissue Expression (GTEx) data, was used for analyzing the expression difference of HSPA2 mRNA between tumorous and normal tissues[17]. The cutoff values for log2FC and p were designated as 0.5 and 0.01, respectively. The 171 normal specimens combined the tissues of TCGA-PAAD and GTEx. Moreover, the association of HSPA2 expression with pathological staging and survival was estimated by the GEPIA2 server.

Assessment of Heat shock protein A2 in relation to immune infiltrates

We evaluated the correlations between HSPA2 with the abundance of infiltrating immune cells such as T cell (CD8+ and CD4+), neutrophil, dendritic cell (DC), macrophage, and B cell in TCGA-PAAD tumor tissues using the Tumor IMmune Estimation Resource version 2.0 (TIMER 2.0; http://timer.cistrome.org/), an open platform for comprehensive estimation of tumor-infiltrating immune cells based on the TIMER algorithm[18]. In addition, we analyzed the associations of HSPA2 with infiltrating stromal cells such as cancer-associated fibroblast (CAF) and endothelial cell based on the EPIC algorithm using the TIMER 2.0 server. The results were displayed as scatterplots. Purity-adjusted Spearman's test was used to obtain correlation (Cor) coefficients.

Acquisition of Heat shock protein A2-related coexpressed genes

LinkedOmics (http://www.linkedomics.org/login.php) is a free access database containing multi-omics data from 32 TCGA tumors, designed with three modules, namely, LinkFinder, LinkInterpreter, and LinkCompare, depending on their functionality[19]. In this study, the LinkFinder module of the LinkedOmics webserver was used to obtain coexpressed genes associated with HSPA2 in PC.

Enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (https://david.ncifcrf.gov/home.jsp) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses on HSPA2-associated coexpressed genes to understand the biological functions of HSPA2 in PC[20]. GO functional annotation includes biological process, cell component, and molecular function.

Statistical analysis

The statistical analyses were conducted using R software version 4.0.3 (https://www.r-project.org/). Differences of gene expression in different pathological stages were estimated by F-test. Survival plots were drawn by the Kaplan-Meier method. Log-rank was used to compare survival differences. Spearman's test was used for correlation analysis. Differences were considered statistically significant at $p<0.05$.

RESULTS

Expression of Heat shock protein A2 in pancreatic cancer tissues

The Cancer Genome Atlas data indicated that HSPA2 mRNA expression was evidently increased in PC specimens compared with nontumor samples ($p<0.05$; Figure 1A). Similarly, the GEPIA2 website, which integrated TCGA and GTEx data, also demonstrated that HSPA2 mRNA was significantly upregulated in tumor samples compared to normal samples ($p<0.05$; Figure 1B). However, no significant differences in HSPA2 expression were observed among the different pathological stages ($p>0.05$). Survival analysis also did not reveal significant differences in disease-free survival (DFS) and overall survival (OS) between patients with high and low HSPA2 expression ($p>0.05$).

Associations of Heat shock protein A2 with immune infiltrates in pancreatic cancer

To explore the immunological role of HSPA2 in PC, we evaluated the correlations between HSPA2 expression and immune infiltrates. First, we evaluated the associations of HSPA2 expression with infiltrating immune cells through the TIMER 2.0 website based on the TIMER algorithm. The results presented that HSPA2 expression was evidently positively interrelated with CD8+ T cell, neutrophil, DC, and macrophage, but not

with CD4+ T and B cells (Figure 2). We then evaluated the correlations of HSPA2 with infiltrating CAF and endothelial cell based on the EPIC algorithm through the TIMER 2.0 platform. The results revealed a remarkable positive associativity between HSPA2 expression and both CAF and endothelial cell infiltration abundance (Figure 2).

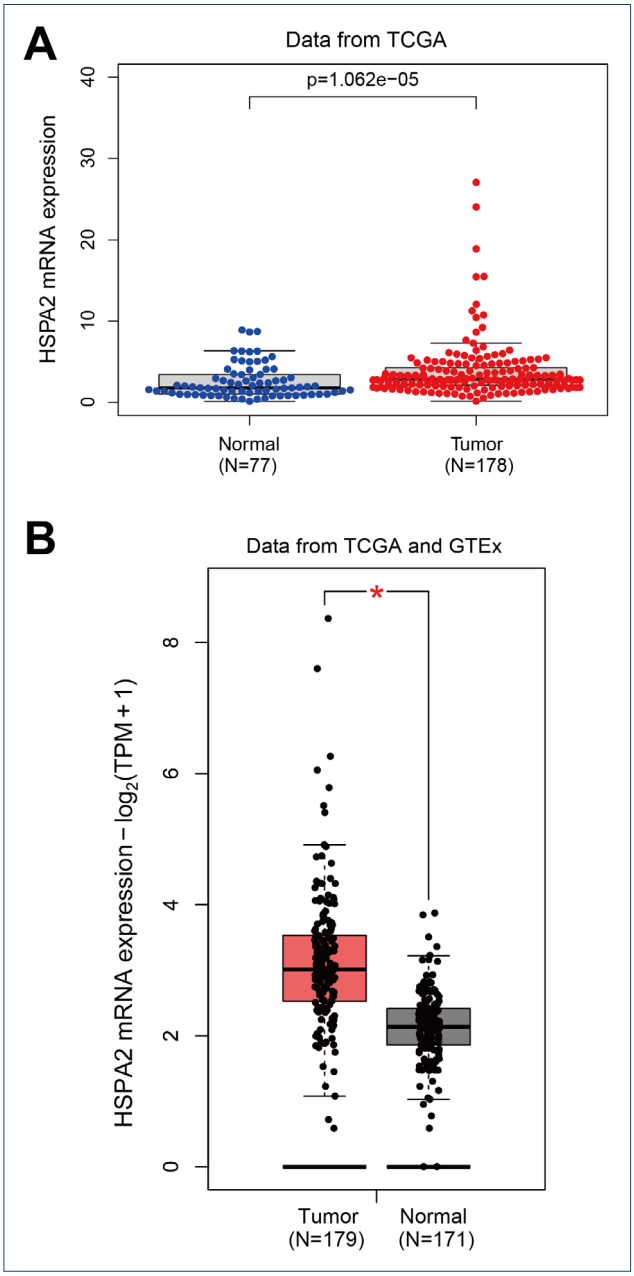

Figure 1. Expression of HSPA2 in PC. (A) Differential expression of HSPA2 between tumor and normal specimens in the TCGA data set. (B) Expression difference of HSPA2 between tumor and normal specimens after combining TCGA and GTEx data sets. HSPA2, heat shock protein A2; PC, pancreatic cancer; TCGA, the Cancer Genome Atlas; GTEx, Genotype-Tissue Expression. *p<0.05.

Gene ontology and Kyoto encyclopedia of genes and genomes pathway analyses data

Coexpression analysis of the LinkedOmics program revealed that there were 1,240 and 342 genes with distinct positive and negative association with HSPA2, respectively (FDR<0.05). The GO functional annotation revealed that HSPA2-related coexpressed genes were principally involved in the extracellular matrix, cell adhesion, focal adhesion, extracellular matrix binding, collagen catabolic process, extracellular space, extracellular region, and so on (FDR<0.001; Figure 3A). The KEGG pathway analysis indicated that these genes were enriched in multiple tumor-related signaling pathways, including PI3K-Akt signaling pathway, MAPK signaling pathway, TGF-β signaling pathway, and so on (FDR<0.05; Figure 3B).

DISCUSSION

In this study, we comprehensively evaluated the expression, clinical significance, immunological role, and biological function of HSPA2 in PC through different bioinformatics platforms. Our results demonstrated that HSPA2 was markedly upregulated in PC specimens compared to non-tumorous specimens. The TIMER algorithm revealed that HSPA2 expression was positively correlative with CD8+ T cell, neutrophil, DC, and macrophage, but not with CD4+ T and B cells. It is worth noting that the EPIC algorithm in the TIMER2.0 server presented a positive correlativity between HSPA2 and infiltrating CAF and endothelial cell. Enrichment analysis revealed that HSPA2 was strongly linked to multiple cancer-related biological processes and signaling pathways.

Many studies have demonstrated that HSPA2 was overexpressed in diverse malignancies, including PC[4-15]. HSPA2 expression has also been reported to increase with progressive tumor stage in PC. Enhanced HSPA2 expression resulted in an obvious reduction of DFS and OS in PC. Consistent with previous reports, the present study confirmed that HSPA2 expression was remarkably elevated in PC samples versus non-cancerous samples. Inconsistently, we did not find any correlation between HSPA2 expression and the pathological stage and survival of PC. The reason for these different results may be that the sample size of PC in the TCGA database is not large enough. Additionally, the gene expression data in the TCGA project were obtained by RNA sequencing, which may differ from the PCR and immunohistochemistry results in the two studies that have been reported[14,15]. Therefore, further investigations are needed to determine the prognostic effect of HSPA2 on PC.

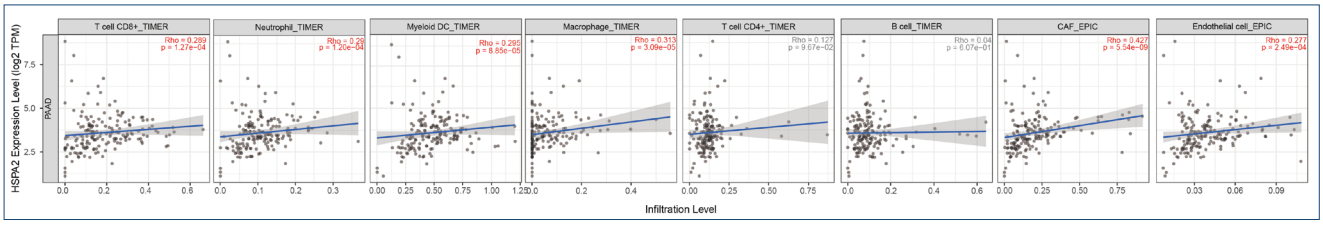

Figure 2. Association of HSPA2 with the abundance of immune infiltrates in PC. HSPA2, heat shock protein A2; PC, pancreatic cancer; DC, dendritic cell, CAF, cancer-associated fibroblast; TIMER, Tumor IMmune Estimation Resource. PAAD represents the TCGA abbreviation for pancreatic cancer.

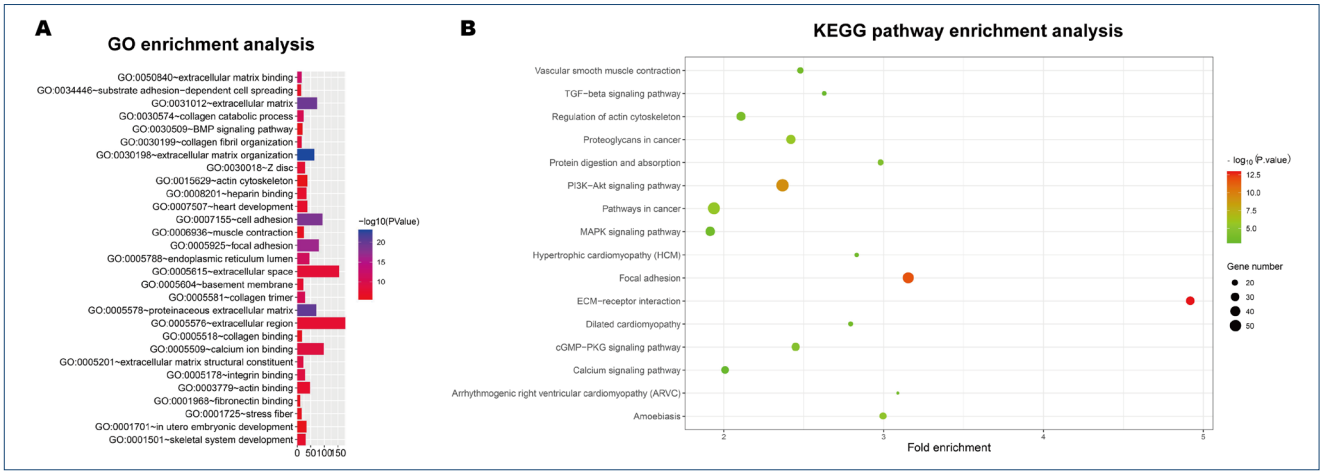

Figure 3. GO and KEGG pathway enrichment analyses of HSPA2-associated coexpressed genes in PC. (A) Bar graph of strongly enriched GO annotation for HSPA2-associated coexpression genes (FDR<0.001). The y-axis indicates the GO annotation item and the x-axis indicates the number of genes enriched in the GO item. (B) Bubble plot of the prominently enriched KEGG pathway for HSPA2-associated coexpressed genes (FDR<0.05). The y-axis indicates the KEGG pathway name and the x-axis indicates the fold enrichment (GeneRatio divided by BgRatio). Higher values of fold enrichment indicate higher levels of enrichment. The size and color of the bubbles in the graph represent the number of enriched genes and the p-value, respectively. HSPA2, heat shock protein A2; PC, pancreatic cancer; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

The components and abundance of immune infiltrates in tumor tissues are known to be strongly related to cancer progression. In this study, we investigated for the first time the relationships between HSPA2 and immune infiltrates in PC. We found that HSPA2 expression was positively linked to CD8+ T cell, neutrophil, DC, and macrophage, but not with CD4+ T and B cells. These different results warrant further in-depth exploration. Of interest, the EPIC algorithm yielded an apparent positive relativity between HSPA2 and CAF and endothelial cell, which are key stromal cells in the tumor microenvironment and contribute to tumor growth and metastasis via immunosuppression[21,22]. Collectively, these findings suggest that HSPA2 is strongly involved in tumor-associated immune responses in PC.

Several studies have demonstrated that HSPA2 regulates the biological behavior of tumor cells. In cervical, bladder, colorectal, and breast cancers, HSPA2 expression deficiency diminished the viability and invasiveness of cancer cells *in vitro* and repressed tumor growth *in vivo*[4,5,10-13,23]. Additionally, in ovarian and lung carcinomas, ablation of HSPA2 led not only to impaired proliferation and motility of cancer cells but also to cell-cycle arrest[24,25]. The role of HSPA2 in the biologic behavior of PC cells is currently unknown. The current study explored for the first time the potential biological functions and mechanisms of HSPA2 in the pathogenesis and progression of PC by enrichment analysis. Enrichment data demonstrated that HSPA2 upregulation was strongly involved in the aggressive behavior of PC. Our data, combined with previous results, reveal a momentous role of HSPA2 in the malignant biologic features of tumor cells.

The present study provides the first meaningful insights into the oncogenic and immunogenic role of HSPA2 in PC. The data also provide a basis for further exploration of the role and molecular mechanisms of HSPA2 as an oncogene in the pathogenesis and progression of PC. However, there are still several limitations. First, the results in this study were obtained

based on bioinformatics and can only be interpreted from the perspective of cancer omics, which requires further verification by biological experiments. Moreover, this study failed to confirm the clinicopathological and prognostic significance of HSPA2 expression, and further studies with large sample sizes are thus needed.

CONCLUSION

In summary, our findings suggest that HSPA2 is upregulated in PC and is strongly associated with tumor immunity and aggressive progression.

AUTHORS' CONTRIBUTIONS

L-LZ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **P-PQ:** Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **Y-SS:** Data curation, Formal Analysis, Validation, Writing – original draft, Writing – review & editing. **T-FJ:** Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Z-GT:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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