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■ RESEARCH ARTICLE

HSPB1 as a Novel Genetic Marker for Predicting Poor Survival of Stomach Cancer Patients

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ABSTRACT: The *HSPB1* gene encodes heat shock proteins and acts as a chaperone by regulating processes and maintaining competent proteins induced in response to stresses and injuries. The expression or alteration of this HSPB1 gene can be associated with poor survival rates by promoting and proliferating metastasis. Although this gene enhances tumor growth in various cancers, gene alteration of *HSPB1* has not been reported for stomach cancer. DNA, mRNA, and clinical data of 1512 patient samples were analyzed from seven stomach cancer studies by cBioPortal. The data showed that gastric cancer patients with the HSPB1 amplified gene had a significantly lower survival rate (median survival = 14.3) than the patients with the HSPB1 non-amplified gene (median survival = 31.0) (p = 0.033). The median survival month of the patient group with high expression of HSPB1 (21.2 months) was lower than the group with a low expression of the *HSPB1* gene (107.7 months). Overall, patients with a non-amplified *HSPB1* gene had a higher percentage of papillary stomach adenocarcinoma.

KEYWORDS: Biology; Genetics; *HSPB1* gene; Stomach Cancer; Survival Rate.

■ Introduction

Stomach cancer starts when cells in the stomach grow out of control. A tumor can be malignant or benign. A malignant tumor is threatening, which can develop and spread to different organs. A benign tumor implies the tumor can grow but would not spread. Cancer can start in any part of the stomach. The cause of gastric cancer is multifactorial.[1]

Heat shock proteins are proteins induced in response to environmental, physical, and chemical stresses, and they limit damage and help the recovery of cells. They protect protein substrates against damage to promote the function of the proteins, prevent aggregation, and prevent the formation of toxic inclusion bodies.[2]

Heat shock proteins strengthen healthy cells by protecting cells against stress and injuries, making them more resistant to diseases. They are significant regulators of cell proliferation and differentiation and implicated in cancer development and progression as they are well-established oncoproteins in many tumor types.[3]

The *HSPB1* gene provides the necessary information for making heat shock proteins; it functions as a chaperone by regulating processes and maintaining competent proteins. Additionally, it helps cells stay secure from many conditions such as injuries and disease. The *HSPB1* gene can enhance or proliferate cell growth and cancer progression. It can also display help in tumor growth and can be found in breast cancer, colorectal cancer, cervical cancer, liver cancer, and lung cancer, in addition to gastric or stomach cancer.[4]

This research aims to find the association between the genetic alterations of the heat shock protein gene *HSPB1* and the overall survival rate of these stomach cancer patients. Because stomach cancer is a common type of cancer with one of the highest death rates, the analysis of both the genetic alteration of the heat shock protein gene and the survival rate can help predict the survival of stomach cancer patients. Therefore, this research would aim to aid these stomach cancer patients by using the *HSPB1* heat shock protein gene as a novel genetic marker.

■ Methods

Patient survival analysis by cBioPortal:

cBioPortal is an open-source cancer genomic database.[5] This database provides a patient's genomic data set published from previous studies. In this study, seven previous studies with stomach cancer patients' data were analyzed for survival. The query "*HSPB1*" was typed for the input command. Then, the selected studies were divided into two groups: the HSPB1 amplified group and the *HSPB1* non-amplified group. The Kaplan Meier plot analyzed the overall survival.

Patient clinical analysis by cBioPortal:

The clinical attributes were analyzed in different categories: cancer type, 15q status, and 19p status. In clinical analysis, seven studies from the previous studies were selected, and the stomach cancer patients were divided into two groups: HSPB1 amplified group vs HSPB1 non-amplified group.

Kaplan-Meier plot survival analysis :

Gene expression data from 875 stomach cancer patients were downloaded from GEO, EGA, and TCGA. The database was analyzed by a PostgreSQL server, which integrates gene expression and clinical data. The prognostic value of the *HSPB1* gene was analyzed by splitting the patient samples into two groups by quantile expression of the *HSPB1*. Two groups (low vs high expression) were compared by Kaplan-Meier survival plot with 95% confidence intervals, and log-rank p-value was calculated. The databases and clinical data are updated regularly in http://kmplot.com.[6]

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Results and Discussion

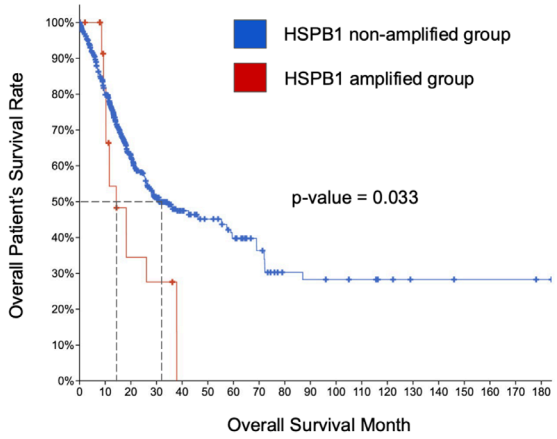

Figure 1: Overall survival rate of *HSPB1* non-amplified (n=1093) and amplified (n=29) stomach cancer patients. The dotted line indicated the median survival month: *HSPB1* non-amplified (median survival month= 31.0) and amplified group (median survival month= 14.3). A log-rank statistical test calculated statistical significance (p= 0.033).

First the copy number variation of the *HSPB1* gene with an amplified and non-amplified group was determined, showing the difference in the survival rate of patients with gastric cancer between the two groups (Figure 1). Because gastric cancer is a type of cancer with a low survival rate, this study decided to evaluate whether there is a correlation between survival in months and amplifications of the *HSPB1* heat shock protein gene. A significant difference according to the given data in this figure was observed, where the patient group with an amplified *HSPB1* gene had a much lower survival rate. In contrast, the non-amplified group had a higher one. In summary, this led to the conclusion of an existing association between the *HSPB1* gene amplification and survival rates of patients affected by gastric cancer.

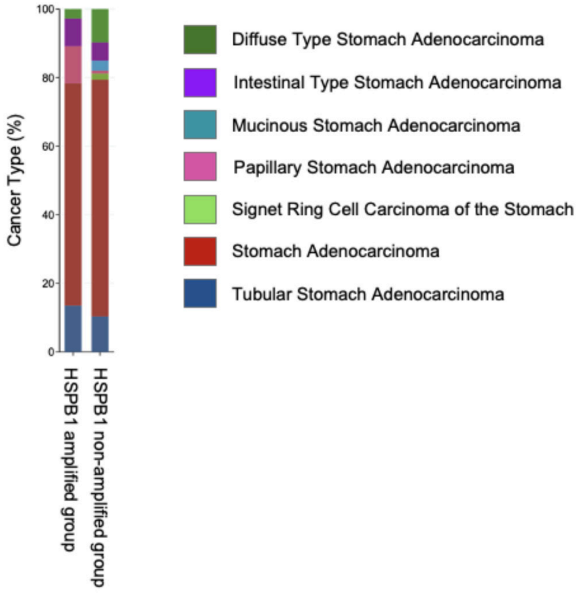

Figure 2: A comparative analysis of *HSPB1* non-amplified and amplified patients based on cancer type. A chi-squared test was used to calculate statistical significance. ($p= 6.12e^{-5}$)

First the difference in types of stomach cancer of patients with an amplified *HSPB1* gene and a non-amplified *HSPB1* gene with the data was determined (Figure 2). The goal was to find an association between amplifications of the heat shock protein gene and specific types of gastric cancer it causes. A notable difference was observed, where 10.8% of patients with the amplified *HSPB1* gene had papillary stomach adenocarcinoma while only 0.81% of patients with the non-amplified *HSPB1*. Papillary gastric adenocarcinoma is a rare histologic entity among gastric adenocarcinomas. Although the 5-year survival rate for the PGC did not differ significantly, death caused by papillary stomach adenocarcinoma was more frequently associated with liver metastasis (62%) than with peritoneal dissemination (5%).[7] This result indicates that both *HSPB1* amplification and papillary stomach adenocarcinoma may be associated with decreased survival rates.

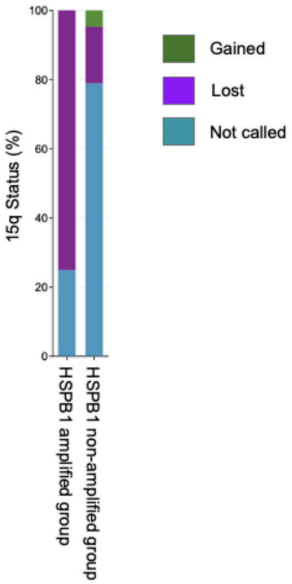

Figure 3: A comparative analysis of *HSPB1* non-amplified and amplified patients based on 15q chromosome status. A chi-squared test was used to calculate statistical significance. ($p= 9.15e^{-5}$)

Next, the 15q status from both *HSPB1* amplified and non-amplified patients was analyzed because chromosomal imbalances in gastric cancer are often correlated with tumor progression. 75% of *HSPB1* non-amplified patients lost chromosome 15q, whereas only 16.27% of *HSPB1* amplified patients lost chromosome 15q (Figure 3). The deletion of 15q has been reported in various steps of cancer, such as ovarian carcinoma and breast cancer.[8] The loss of 15q is known to be discovered in a late event in most high-grade tumors and metastatic breast carcinomas.[9] This result suggested that both 15q loss and *HSPB1* amplification are associated with the decreased patient survival rate.

Additionally, this data set of 19p chromosome status of both the *HSPB1* amplified and non-amplified patients was analyzed with the goal of seeing an association between the 19p status and cancer development. It was observed that 77.78% of patients with the non-amplified *HSPB1* gene lost the 19p chromosome, while only 27.27% of the

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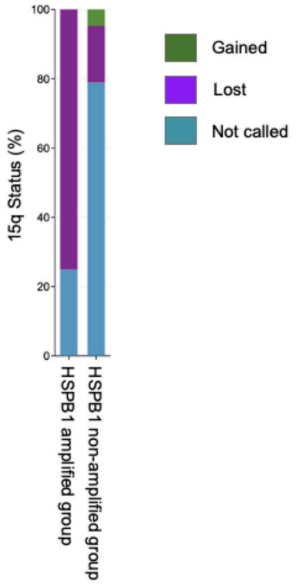

Figure 4: A comparative analysis of *HSPB1* non-amplified and amplified patients based on 19p chromosome status. A chi-squared test was used to calculate statistical significance. ($p= 4.60e^{-3}$)

amplified gene lost chromosome 19p. The data also shows that the *HSPB1* gene amplified group gained 5.43% of the chromosome. Similar losses of this chromosome 19p can be found in different types of cancer, such as ovarian cancer, lung cancer, breast cancer, and neuroblastoma.[10] Chromosome 19p plays a vital role in ovarian carcinogenesis, correlates with poor prognosis in neuroblastoma, and lung oncogenesis or remote metastasis in breast cancer.[11] The result of this figure shows an association between the loss of chromosome 19p and decreased patient survival (Figure 4).

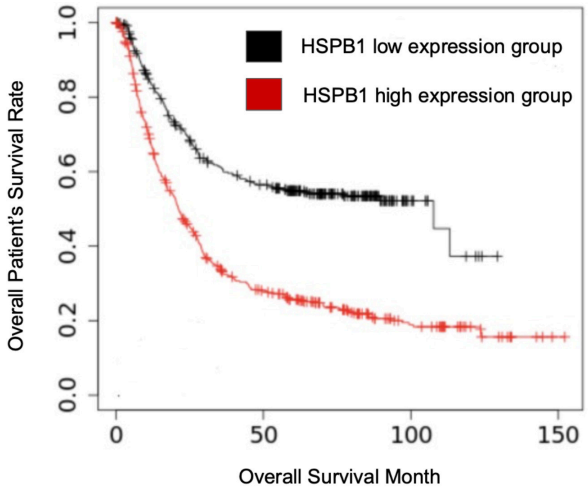

Figure 5: The overall survival rate of low (n= 557) and high (n=665) HSPB[1] mRNA expression of stomach cancer patients. The median survival of the low *HSPB1* expression group was 107.7 months. The median survival of the high *HSPB1* expression group was 21.2 months. A log-rank statistical test was used to calculate statistical significance. (p< 1e-16)

Since gene amplification promotes over-expression of genes, the patient survival rate was further analyzed among two different *HSPB1* expression groups: high and low *HSPB1* expression groups. These two groups were selected according to various quantile expressions of *HSPB1*. The public databases provided these patient survival cohorts: GEO, EGA, and TCGA, and compared by a Kaplan-Meier survival plot (kmplot.com). As shown in Figure 5, stomach cancer patients with high *HSPB1* mRNA expression levels had significantly poorer survival rates than patients with low *HSPB1* expression levels.

Conclusion

Accurately predicting the prognosis of cancer states and identifying cancer stages may benefit cancer patients. For example, molecular markers with clinicopathological parameters are often used to predict cancer patients' survival rates. However, due to the complexity of cancer, the molecular marker for predicting the survival of cancer patients has not been well established. This research identified that amplification of the heat shock protein gene, *HSPB1*, can be used as a novel marker for predicting a patient's overall survival rate. When the *HSPB1* amplified patient's clinical status was further analyzed, they were also associated with a high percentage rate of papillary cancer type and chromosomal loss on 15q and 19p. It was also expected that an increased mRNA expression level of *HSPB1* may decrease patient survival. This expectation was consistent with the survival analysis performed by the Kaplan-Meier survival plot (Figure 5).

Some overexpressed genes in stomach cancer are the HER-2/neu oncogene, the TOP2A gene, the EGFR gene, and chromosome 17q, which decrease survival rates.[12] The amplification of the HER-2/neu oncogene affects the progression of gastric cancer, as it amplifies the amount of tumor tissue and therefore reduces the survival rate. There is also a correlation between gene amplification and protein overexpression of the TOP2A gene shown in gastric cancer.[13] Additionally, the overexpression of the EGFR gene has been implicated in the process of tumor cell motility and metastasis, which is associated with decreased patient survival. Chromosome 17q also is frequently amplified in patients with gastric cancer. The increased copy number of many genes with high expression levels within this chromosome has been identified.[14]

Many studies indicate that heat shock protein (Hsp) overexpression in many types of cancer cells. This phenomenon can be associated with the tumor microenvironment's various stress conditions, including low pH, high oxidative stress conditions, and low glucose levels. Cancer cells reprogram many biological pathways to survive harsh tumor microenvironments in response to this stress condition. Hsps prevent protein aggregation, which is induced by cellular stress.[15] Recently, *HSPB1*, one of the small Hsps groups, has been described as ATP-independent molecular chaperones. Many studies indicate that HSPB1 interacts with many pro-oncogenic proteins that play an essential role in human cancer progression. The high expression level of *HSPB1* usually results in a poor survival rate in many cancers: stomach, uterine, breast, ovarian, and kidney.[16]

Overexpression of *HSPB1* has been identified in both lung and breast cancer. The overexpression of the *HSPB1* gene can also be found in lung cancer, where *HSPB1* helps lead to tumor invasion and metastasis by enhancing the EMT in

lung cancer. The *HSPB1* gene may cause deleterious effects by allowing cancer cells to evade immune surveillance. The overexpression of the *HSPB1* gene has also been identified in breast cancer. It can enhance cell proliferation, tumor development, and the growth of breast cancer cells. It is also associated with resistance to apoptosis in human breast cancer cells; high levels of *HSPB1* expression affect tumor susceptibility to cancer treatments and therefore decrease survival rates.[17]

The next goal of this research is to find the functional role of a high expression level of *HSPB1* on cancer cell progression. *In vitro* experiments will be performed to find the novel function of *HSPB1* on stomach cancer. These findings may lead to the development of a novel stomach cancer therapy.

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