Distinct prognostic roles of HSPB1 expression in non-small cell lung cancer

Z.C. HUANG1,*, H. LI1,*, Z.Q. SUN1, J. ZHENG1, R.K. ZHAO1, J. CHEN3, S.G. SUN1,*, C.J. WU4,*

1Department of Radiology, 2Department of Thoracic Oncology, 3Department of Interventional Radiology, 4Office of National Drug Clinical Trials, Jilin Province Cancer Hospital, Changchun, Jilin, China

*Correspondence: sunshuangyan007@163.com; chunjiaowu216735@gmail.com
#Contributed equally to this work.

Received May 5, 2017/Accepted June 28, 2017

Lung cancer is the leading cause of cancer morbidity and mortality around world. Heat shock protein beta-1 (HSPB1) expression is aberrantly increased in non-small cell lung cancer (NSCLC) patients. However, the roles of HSPB1 expression in the prognosis of NSCLC are still elusive. In this study, we investigated the prognostic roles of HSPB1 in NSCLC by using "The Kaplan-Meier plotter" (KM plotter) database. Our data indicated that HSPB1 mRNA low expression was correlated to better overall survival (OS) for all NSCLC patients, hazard ratio (HR) 1.41 (1.24–1.61), p=1.1e-7, and better OS in lung adenocarcinoma (LUAD) patients, HR 1.81 (1.42–2.32), p=1.5e-06, but not in lung squamous cell carcinoma (LUSC) patients, HR 1.21 (0.94–1.55), p=0.14. In addition, mRNA low expression of HSPB1 is also significantly associated with better OS of NSCLC patients in different smoking status, in different chemotherapy status, in clinical stage I & II, as well as patients with successful surgery treatment. Our results indicated that HSPB1 expression may have distinct prognostic values in NSCLC patients, and may provide an effective clinical strategy to accurately predict the prognosis of NSCLC patients.

Key words: HSPB1, NSCLC, KM plotter, prognostic roles

Lung cancer (LC) is the most common causes of cancer induced deaths in China and worldwide [1]. The 5-year survival rate of LC is very low, which is below 50% in patients with early-stage cancer and is less than 5% in patients with advanced-stage cancer [2]. And the prognosis of LC is also very poor. Non-small cell lung carcinoma (NSCLC), accounting ~85% of LC associated mortalities worldwide [1], includes two major types, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). Although, remarkable progress in LC treatment has been made recently, only limited efforts have been made in comprehensive to identify potential prognostic markers and potential drug targets of lung cancer patients, especially in NSCLC. Therefore, it is critical to develop a more accurate prognostic assessment and molecular biomarkers for NSCLC.

HSPB1 (heat shock protein beta-1) is a gene coding the protein called heat shock protein beta-1 (HSPB1), which is also called heat shock protein 27 (HSP27) [3]. Accumulated evidences indicated that HSPB1 was involved in the pathogenesis of various cancers [4–6]. HSPB1 was highly expressed in mouse lung cancer tissues and stimulated cell proliferation through activator protein-1 (AP-1) related pathway [7]. In hepatocellular carcinoma (HCC) patients, the high expression of HSPB1 indicated poor prognosis [6]. And in prostate cancer and colon cancer patients, the HSPB1 is critical to the initiation of chemoresistance [8]. However, there are no reports on the prognostic roles of HSPB1 in NSCLC patients.

The Cancer Genome Atlas (TCGA) is a project supervised by the National Cancer Institute's Center and the National Human Genome Research Institute funded by the US government from 2005. By using the genome sequencing and bioinformatics approaches, TCGA catalogues genetic mutations responsible for various cancers, and this provides a rich source for investigators to analyze the potential mechanism of cancer pathogenesis. The “Kaplan-Meier plotter” (KM plotter), established using the gene expression data and relapse free and overall survival information downloaded from TCGA, as well as The European Genome-phenome Archive (EGA) and Gene Expression Omnibus (GEO) [9]. KM plotter has been reported to analyze the prognostic value of a particular gene and potential drug targets in NSCLC and other cancers [10–14]. Here, we used KM plotter database to investigate the prognostic roles of HSPB1 expression in NSCLC patients.
Materials and methods

To analysis the prognostic roles of HSPB1 expression in NSCLC, we used a public database to study the relevance and significance of the mRNA level of HSPB1 to overall survival (OS) in different analysis section. The data from NSCLC patients used for KM plotter analysis were pooled from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov), Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and Cancer Biomedical Informatics Grid (caBIG, https://biospecimens.cancer.gov/relatedinitiatives/overview/caBig.asp) [15]. And the whole database included the gene expression (most of samples including the mRNA level) and survival information (20 years) from 1928 NSCLC patients.

To obtain Kaplan-Meier plots for the patients OS rate, we used the KM plotter, and HSPB1 was entered into the database (http://kmplot.com/analysis/index.php?p=service&cancer=lung). And the number-at-risk displayed under the main panel of the OS. Based on the expression of HSPB1, the patients were divided into two groups. Patients with HSPB1 expression higher than the median separates were pooled into the group with high expression, while the patients with HSPB1 expression lower than the median separates were pooled into the group with low expression. Other statistic outcomes, including hazard ratio (HR), 95% confidence intervals and log rank P, calculated from the database were also included in the figures and tables in this manuscript. Values of p<0.05 were used to indicate a statistically significant difference [16].

Results

We used KM plotter and determined the prognostic value of HSPB1 in the database. The Affymetrix IDs is valid: 201841_s_at (HSPB1). Survival curves are drafted for all NSCLC patients (n=1,926) (Figure 1A), for LUAD patients (n=720) (Figure 1B), and for LUSC patients (n=524) (Figure 1C). HSPB1 mRNA low expression was correlated to better overall survival (OS) for all NSCLC patients who were followed for 20 years, hazard ratio (HR) 1.41 (1.24–1.61),
Figure 3. Prognostic value of HSPB1 mRNA expression in NSCLC patients in different stages. The Affymetrix IDs is valid: 201841_s_at (HSPB1). A) Survival curves are plotted for patients in stage 1 (n=577). B) Survival curves are plotted for patients in stage 2 (n=244). C) Survival curves are plotted for patients in stage 3 (n=70).

Figure 4. Determination of prognostic value of HSPB1 expression in NSCLC patients with or without chemotherapy. The Affymetrix IDs is valid: 201841_s_at (HSPB1). A) Survival curves are plotted for patients with chemotherapy (n=176). B) Survival curves are plotted for patients without chemotherapy (n=310).

Figure 5. Prognostic value of HSPB1 mRNA expression in NSCLC patients with surgery. The Affymetrix IDs is valid: 201841_s_at (HSPB1). Survival curves are plotted for patients after surgery only with negative surgical margins (n=726).

HSPB1 mRNA low expression was also correlated to better OS in LUAD patients, HR 1.81 (1.42–2.32), p=1.5e-06, but not in LUSC patients, HR 1.21 (0.94–1.55), p=0.14.

For further assess the association of HSPB1 with other clinicopathological profiles, we determined the correlation with the patients’ smoking status (Table 1, Figure 2), different clinical stages (Table 2, Figure 3), different chemotherapeutic treatments (Table 3, Figure 4) and different surgical treatments (Table 4, Figure 5). As shown in Figure 2 and Table 1, HSPB1 mRNA low expression was correlated to better OS in smoked patients HR 1.32 (1.06–1.63), p=0.0123 (Figure 2A) and in never smoked patients HR 3.58 (2.04–6.25), p=1.9e-06 (Figure 2B). As shown in Figure 3 and Table 2, HSPB1 mRNA low expression was correlated to better OS in stage I patients HR 2.22 (1.69–2.9), p=3e-9 (Figure 3A) and stage II patients HR 1.96 (1.36–2.83), p=0.0003 (Figure 3B). And the HSPB1 mRNA low expression was also marginally correlated to better OS in patients with chemotherapeutic treatment HR 1.58 (1–2.51), p=0.0522 (Figure 4A) and significantly corre-
Table 1. Correlation of HSPB1 mRNA expression with smoking status of NSCLC patients.

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Cases</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never smoked</td>
<td>205</td>
<td>3.58</td>
<td>2.04–6.25</td>
<td>1.9e-6</td>
</tr>
<tr>
<td>Smoked</td>
<td>820</td>
<td>1.32</td>
<td>1.06–1.63</td>
<td>0.0123</td>
</tr>
</tbody>
</table>

Table 2. Correlation of HSPB1 mRNA expression with clinical stages of NSCLC patients.

<table>
<thead>
<tr>
<th>Clinical stages</th>
<th>Cases</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>677</td>
<td>2.22</td>
<td>1.69–2.9</td>
<td>3e-9</td>
</tr>
<tr>
<td>II</td>
<td>244</td>
<td>1.96</td>
<td>1.36–2.83</td>
<td>0.0003</td>
</tr>
<tr>
<td>III</td>
<td>70</td>
<td>1.47</td>
<td>0.82–2.64</td>
<td>0.1918</td>
</tr>
</tbody>
</table>

Table 3. Correlation of HSPB1 mRNA expression with chemotherapy of NSCLC patients.

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Cases</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>310</td>
<td>1.66</td>
<td>1.16–2.36</td>
<td>0.0046</td>
</tr>
<tr>
<td>Yes</td>
<td>176</td>
<td>1.58</td>
<td>1–2.51</td>
<td>0.0522</td>
</tr>
</tbody>
</table>

Table 4. Correlation of HSPB1 mRNA expression in NSCLC patients with negative surgical margins.

<table>
<thead>
<tr>
<th>Cases</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>726</td>
<td>1.7</td>
<td>1.35–2.16</td>
</tr>
</tbody>
</table>

Discussion

Heat shock proteins (HSPs) are produced by cells and tissues in response to various stressful conditions, including cold [17], UV light [18], wound healing or tissue remodeling [19]. Recently, accumulated evidences indicated that HSPs are aberrantly overexpressed in many types of human cancers and play critical roles in tumor progression. HSPs are involved in the cancer cell differentiation, proliferation, metastasis and invasion [20]. HSPB1 is one of important components in HSP family [21]. Recent studies have indicated that the expression of HSPB1 is associated with poor prognosis of a wide range of human cancers [20, 22], including gastric [23], osteosarcomas [24], prostate [25, 26] and liver carcinoma [27]. However, the prognostic roles of HSPB1 in NSCLC have not been studied.

HSPB1 is expressed at high levels in breast cancer [28], HCC cancer [6, 27] and prostate cancer [4, 8], and it is essential for tumor development through different mechanisms. In breast cancer cells, HSPB1 was required for cell migration and cancer stem cell maintenance, which may through the interaction of NF-κB dependent pathway [28]. And the phosphorylated HSPB1, which is formed through the kinases catalyzed phosphorylation of HSPB1, is essential to the progression of various cancers. Recently, phosphorylated HSPB1 has been proved to suppress apoptosis, enhance invasion and survival in cancer cells [29]. Additionally, recent studies indicated that phosphorylation of HSPB1 could modify the subcellular localization of HSPB1 in HCC cells [30]. And abolish the phosphorylation of HSPB1 through mutating the phosphorylation site from serine residues to alanine residues blocks the phosphorylation of HSPB1 and attenuates the translocation of HSPB1 to nuclei in HCC cells, and suggested that the subcellular localization of HSPB1 may play essential biological roles in liver cancer [30]. Accumulating evidence links rising HSPB1 expression levels with the progression of prostate cancer [31]. In prostate cancer cells, HSPB1 has been shown to bind with androgen and androgen receptor to form a complex, and induced the activation of androgen receptor, stimulated translocation of the complex to nuclei and further regulated gene expression and tumor progression [31].

Recent investigation indicated that the protein expression of HSPB1 was increased in the mouse lung tissue with lung cancer [7], and predicted the relationship between the expression of HSPB1 and lung cancer progression. In this study, we investigated the prognostic values of HSPB1 expression in NSCLC patients through analyzing the OS of NSCLC patients by using the KM plotter database. Our current studies indicated that HSPB1 is a predictor of NSCLC prognosis, and it is functional as an oncogene during NSCLC progression. The NSCLC patients with high expression of HSPB1 associated with the poor OS. And recent investigations in animal models and cell culture system also suggested that HSPB1 expression is associated with the tumor progression in various human cancers. Molecular biological investigations to study the expression of HSPB1 in tumor tissues and cells from human cancer patients have proved that the expression of HSPB1 is significantly higher in tumor tissues than control tissues from human hepatocellular carcinoma [6] and prostate cancer [4, 8, 32] and mouse lung cancer [7]. In human hepatocellular carcinoma, HSPB1 stimulates tumor progression through facilitating the hepatocellular carcinoma cells metastasis via Akt signaling [6, 22]. Both in vivo and in vitro studies of the prostate cancer indicated that HSPB1 regulates human prostate cancer cell motility and metastatic progression via increased the expression of matrix metalloproteinase 2 (MMP-2) [29], which is a key stimulator for cancer cell invasion [33]. In mouse lung cancer development, HSPB1 was proved to induce the lung cancer cells proliferation via activator protein-1 (AP-1) dependent pathway [7]. Additionally, HSPB1 also plays critical roles to the initiation of the chemotherapeutic resistance in prostate cancer [8] and colon cancer [34], and this may through the
phosphorylation of HSPB1 protein. Notably, the HSPB1 inhibitor, OGX-427, has been used as a drug candidate to treat the patients with castration-resistant prostate cancer and other advanced cancers [32]. Together, HSPB1 was proved to involve in the tumor progression in various kinds of cancers, and its inhibitor would be a potential therapy to patient with advanced cancers.

In conclusion, our investigations demonstrated the distinct prognostic roles of HSPB1 mRNA expression in patients with NSCLC, and HSPB1 mRNA low expression was correlated to better OS for all NSCLC patients and in LUAD patients, but not in LUSC patients. Thus, our results suggested that HSPB1 might be potential drug target for NSCLC patients, and its expression may have distinct prognostic values in NSCLC patients.

Acknowledgements: This study was supported by the China National Natural Science Foundation (NSFC-81400047), the Science and Technology Department of Jiangsu Province (BK20150213). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References


