Diagnostic Value of HSP90α and Related Markers in Lung Cancer

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Abstract

Purpose
To investigate the expression of heat shock protein 90α (HSP90α) in patients with lung cancer and the clinical value of HSP90α and other related markers in the diagnosis of lung cancer.

Methods
The plasma levels of HSP90α and related markers (CEA, NSE, CF211 and ProGRP) were detected in the blood of 560 patients with lung cancer by ELISA (enzyme-linked immunosorbent assay). Groups were divided according to the gender (male/female), age (age≤40, 41<age≤50, 51<age≤60, 61<age≤70 and age>70), types of lung cancer (small-cell, squamous carcinoma, adenocarcinoma, hybrid and other type), staging (Ⅰ, Ⅱ, Ⅲ and Ⅳ) and metastasis (metastasis and non-metastasis) separately. Wilcoxon Mann-Whitney test and Kruskal-Wallis test were used to compare statistical differences between two groups/among the multiple groups for each factor of HSP90α.

Results
No statistical difference was found in plasma level of HSP90α among different age and gender groups (P>0.05). In the group divided by lung cancer type, staging and metastasis status, there were statistical differences among different groups in HSP90α level (P<0.05). R values of HSP90α correlated with other related markers in the diagnosis of lung cancer (P<0.05). Although HSP90α and other related markers didn't fit the satisfactory conformance, in terms of the positive rate of diagnosis, it was statistically differences in the diagnostic positive rate between HSP90α and each marker (P<0.01). Reduced cut-off value of HSP90α in lung cancer can effectively improve the positive rate of diagnosis when combined with other tumor biomarkers.

Conclusions
HSP90α has significant clinical value on early screening and diagnosis of lung cancer. The combined application of HSP90α and related markers can improve the positive rate of early diagnosis of lung cancer effectively.

Introduction
Lung cancer (LC) is one of the malignant tumors with the highest morbidity and mortality that greatly threatening human health and life. According to the global cancer statistics report of 2018 published by Bray et al. lung cancer remains the most common cancer (11.6% of the total cases) and the leading cause of death (18.4% of the total deaths) globally [1]. In China, the lung cancer has the highest morbidity (57.26/1000,000) and mortality (47.87/1000,000) compared to other malignant tumors [2]. As there were no typical symptoms and/or discomfort in early lung cancer, most were in middle-late or terminal stage when being diagnosed. Therefore, it would be important to find more meaningful biomarkers in improving the early diagnosis of lung cancer.
Heat shock proteins (HSPs), also known as stress proteins, is a group of proteins that are highly expressed by body cells after being stimulated by several physical and chemical factors [3]. HSP90α is actively secreted to the extracellular domain and plays a role by tumor cells when stress or malignant transformation occurs [4, 5]. In 2009, Wang et al. found patients with liver cancer had higher level of HSP90α in plasma, and the expression level correlated with the stage of liver cancer, which suggested HSP90α can be used as a tumor marker for early screening [6]. Previous studies of HSP90α mainly involved in liver cancer and colorectal cancer et al [7-11], while studies on the expression level of HSP90α on patients with lung cancer were really rare, and the correlations between HSP90α and other tumor biomarkers were not well described.

Based on the level of HSP90α and other related markers in blood from 560 patients with lung cancer, the goals of this study were I) to explore the expression level among different groups divided on age, gender, pathological types, staging and metastasis status respectively. II) to compare the diagnostic performance between HSP90α and other related markers. Here, we hypothesized that combined HSP90α with other tumor biomarkers such as CEA, NSE, CF211 and ProGRP can effectively improve the early diagnosis rate of lung cancer.

**Materials And Methods**

**Patients and methods**

In this retrospective case-control study, 560 LC patients (400 males, 160 females, and age ranging from 31 to 86 years old) and 78 healthy controls were enrolled in this study from December 2016 to December 2018 at Shaanxi Provincial Cancer Hospital. Clinicopathological variables such as gender, age, pathological types, tumor stage and metastasis status were collected from the database of Shaanxi Provincial Cancer Hospital. Groups were divided according to the gender (male/female), age (age ≤ 40, 41<age ≤ 50, 51<age ≤ 60, 61<age ≤ 70 and age > 70), types of lung cancer (small-cell, squamous carcinoma, adenocarcinoma, hybrid and other type), staging (Ⅰ, Ⅱ, Ⅲ and Ⅳ) and metastasis (metastasis and non-metastasis) separately. The staging of LC were classified according to American Joint Committee on Cancer classification (AJCC 7th edition, 2010). This study was approved by the ethics committee and review committee of Shaanxi Provincial Cancer Hospital, and the methods were carried out in accordance with the approved guidelines. For this type of study, informed consent is not required.

**Testing of blood samples**

Peripheral blood samples were collected into a 2ml EDTA-K2 anticoagulant tubes for detection of HSP90α and other related markers. Plasma were separated from the whole blood cells by centrifugation at 3000 RPM for 10 min, then stored the plasma at -20°C until use. Plasma HSP90α was measured with commercially ELISA Kit (Progy Biotechnology Development Co. Ltd, Shenzhen, China). Briefly, samples were added to the 96-well microplate pre-coated with HRP labeled monoclonal antibody to HSP90α, then incubated at 37°C for 1 hour. The reaction was visualized by adding 50 μL chromogen 3,3,5,5- tetramethylbenzidine (TMB) solution A and 50 μL chromogen TMB solution B to each well and incubated for 20 minutes at 37°C. Finally, the reaction was stopped by adding with 50 μL stop solution to each well. The optical density was measured at 450 nm and referenced to 620 nm on Rayto RT-6100 Micro-plate Spectrophotometer (Rayto Co. Ltd, Shenzhen, China).
The standard curve was generated by plotting the logarithm of average O.D. obtained for each of the six standard samples on the vertical (Y) axis versus the logarithm of corresponding concentrations on the horizontal (X) axis. The absorbance of samples was calculated with the method of substitution in the standard curve. Double logarithmic curve fitting was recommended, and the coefficient of correlation ($R^2$) was required to be greater than 0.980. Commercially available ELISA kit was used for the quantitative assessment of plasma HSP90α concentrations according to the manufacturer’s recommendations.

The levels of serum CEA, NSE, CF211 and ProGRP were tested with commercially available ELISA kit (Roche Life Science) following the manufacturer’s recommendations using Cobas E411 automatic analyzer (Roche Diagnostics, IN, USA).

**Statistical methods**

Wilcoxon Mann-Whitney test and Kruskal-Wallis test were used to compare statistical differences between two groups/among the multiple groups for each factor of HSP90α. In addition, the correlation coefficient $r$, kappa value of HSP90α and related markers in lung cancer were calculated to compare the differences between HSP90α and various markers separately in lung cancer diagnosis and the combined diagnosis efficiency as well as to analyze the correlation between HSP90α and related markers in lung cancer. The application SPSS21.0 was used for all statistical comparisons and the significantly statistical level was set at the threshold $P< 0.05$.

**Results**

A total number of 638 cases were enrolled in this study, including 560 cases with lung cancer and 78 healthy controls. The mean and quartiles of HSP90α in LC groups and healthy controls were showed in Table 1, levels of HSP90α in LC groups were significantly higher than healthy controls ($P< 0.01$). No significant difference in HSP90α were observed between male and female subjects ($P= 0.587$) and between different age groups ($P= 0.924$), as shown in Table1 and Table 2.

For different types of lung cancer, plasma levels of HSP90α were significantly different ($P= 0.021$). HSP90α level is higher in SCLC groups than SLC group ($P< 0.05$), and there were no statistically significant differences between other two groups ($P> 0.05$), as shown in Figure 1 and Table 2.

Plasma levels of HSP90α in late-stage lung cancer patients (TNM stage III+IV) were significantly higher than patients with early-stage lung cancer (TNM stage I+II, $P< 0.001$, Figure 2, Table 2), which suggested the plasma levels of HSP90α positively correlated with malignancy of lung cancer.

For the metastasis status of lung cancer patients, patients with lymph node and/or distant metastasis status had a higher plasma levels of HSP90α compared with patients with non-metastatic status ($P< 0.001$), as shown in Table 2.

Pearson correlation analysis was used to compare the correlation coefficient $r$ and $p$ values of HSP90α, CEA, NSE, CF211 and ProGRP, and Kappa method was used to compare the consistency of HSP90α, CEA, NSE, CF211 and ProGRP, as shown in Table 3. In diagnosis of lung cancer, $r$ values of HSP90α and various markers
was: NSE>CEA>ProGRP>CF211, which showed that HSP90α and various markers were correlated in lung cancer diagnosis ($P<0.05$). The Kappa values of HSP90α with CEA, NSE, CF211 was 0.129, 0.293, 0.121 ($P<0.05$), and the Kappa value of HSP90α and ProGRP was 0.055 ($P>0.05$).

To analyze the difference between HSP90α and various markers in the diagnosis of lung cancer, we calculate the positive rate at different critical value of HSP90α combined with other tumor biomarkers, as shown in Table 4, when the critical value of HSP90α decrease, the positive rate of HSP90α gradually increase, and the total positive rate combined with other biomarkers gradually increases.

**Discussion**

HSP90α is one type of homologous hypotype molecular chaperone proteins encoded by the gene HSP90AA1 [12]. Cheng et al. demonstrated that HSP90α present in the cytoplasm and can be secreted by tumor cells [13]. The extracellular HSP90α participates in invasion and metastasis of malignant tumor cells [14, 15], it can promote metastasis and invasion of the tumor cells via activating plasma fibrinolysin. HSP90α can promote and induce the growing of tumor cells, angiogenesis, cell proliferation, metastasis and local invasion [16, 17]. In this study, plasma level of HSP90α in LC patients had a higher level compared with healthy controls, while not affected by the gender and age. The expression of HSP90α in SCLC was higher than other pathology subtypes, which indicated HSP90α can be used as an auxiliary indicator for identification the pathology subtypes of lung cancer. In the study of Shi et al., the plasma level of HSP90α was found to be associated with the staging of tumor, therapeutic response, preoperative and postoperative of the surgery, disease progression in patients with lung cancer [18], our data also suggested that HSP90α showed a positive correlation in staging and pathology subtypes. Cheng et al. reported the abnormally elevated of HSP90α indicate poor prognosis or metastasis in patients with breast cancer [19, 20], our study also showed a significant increase of HSP90α levels in patients with lymph node and/or distant metastasis which correlated with previous studies [10, 21-23].

HSP90α was considered to have differences in correlation with various markers in the diagnosis of lung cancer by comparison ($P<0.05$). In the consistency comparison of HSP90 with CEA, NSE, CF211 and ProGRP, according to the standard that Kappa<0.75 was considered consistency unsatisfactory, it can be found the consistency of HSP90α with each marker is not satisfactory. Therefore, we further discussed whether lowering the HSP90α threshold could improve the overall diagnostic positive rate. With the decrease of HSP90α critical value, the positive rate of HSP90α gradually increased, and the total positive rate of combined diagnosis gradually increased. Therefore, while reducing the cut-off value of HSP90α, the positive combined diagnosis rate of the early lung cancer can be effectively improve [6].

The overexpression of HSP90α in tumors also provides a new therapeutic target for treatment. Alarcon et al. found that HSP90α inhibitor had specificity and pleiotropic effect obviously for the treatment of malignant tumors [24]. The expression of HSP90 is proportional to that of STAT5b in hypoxia and the Jak2/STAT5b pathway is a new target for solid tumor therapy and it can regulate the expression of HSP90α [25]. The HSP90 carboxyl terminal inhibitors played an important role in cell apoptosis and metastasis by blocking the complex activity of HSP90α/Aha1 and pc3-mm cells [26]. It can be interfered with the invasion and metastasis of pancreatic ductal adenocarcinoma (PDAC) by regulating HSP90α/uPA mmp-2 protein
hydrolysis axis [27]. These studies strongly demonstrate that HSP90 plays an important role in tumor metabolism, and levels of HSP90α in plasma is important in tumor treatment and monitoring.

Rong et al. explored the significance of HSP90α as a potential biomarker in liver cancer in their latest study [28]. While, McDowell et al. obtained the diagnostic value of tumor markers in bronchoalveolar lavage fluid for peripheral lung cancer, and HSP90α has been proved to have clinical value in the diagnosis of peripheral lung cancer [29]. Sourbier et al. conducted a systematic summarize on the up-regulation of heat shock protein 90α (HSP90α) in cancer cells, tissues and serum of lung cancer patients, and its close correlation with the occurrence, development and outcome of lung cancer [30]. These recent studies further demonstrate that HSP90α is not only a potential biomarker for liver cancer, but also a potential biomarker for lung cancer. McDowell et al. found that in the of detection proteomic HSP90α and HSP90β, at least 10 of 17 human tumors had one significantly up-regulated HSP90 hypotype or HSP90 synergistic partner [31].

**Conclusion**

In conclusion, HSP90α has a significant diagnostic value in classification, staging and metastasis of lung cancer. As a potential tumor biomarker, HSP90α has important clinical significance in early screening, diagnosis, treatment and prognosis evaluation of lung cancer. Combined HSP90α with other tumor biomarkers such as CEA, NSE, CF211 and ProGRP can improve the early diagnosis rate of lung cancer effectively.

**Declarations**

**Authors contributions**

Study conception and design: Bin Yuan, Zhimin Yuan, Lin Li, Ting Tang and Changbei Shi; Acquisition of data: Zhimin Yuan; Analysis and interpretation of data: Zhimin Yuan, Longhao Wang and Changbei Shi; Drafting of manuscript: Zhimin Yuan and Songlin Hong; Critical revision: Longhao Wang; Final approval of the article: all authors.

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**Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

**Consent for publication**

Not applicable.

**Competing interests**
All authors declare no conflicts of interest.

References


Tables

**Table 1 Mean and Quartile of HSP90α in Lung Cancer and Healthy Control**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean±SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Percentiles</th>
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<td></td>
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<td></td>
<td>25th</td>
<td>50th (Median)</td>
<td>75th</td>
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<tr>
<td>LC</td>
<td>560</td>
<td>97.64±103.36</td>
<td>2.56</td>
<td>852.30</td>
<td>40.85</td>
<td>65.69</td>
<td>110.95</td>
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<tr>
<td>HC</td>
<td>78</td>
<td>38.44±15.40</td>
<td>1.56</td>
<td>79.10</td>
<td>29.11</td>
<td>39.78</td>
<td>48.46</td>
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</table>

Note: P<0.05, indicates statistical difference.

**Table 2 Mean and Quartile of HSP90α in Gender, Age, Classified, staging and metastasis Groups**
<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Mean±SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Percentiles 25th</th>
<th>50th (Median)</th>
<th>75th</th>
<th>Z</th>
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<tr>
<td>≤40y</td>
<td>16</td>
<td>105.73±98.96</td>
<td>9.69</td>
<td>363.60</td>
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<td>73.56</td>
<td>134.83</td>
<td>0.907</td>
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<tr>
<td>41-50y</td>
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<td>92.24±86.20</td>
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<td>512.60</td>
<td>39.62</td>
<td>68.40</td>
<td>117.80</td>
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<td>51-60y</td>
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<td>8.16</td>
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<td>41.47</td>
<td>66.92</td>
<td>113.70</td>
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<td>61-70y</td>
<td>239</td>
<td>100.41±119.89</td>
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<td>852.30</td>
<td>37.61</td>
<td>63.59</td>
<td>109.10</td>
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<tr>
<td>&gt;70y</td>
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<td>92.36±83.94</td>
<td>5.62</td>
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<td>45.11</td>
<td>63.83</td>
<td>100.90</td>
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<td>SCLC</td>
<td>117</td>
<td>110.48±101.86</td>
<td>11.43</td>
<td>573.40</td>
<td>46.72</td>
<td>73.27</td>
<td>131.35</td>
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<td>.021</td>
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<tr>
<td>SLC</td>
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<td>54.90</td>
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<td>ALC</td>
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<td>16.12</td>
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<td>Ⅰ</td>
<td>37</td>
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<td>222.30</td>
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<td>42.01</td>
<td>75.82</td>
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<td>10.48</td>
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<td>47.58</td>
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<tr>
<td>Ⅲ</td>
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<td>74.91</td>
<td>121.18</td>
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<tr>
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<td>333</td>
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<td>2.58</td>
<td>852.30</td>
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<td>70.99</td>
<td>129.85</td>
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<td>37.12</td>
<td>56.66</td>
<td>85.96</td>
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</table>

Note: SCLC: small cell lung cancer; SLC: squamous cell lung carcinoma; ALC: adenocarcinoma of lung; MLC: mixed lung cancer; OLC: other type lung cancer

P<0.05, indicates statistical difference.
Table 3 Correlation and consistency between HSP90α and related markers in lung cancer

<table>
<thead>
<tr>
<th></th>
<th>CEA</th>
<th>NSE</th>
<th>CF211</th>
<th>ProGRP</th>
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<tr>
<td>n</td>
<td>557</td>
<td>521</td>
<td>548</td>
<td>185</td>
</tr>
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<td>HSP90α</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.224</td>
<td>0.305</td>
<td>0.117</td>
<td>0.194</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>+</td>
<td>72</td>
<td>143</td>
<td>81</td>
<td>116</td>
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<td>-</td>
<td>73</td>
<td>269</td>
<td>45</td>
<td>279</td>
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<tr>
<td>Kappa value</td>
<td>0.129</td>
<td>0.293</td>
<td>0.121</td>
<td>0.055</td>
</tr>
</tbody>
</table>

Note: the normal reference values were HSP90α<82ng/ml, CEA<20ng/ml, NSE<13ng/ml, CF211<7ng/ml, ProGRP<60ng/ml.

Kappa value>0.75, indicates the satisfactory consistency.

P<0.05, indicates statistical difference.

Table 4 Positive rate of combined diagnosis of HSP90α and related markers in lung cancer

<table>
<thead>
<tr>
<th>Hsp90α ng/ml</th>
<th>n</th>
<th>Positive%</th>
<th>Total</th>
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<tbody>
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<td>Hsp90α</td>
<td></td>
<td>Hsp90α</td>
<td>CEA</td>
</tr>
<tr>
<td>35</td>
<td>451</td>
<td>80.54%</td>
<td>27.84%</td>
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<td>50</td>
<td>370</td>
<td>66.07%</td>
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<tr>
<td>65</td>
<td>282</td>
<td>50.36%</td>
<td>30.96%</td>
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<tr>
<td>82</td>
<td>214</td>
<td>38.21%</td>
<td>33.18%</td>
</tr>
</tbody>
</table>

Figures
Figure 1

In the pathological types of lung cancer, HSP90α increased significantly in SCLC, moderately in ALC, and slightly in SLC, Mixed LC and Other LC. Note: lung cancer types (SCLC: small cell lung cancer; SLC: squamous cell lung carcinoma; ALC: adenocarcinoma of lung; MLC: mixed type lung cancer; OLC: other types of lung cancer.)
In accordance with the staging system of lung cancer (TNM), level of HSP90α is positively correlated with the progress of staging. Note: ✖ phase i, ☑ phase ii, ☐ phase iii, ☐ phase iv.