Diagnostic value of HSP90 α and Related Markers in Lung Cancer

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Abstract

Aim: To prove the expression of heat shock protein 90 α (HSP90 α) in lung cancer and the clinical value of HSP90 α and related markers in the diagnosis of lung cancer. **Methods:** The concentrations of HSP90 α and related markers were detected in the blood of 560 lung cancer patients by enzyme-linked immunosorbent assay for analyzing the statistical differences of HSP90 α in patients' age, gender, pathological types, tumour staging and metastasis status, as well as the differences and evaluate the value of HSP90 α and related markers in lung cancer diagnosis. **Results:** The results showed no statistical difference in HSP90 α among age and gender groups ($P>0.05$); In the group by lung cancer type, statistical differences were found in the HSP90 α level between the small cell lung cancer (SCLC) group and the squamous carcinoma (SLC) group ($P<0.05$); In the group by staging, the HSP90 α level of high staging was significantly higher than that low staging, and the HSP90 α level at I/II/III/IV shows statistical differences among the groups ($P<0.05$); The test result of HSP90 α was higher in the metastatic group than in the non-metastatic group significantly, and the significant difference between the two groups ($P<0.05$). The r value of the HSP90 α and related markers in the diagnosis of lung cancer: $\text{NSE}>\text{CEA}>\text{ProGRP}>\text{CF211}$ ($P<0.05$). Although HSP90 α and related markers didn't fit the satisfactory conformance, in terms of the positive rate of diagnosis, it were statistically differences in the diagnostic positive rate between HSP90 α and each marker ($P<0.01$). Reducing HSP90 α clinical references in lung cancer combined diagnosis can effectively improve the positive rate of the combined diagnosis. **Conclusion:** HSP90 α has significant value on early screening and diagnosis of lung cancer. The combined application of HSP90 α and related markers can improve the positive rate of early diagnosis of lung cancer effectively.

Background

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 Brsy[1] 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. Yet China has become the country with the highest morbidity and mortality of cancer comparing to other countries. Because the early symptoms of lung cancer are often mild and lack of typical symptoms, even without any discomfort, most of the cancer has been found in the middle-late stage of cancer. Therefore,

finding more meaningful biomarkers is of particular importance 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 some physical and chemical factors [2]. According to the molecular weight of proteins, they are divided into five categories, namely HSP110, HSP90, HSP70, HSP60 and small molecule heat shock protein (sHSPs). In these proteins, it was found that HSP90 only exists in the extracellular domain, with two subtypes of HSP90 α and HSP90 β . Under normal physiological

conditions, HSP90 α is not secreted to the extracellular domain, but can be actively secreted to the extracellular domain and play a role by tumor cells when stress or malignant transformation occurs [3-4]. In 2009, WANG et al. [5] first found that HSP90 α was highly expressed in the blood of patients with liver cancer, and the plasma content was positively correlated with the degree of malignancy of liver cancer, which could be used as a tumor marker for early screening. In the previous researches, HSP90 α content was increased in studies such as Burgess's [6] on non-small-cell lung cancer, Jiang's[7] on pancreatic cancer, Alexiou's[8] on glioma and meningioma, FU's[9] on liver cancer, Kasanga's[10] on colorectal cancer,etc. Studies have shown that the presence of HSP90 α on the cell surface is associated with tumor growth and development, including age, tumor volume, estrogen receptor expression, and metastasis [11]. Which means that HSP90 α , as an important biomarker [12],is of great significance in the diagnosis and prognosis of tumors.

In this study, the HSP90 α and related markers of the blood were collected among 560 lung cancer patients, in order to explore the clinical expression on age, gender, different pathological types, lung cancer staging, and metastasis status respectively. Also by comparing the diagnostic performance on HSP90 α and other related markers, this paper demonstrated the clinical value of HSP90 α in early diagnosis, treatment monitoring and prognosis evaluation of lung cancer.

Methods

1.1 Patients and methods: The clinical data which contained 560 LC patients with HSP90 α and related markers was collected from the Shaanxi Provincial Cancer Hospital during the period of December 2016 to December 2018. 400 males' cases and 160 females' cases were included in this dataset. Their ages range from 31 to 86 and the average age was 73 years with a standard deviation of 6.4. Clinicopathological variables such as gender, age, pathological typing, tumor stage and metastasis were all retrieved from the database of Shaanxi provincial Cancer Hospital. All lung cancer patients were staged and classified according to the type of Tumor, Node, Metastasis, TNM in AJCC 7th edition 2010. This study was approved by the ethics committee and review committee of Shaanxi provincial Cancer Hospital.

1.2 Instruments and Methods: Plasma HSP90 α was measured with ELISA Kit (Progy Biotechnology Development Co. Ltd, ShenZhen, China), and the detection instrument was Rayto RT-6100 labelmeter of ShenZhen redu company. The assessment of serum CEA NSE, CF211 and ProGRP levels were assessed with an electrochemiluminescence immunoassay. The samples were collected in EDTA-K2 anticoagulant tubes for the detection of HSP90 α and the clot activator, clear vacuum tube with separating glue for detection of CEA, NSE, CF211 and ProGRP. Plasma and serum were separated from the whole blood cells by centrifugation of blood samples at 3000 RPM for 10 min at room temperature within 2 h after sample

collection. All methods were performed as per the relevant guidelines. The specimens were qualified and no hemolysis or clotting occurred.

Take 2ml edta-k2 sample and diluted by 20 times, 50µl standard and diluted samples were added to HSP90α solid phase plate. Add HSP90α labeled liquid 50µl, Oscillating mixing; and then subsequently, the plate was incubated at 37°C for 60 minutes. Add 300µl of lotion per hole, Wash the board 6 times.

Adding color developing agent 50µl, Oscillating mixing; and the plate was incubated at 37°C for 20 minutes. Add 50µl of termination liquid, the OD value was read at the wavelength of 450nm/620nm according to the standard curve was used to calculate plasma HSP90α. The standard curve was produced by plotting the logarithm of average optical density obtained for standard samples, and the levels of plasma HSP90α were analyzed using commercially available ELISA kit following the manufacturer's recommendations.

1.3 Statistical methods: Classify 560 cases of HSP90α testing results in lung cancer according to gender (male/female), age (age≤40, 41<age≤50, 51<age≤60, 61<age≤70, 70>age), lung cancer type (small cell, squamous carcinoma, adenocarcinoma, hybrid, other type), stage (Ⅰ/Ⅱ/Ⅲ/Ⅳ) and metastasis (metastasis/non-metastasis), and the results were indicated with quartiles of HSP90α, Mann-Whitney Test was used for comparison between two groups, Kruskal-Wallis was used for comparison among multiple groups, to compare the statistical differences among the groups for each factor of HSP90α. The correlation coefficient r, kappa value and positive rate 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 and to analyze the correlation between HSP90α and related markers in lung cancer. The application SPSS21.0 was used for all statistical comparisons P<0.05 was considered statistically significant.

Results

The test results and quartiles of 560 cases of HSP90α in lung cancer were shown in the figure below, with the HSP90α>82ng/ml as the clinical reference value, and the test results of the positive rate of HSP90α was 38.2%.

In the group by gender (male/female), the test results and quartiles of 400 cases in males and 160 cases in females of HSP90α were shown in the figure below, Mann-whitney U Test showed U=31060.5, Z=-0.543 with P=0.587>0.05, indicating that there was no significant difference in HSP90α test results between the gender groups(P>0.05). In the group by age (age≤40, 41<age≤50, 51<age≤60, 61<age≤70, 70>age), the test results and quartiles of HSP90α in each age group were shown in the figure below. Kruskal-Wallis Test showed H=0.907 with P=0.924>0.05, these results indicated that there was no statistical difference among groups by age(P>0.05).

In the group by lung cancer type (small cell, squamous carcinoma, adenocarcinoma, hybrid, other type), according to the diagnosis results of the cases, 545 cases with definite diagnostic type were obtained, and the test results and quartiles of HSP90 α were performed separately according to the type. In the box plot of Kruskal-Wallis Test result, the Grand Median=64.91, H=11.520 and p=0.021 which was less than 0.05, indicating that there were statistical differences among the groups(P<0.05). Each groups were compared with a pair-wise comparison, between the SLC group and the SCLC group,

H=57.150, P=0.035<0.05, showed a statistically significant difference in test results of HSP90 α (P<0.05), and there was no statistically significant difference in test results of HSP90 α between any other two groups (P>0.05), as shown in the figure below, there was a statistically significant difference in test results of HSP90 α between the groups which connected by yellow line (P<0.05).

In the group by cancer staging (I/II/III/IV), according to the diagnosis of the cases, 516 cases with definite diagnosis in stage were obtained, and the test results and quartiles of HSP90 α were performed separately according to the stage, in the box plot of Kruskal-Wallis Test result, the Grand Median= 62.94, H=34.076 and p<0.001, suggesting that there were statistical differences in HSP90 α among the lung cancer staging groups(P<0.05). Each groups were compared with a pair-wise comparison, the results of Kruskal-Wallis test in staging I respectively tested with staging II, III, IV were as follows: H=-93.743 with P=0.001<0.05; H=86.525 with P<0.001; H=-46.799 with P=0.036<0.05, indicating that there were statistically significant differences in the test results of HSP90 α from the results in staging I respectively tested with staging II, III, IV (P<0.05). And there was no statistically significant difference in test results of HSP90 α between any other two groups (P>0.05), as shown in the figure below, there was a statistically significant difference in test results of HSP90 α between the groups which connected by yellow line (P<0.05).

In the group by metastatic, according to the diagnosis of the cases, 556 cases with definite diagnosis in stage were obtained. Among them, the test results and quartiles of HSP90 α , including 333 patients in the metastatic group and 223 patients in the non-metastatic group, were shown in the figure below. The Mann-Whitney U test results showed that U=29600.5, Z=-4.055 with P<0.001, indicating that there was a statistical differences between the test results of HSP90 α in the metastatic group and the non-metastatic group(P<0.05).

As for the correlation analysis of HSP90 α and related markers in lung cancer diagnosis, Pearson correlation analysis was used to compare the correlation coefficient r and p values of HSP90 α , CEA, NSE, CF211 and ProGRP, and Kappa method was used to compare the consistency of HSP90 α , CEA, NSE, CF211 and ProGRP. In lung cancer diagnosis, the sequence of r values of HSP90 α and various markers was: NSE>CEA>ProGRP>CF211, which shows that HSP90 and various markers are correlated in lung cancer diagnosis (p<0.05). In the comparison of Kappa, the Kappa values of HSP90 α respectively with CEA, NSE, CF211 was (Kappa=0.129, 0.293, 0.121 with

P<0.05). And the Kappa value of HSP90 α and ProGRP was (Kappa=0.055, P>0.05).

Analysis of the difference between HSP90 α and various markers in the diagnosis of lung cancer, and calculate the positive rate of various makers at different concentration thresholds of HSP90 α . As shown in the figure below, when the critical value of HSP90 α increases, the positive rate of HSP90 α gradually decreases, while the positive rate of other markers increases, and the total positive rate of combined diagnosis gradually decreases. In the combined diagnosis, considering HSP90 α =50ng/ml as a clinical reference value can effectively improve the accuracy of combined diagnosis and reduce the false negative rate.

Discussion

Heat shock protein 90 α (HSP90 α) is one type of homologous hypotype molecular chaperone proteins encoded by the gene HSP90AA1 [13]. CHENG et al. [14] demonstrated that the HSP90 α in tumor cells is not only present in the cells, but more importantly than all of that, it can be actively secreted by tumor cells and play a role outside the cells. The content of Hsp90 α in normal cells is about 1%, which has a good buffering function. The content of Hsp90 α in tumor cells is as high as 2-7%, which has a more significant buffering function. The extracellular Hsp90 α , like the intracellular Hsp90 α , plays the role of molecular chaperone and participates in almost all activities of the tumor. The proto-oncogene her-2 and metalloproteinase 2 acting on the cell surface promote the invasion and metastasis of the tumor cells [15-16]; activate plasma fibrinolysin to promote metastasis and invasion of the tumor cells [17], promote and induce the growing of tumor cells, angiogenesis, cell proliferation, metastasis, local invasion and other activities [18]. Thus it can be seen that Hsp90 α has an extremely active expression in tumor diseases.

In this paper, we found that there was no statistically significant difference in HSP90 α level between lung cancer patients in terms of gender and age ($P>0.05$), indicating that gender and age were not the affecting factors for the increase of HSP90 α . In the group by lung cancer type, a statistically significant difference were detected in test results of HSP90 α between SCLC group and SLC group ($P<0.05$). It was found from the median of each type group that the HSP90 α expression of SCLC was more significant in lung cancer typing, which could be used as an auxiliary indicator for the differential diagnosis of early lung cancer. In the group by cancer staging, the level of HSP90 α showed an increasing trend with the increase of staging, and there were statistical differences in the test results of HSP90 α from the results in staging χ^2 respectively tested with staging χ^2 , χ^2 ($P<0.05$), indicating that the active expression of HSP90 α gradually increased during the development of lung cancer. In the studies of Shi[19] et al.the content of Hsp90 α was found to be associated with the stage of tumor, therapeutic response, preoperative and postoperative of the surgery, disease progression and stationary stage in the treatment of lung cancer patients. Moreover, our data also suggests that HSP90 α showed a positive correlation in stage and typing,the content of Hsp90 α increases with the severity of the disease. In the studies of breast cancer, Pick[20],Cheng[21] et al. also indicated that expression of HSP90 α was normally distributed with the development of disease. This suggests that when HSP90 α is abnormally elevated suddenly, which may

indicate the further deterioration of the disease, such as the occurrence of poor prognosis or metastasis. Our study also showed a significant increase in HSP90 α levels in the metastatic group, with a statistically significant difference from the results in the non-metastatic group ($p < 0.01$), suggesting that a significant increase in HSP90 α levels in lung cancer disease surveillance may indicate metastasis Gallegos[22], Chen[23], Fu[24], Ono[25] et al. also showed that the overexpression of Hsp90 α was positively correlated with tumor metastasis and poor prognosis in the studies of various tumors.

We further discuss the variation analysis of HSP90 α and related markers (CEA, NSE, CF211, ProGRP) in lung cancer diagnosis. In the analysis of the correlation between HSP90 α and related markers in lung cancer diagnosis, the sequence of r values of HSP90 α and the various markers was:

NSE>CEA>ProGRP>CF211. HSP90 α was considered to have differences in correlation with various markers in the diagnosis of lung cancer by comparison ($p < 0.05$). NSE and ProGRP have high specificity (about 80%) in SCLC. CEA has high specificity (about 40-62%) in ALC. CF211 has high specificity (about 40-62%) in SLC. In this study, there were 261 cases of ALC, 144 cases of SLC and 117 cases of SCLC, which may have an impact on the calculation of correlation coefficient. In the consistency comparison of HSP90 with CEA, NSE, CF211 and ProGRP. According to the standard that Kappa<0.75 considers the consistency unsatisfactory, it can be thought of as the consistency of HSP90 α with each marker is not satisfactory. Therefore, we further discussed whether lowering the HSP90 α threshold could improve the overall diagnostic positive rate. When the HSP90 α critical value was 65ng/ml, the positive rate of HSP90 α was 50.36%. In these 282 patients, the positive rates of CEA, NSE, CF211 and ProGRP are 30.96%, 36.02%, 23.38% and 16.84% respectively, and the positive rates of combined diagnosis are 36.88%. When the HSP90 α critical value was 35ng/ml, the positive rate of HSP90 α was 80.54%. In these 451 patients, the positive rates of CEA, NSE, CF211 and ProGRP are 27.84%, 28.10%, 21.04% and 16.88% respectively, and the positive rates of combined diagnosis are 40.15%. With the decrease of HSP90 α critical value, the positive rate of HSP90 α gradually increased, the relative positive rate of each marker decreased, and the total positive rate of combined diagnosis gradually increased. Therefore, according to the data of combined diagnosis data, considering HSP90 α =50ng/ml as a clinical reference value can effectively improve the positive rate of the combined diagnosis and help improve the diagnosis of early clinical lung cancer [11]. In this study, the detection results of HSP90 α and related markers from patients with lung cancer were obtained from cases, it was considered to be effectively incorporated if the five indicators were detected at the same period, and we didn't consider any affecting factors, so there's a wedge there between the clinical results and the theoretical data, but clinical data is the only criterion to verify the theory. The presence of affecting factors cannot be avoided, which is also one of the main reasons that the positive rate of a single marker cannot be used as a diagnostic index in clinical practice. We calculated the combined positive rate by combining the joint detection with stratified analysis of HSP90 α value, which can effectively improve the diagnostic rate of lung cancer.

To sum up, HSP90 α has a significant diagnostic value in the classification, staging and metastasis of lung cancer, but single biomarker is particularly inadequate for the early diagnosis of lung cancer. It is the embodiment of clinical value that can get better utilization of HSP90 α by combining relevant tumor

markers to improve the diagnostic efficiency. The overexpression of HSP90 α in tumors also provides a new entry point for tumor treatment. Alarcon et al. [26] found that heat shock protein 90 α (Hsp90 α) inhibitor, as the new target spot for clinical treatment, had specificity and pleiotropic effect obviously. 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 Hsp90 α [27]. The Hsp90 carboxyl terminal inhibitors played an important role in cell apoptosis and metastasis by blocking the complex activity of Hsp90 α /Aha1 and pc3-mm cells[28]. It can be interfered with the invasion and metastasis of pancreatic ductal adenocarcinoma(PDAC) by regulating Hsp90 α /uPA mmp-2 protein hydrolysis axis[29]. These studies strongly demonstrate that HSP90 plays an important role in tumor metabolism, and detection of HSP90 α expression levels is equally important in tumor treatment and monitoring.

Sourbier[30] et al. systematically explored the significance of HSP90 α as a potential biomarker in liver cancer in their latest study. While, Zhang[31] et al. obtained the diagnostic value of tumor markers in bronchoalveolar lavage fluid for peripheral lung cancer, and HSP90 α has been proved to have more the clinical value in the diagnosis of peripheral lung cancer. Rong[32] et al. conducted a systematic summarize on the up-regulation of heat shock protein 90 α (HSP90 α) in cancer cells, tissues and serum of lung cancer patients, and its close correlation with the occurrence, development and outcome of lung cancer. These recent studies further demonstrate that HSP90 α is not only a potential biomarker for liver cancer, but also a potential biomarker for lung cancer. McDowell[33] et al. found that in the of detection proteomic HSP90 α and HSP90 β , at least 10 of 17 human tumors had one significantly up-regulated HSP90 hypotype or HSP90 synergistic partner. It is demonstrated that HSP90 α is an overexpressed molecular chaperone in malignant tumors. As a potential biomarker, it has important guiding significance in early screening, diagnosis, treatment and prognosis evaluation of lung cancer, and it is the main monitoring and evaluation index of clinical HSP90 α inhibitors.

Declarations

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Conflict of interest The all authors declare no conflicts of interest.

Ethical standard In accordance with the Helsinki Declaration of 1975, source data were obtained during routine clinical practice at the Shaanxi Provincial Cancer Hospital approved by the appropriate ethics committee.

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Tables

Table-1 Mean and Quantile of HSP90α in Lung Cancer

	N	Mean±SD	Minimum	Maximum	Percentiles		
					25th	50th (Median)	75th
HSP90α	560	97.64±103.36	2.56	852.30	40.85	65.69	110.95

Table-2 Mean and Quantile of HSP90α in Gender and Age Groups

	n	Mean±SD	Mini mum	Maxi mum	Percentiles				
					25th	50th (Median)	75th		
sex								Z	P
male	400	93.43±96.64	2.56	852.30	40.98	65.59	104.10	0.543	0.587
female	160	108.12±118.22	4.99	682.30	40.27	66.02	117.60		
year								H	P
≤40y	16	105.73±98.96	9.69	363.60	48.25	73.56	134.83	0.907	0.924
41-50y	79	92.24±86.20	2.58	512.60	39.62	68.40	117.80		
51-60y	135	98.48±93.94	8.16	524.40	41.47	66.92	113.70		
61-70y	239	100.41±119.89	2.56	852.30	37.61	63.59	109.10		
>70y	91	92.36±83.94	5.62	429.40	45.11	63.83	100.90		

Table-3 Mean and Quantile of HSP90α in Classified Groups

Pathological type	n	Mean±SD	Mini mum	Maxi mum	Percentiles			H	P
					25th	50th (Median)	75th		
SCLC	117	110.48±101.86	11.43	573.40	46.72	73.27	131.35		
SLC	144	88.59±93.83	2.66	539.10	38.48	54.90	90.48		
ALC	261	95.65±110.00	2.56	852.30	37.12	64.41	106.00	11.520	0.021
Mixed LC	15	87.41±34.50	25.99	136.60	68.07	87.58	117.60		
Other LC	8	87.37±119.42	12.03	372.80	16.12	50.44	87.32		

Note: lung cancer type (small cell, squamous carcinoma, adenocarcinoma, hybrid, other type) abbreviated as (SCLC, SLC, ALC, Mixed LC, Other LC)

P<0.05, indicates statistical difference

Table-4 Mean and Quantile of HSP90α in Phase Grouping

staging	n	Mean±SD	Mini mum	Maxi mum	Percentiles			H	P
					25th	50th (Median)	75th		
I	37	53.38±40.08	2.56	222.30	31.53	42.01	75.82		<0.001
II	58	58.99±47.30	10.48	246.70	30.89	47.58	69.53	34.075	
III	114	74.45±61.28	2.66	362.10	37.03	56.18	95.42		
IV	246	113.41±120.14	4.99	852.30	47.27	74.91	121.18		

Note: P<0.05, indicates statistical difference

Table-5 Mean and Quantile of HSP90α in Metastasis Groups

metastasis	N	Mean±SD	Minimum	Maximum	Percentiles		
					25th	50th (Median)	75th
yes	333	112.39±117.76	2.58	852.30	45.37	70.99	129.85
no	223	73.75±67.90	2.56	539.10	37.12	56.66	85.96

Table-6 correlation and consistency between HSP90α and related markers in lung cancer

	CEA		NSE		CF211		ProGRP	
n	557		521		548		185	
HSP90α								
r	0.224		0.305		0.117		0.194	
p	<0.01		<0.01		<0.01		<0.01	
	+	-	+	-	+	-	+	-
+	72	143	81	116	55	156	13	61
-	73	269	45	279	51	286	14	97
Kappa value	0.129		0.293		0.121		0.055	

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.

Table-7 Positive rate of combined diagnosis of HSP90α and related markers in lung cancer

Hsp90α	n	Positive[%]					Total
		Hsp90α	CEA	NSE	CF211	ProGRP	Positive[%]
≤35ng/ml	451	80.54%	27.84%	28.10%	21.04%	16.88%	40.15%
35-50	370	66.07%	29.81%	31.30%	21.70%	16.54%	38.98%
50-65	282	50.36%	30.96%	36.02%	23.38%	16.84%	36.88%
≥82	214	38.21%	33.18%	41.12%	26.19%	17.81%	34.61%

Figures

Pairwise Comparisons of Pathological

Figure 1

Pathological type groups compared and pairwise comparisons

Pairwise Comparisons of staging

Figure 2

Staging groups compared and pairwise comparisons