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RESEARCH ARTICLE

# Four-protein model for predicting prognostic risk of lung cancer

Xiang Wang[1], Minghui Wang[1], Lin Feng[1], Jie Song[1], Xin Dong (✉)[2], Ting Xiao (✉)[1], Shujun Cheng (✉)[1]

*[1]State Key Laboratory of Molecular Oncology, Department of Etiology and Carcinogenesis, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China; [2]Department of Clinical Laboratory, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China*



**Abstract** **Patients with lung cancer at the same stage may have markedly different overall outcome and a lack of specific biomarker to predict lung cancer outcome. Heat-shock protein 90 β (HSP90β) is overexpressed in various tumor cells. In this study, the ELISA results of HSP90β combined with CEA, CA125, and CYFRA21-1 were used to construct a recursive partitioning decision tree model to establish a four-protein diagnostic model and predict the survival of patients with lung cancer. Survival analysis showed that the recursive partitioning decision tree could distinguish the prognosis between high- and low-risk groups. Results suggested that the joint detection of HSP90β, CEA, CA125, and CYFRA21-1 in the peripheral blood of patients with lung cancer is plausible for early diagnosis and prognosis prediction of lung cancer.**

**Keywords** lung cancer; HSP90β; decision tree model; prognosis

## Introduction

Lung cancer is currently the leading cause of cancer-related death worldwide [1]. Most of patients with lung cancer have an advanced stage disease at diagnosis due to a lack of symptoms in the early stage and a lack of effective biomarkers for early detection. Therefore, the prognosis of those with advanced-stage lung cancer is still dismal even multimodal treatment has been applied. Finding useful biomarkers for early detection, prognosis prediction, and recurrence monitoring is necessary and urgent to improve the survival outcome of patients with lung cancer. Recursive partitioning analysis is a method of classification that is intended to provide a method to divide patients into homogenous groups [2]. This method could lead to lessened modeling assumptions and establish procedures that may be used as a stratified tool for clinical research in previous research [3,4].

Heat-shock protein 90 (HSP90) is a highly conserved molecular chaperone that facilitates the maturation of a wide range of proteins (known as clients). Clients are enriched in signal transducers, protein folding, protein degradation, and morphological evolution [5–8]. Aberrant expression of the molecular chaperone HSP90 may result in developmental malformations, diseases, or even cell death [9,10]. HSP90 clients include receptor tyrosine kinases; cytosolic signaling proteins, such as AKT and RAF-1; and a number of cell cycle regulators, such as CDK4 and polo-like kinase [11,12]. HSP90 client proteins are frequently mutated, overexpressed, or persistently activated in cancer cells, and the chaperone has received increasing interest as a target for anticancer drugs [13]. Besides, HSP90 itself has been reported to be over-expressed in advanced tumors, such as pancreatic carcinoma, ovarian cancer, and prostate carcinomas [14–17].

The two major cytoplasmic isoforms of HSP90 are HSP90α (HSP90AA1, inducible form/major form) and HSP90β (HSP90AB1, constitutive form/minor form) [18]. Multiple differences exist between HSP90 isoforms in terms of cell differentiation and embryonic development in various organisms [19]. HSP90β may be correlated to structural conformation by forming complexes with the actin and tubulin that constitute the cytoskeleton [20]. HSP90β is a major microtubule-interacting protein [21] involved in normal cellular functions, such as maintenance of the cytoarchitecture [22], differentiation [23], and cytoprotection [24]. HSP90β expression was shown to be

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Correspondence: Xin Dong, dongxin_ab@163.com;
Ting Xiao, xiaot@cicams.ac.cn;
Shujun Cheng, chengshj@cicams.ac.cn

associated with the development of drug resistance [25].

Thus far, little research has been conducted on the role of HSP90β in lung cancer, especially in its diagnosis and prognosis. Carcinoembryonic antigen (CEA) is a classic tumor marker of colorectal cancer, breast cancer, and ovarian cancer. In lung cancer, CEA was considered to be a marker of adenocarcinoma subtype [26]. Cancer antigen 125 (CA125) is a glycoprotein that plays an important role in the diagnosis of patients with ovarian cancer, and it is used to monitor patient's response to treatment and prognosis [27]. Cytokeratin fragment 19 (CYFRA21-1) is a cytokeratin protein expressed in bronchial epithelium and malignant tumors derived from these epithelial cells [28]. The prognostic value of these tumor markers in lung cancer has always been controversial, and the relationship between them is unclear. The present study aimed to examine the HSP90β plasma protein levels in patients with lung cancer to analyze its relationship with the diagnosis and prognosis of lung cancer combined with the serum protein levels of CEA, CA125, and CYFRA21-1 to establish a prognostic model for lung cancer.

## Material and methods

### Clinical samples

Peripheral blood samples were collected from July 2007 to August 2013. A total of 1162 patients with lung cancer, including 371 cases of lung squamous cell carcinoma (LUSC), 705 cases of lung adenocarcinoma (LUAD), six cases of unspecific lung cancer types, and 80 cases of small-cell lung cancer (SCLC), who underwent surgery at the Cancer Hospital of the Chinese Academy of Medical Sciences and Peking Union Medical College, were included in the study. The clinical information of SCLC is shown in Table S1. All patients provided a written informed consent before surgery, and the treatments were performed in accordance with present ethical principles. A total of 282 healthy persons (182 males and 100 females), whose peripheral blood samples were collected from cancer prevention department at the Cancer Hospital of the Chinese Academy of Medical Sciences and Peking Union Medical College, were also included in the study. The clinical data of LUSC, LUAD, and healthy persons have been published before [29,30].

Peripheral blood samples were collected prior to surgery by venipuncture and preserved in EDTA-coated tubes. The samples were centrifuged at 4 °C for 10 min at 1000× *g* to separate plasma from blood cells. Supernatants were collected and stored at –80 °C until use.

### ELISA

Plasma protein concentrations were assessed using enzyme linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions. The ELISA kit for HSP90β was purchased from USCN, China. In brief, 100 μL of diluted plasma was added into wells of anti-HSP90β microplate and incubated at 37 °C for 2 h. Then, 100 μL of prepared biotinylated HSP90β detector antibody was added to each well and incubated at 37 °C for 1 h. After washing three times, 100 μL of prepared conjugate was added to each well and incubated at 37 °C for 1 h. After washing for five times, the absorbance at 450 nm was measured immediately using a microplate reader (Bio-Rad Laboratory, Hercules, CA, USA). The level of HSP90β was divided into two groups on the basis of median concentration of HSP90β in all the patients; those levels higher than the median were considered high, while those lower than the median were considered low (Table S2).

The serum protein levels of CEA, CA125, and CYFRA21-1 were tested using a commercial electrochemiluminescent immunoassay kit (Roche Diagnostics, Mannheim, Germany) at the Clinical Diagnostic Laboratories in the Cancer Hospital of the Chinese Academy of Medical Sciences and Peking Union Medical College. The corresponding serum concentrations are listed in Table S2.

### Immunohistochemistry (IHC) and its scoring

Lung tumor tissues and adjacent normal lung tissues were sliced into 4 mm slides for IHC staining. The slides were stained with HSP90β antibody (1:50, ABGENT, catalog No. AP7867D). They were processed using an automated Leica Bond staining system in accordance with the manufacturer's protocol as previously described [29]. H-score was used to score the IHC result. It was calculated by multiplying the proportion of positive cells in the sample (0–100%) by the average intensity of the positive staining (0, 1+, 2+, or 3+) to obtain a score ranging between 0 and 300 as previously described [29]. The expression of HSP90β was classified into negative (H-score = 0), low (H-score: 1–50), medium (H-score: 51–150), or high (H-score: 151–300, Table S3).

### Risk prediction model for lung cancer by using recursive partitioning decision tree algorithm

First, 229 patients with bipolar prognosis were selected from the 671 patients with non-SCLC (NSCLC) with complete expression of HSP90β, CEA, CA125, and CYFRA21-1, including 83 high-risk patients (survival time $< 30$ months) and 146 low-risk patients (survival time $> 60$ months, Table S2). A total of 150 cases were randomly selected from 229 patients as training set. On the basis of the protein concentration of HSP90β, CEA, CA125, and CYFRA21-1, the risk prediction decision tree model was established using the recursive partitioning decision tree algorithm of "rpart" package, one of the

supervised machine learning algorithms. The tree was built by the machine learning through the following process: first, the single variable, which best splits the data into two groups ("best" was defined below), is found. Data are separated, and then this process is applied separately to each sub-group recursively until the subgroups either reach a minimum size or until no improvement could be made [31]. In the present study, the protein results of each case were entered into the model from the top of the decision tree (root). Then, the protein level of the single protein marker indicated at each node was tested, selected into the next level of inspection in accordance with the decision criteria, and continued in accordance with the decision criteria to determine until the tree reached the end of the "end point" and on the basis of the model to predict the risk of prognosis. The prognostic accuracy of the model in the training set was 146/150 (97.3%). For the remaining 442 patients, the risks of prognosis were first evaluated as high or low on the basis of overall survival (OS) and disease-free survival (DFS). High risk indicated that the OS and DFS had the end event; otherwise, it was low risk. Then, the prediction model was applied into the patients to obtain the prediction risk, and the prediction accuracy was calculated.

### Statistical analysis

Mann–Whitney U test was used to verify the significance between the groups of patients with lung cancer. Pearson's $\chi^2$ test was used to compare dichotomous variables. Fisher's exact test was used to compare the different levels of H-score between the tumor and normal tissue. The Kaplan–Meier estimation method was used for OS analysis, and log-rank test was used to compare differences. The level of significant statistical difference was set at 0.05.

## Results

### The plasma protein level of HSP90β in patients with lung cancer was higher than that in healthy population

The correlation between HSP90β protein concentration and clinical information of 1162 patients with lung cancer is shown in Table 1. The results showed that the protein concentration of HSP90β in patients with lung cancer was significantly higher than that in healthy persons (Fig. 1A, $P < 0.001$). Notably, the protein level of HSP90β in SCLC

**Table 1** Correlation between HSP90β protein concentration and clinical information of 1162 patients with lung cancer

| Group | Number | HSP90β levels (ng/mL) | | | *P* value |
|---|---|---|---|---|---|
| | | Mean±SD | Range | Median | |
| Healthy group | 282 | 52.15±50.61 | 14.22–293.61 | 30.41 | <0.001[a] |
| Age (year) | | | | | <0.001 |
| >60 | 93 | 71.47±58.19 | 14.37–293.61 | 50.17 | |
| ⩽60 | 189 | 42.65±43.55 | 14.22–277.25 | 23.17 | |
| Gender | | | | | 0.394 |
| Male | 182 | 53.07±50.30 | 14.37–293.61 | 32.09 | |
| Female | 100 | 50.82±51.55 | 14.22–277.25 | 29.57 | |
| Lung cancer | 1162 | 196.65±127.72 | 15.96–686.28 | 162.02 | |
| SCLC | 80 | 247.11±123.38 | 40.71–661.23 | 221.80 | <0.001[b] |
| Age | | | | | 0.528 |
| >60 | 26 | 227.25±98.73 | 70.80–505.89 | 207.00 | |
| ⩽60 | 54 | 256.68±133.44 | 40.71–661.23 | 231.15 | |
| Gender | | | | | 0.158 |
| Male | 58 | 237.63±126.53 | 40.71–661.23 | 214.62 | |
| Female | 22 | 272.10±113.60 | 134.26–530.86 | 231.37 | |
| NSCLC | 1082 | 192.91±127.30 | 14.93–686.28 | 157.06 | 0.973 |
| Age | | | | | |
| >60 | 553 | 191.35±124.61 | 14.93–681.09 | 158.00 | |
| ⩽60 | 529 | 194.55±130.15 | 15.54–686.28 | 155.01 | |
| Gender | | | | | |
| Male | 681 | 195.51±128.19 | 14.93–686.28 | 156.99 | 0.295 |
| Female | 401 | 188.50±125.81 | 16.83–681.09 | 157.37 | |
| Stage | | | | | 0.130 |
| I | 510 | 187.81±132.76 | 15.96–686.28 | 147.22 | |

(*Continued*)

| Group | Number | HSP90β levels (ng/mL) | | | *P* value |
|---|---|---|---|---|---|
| | | Mean±SD | Range | Median | |
| II | 204 | 195.67±118.11 | 16.66–663.66 | 169.90 | |
| III–IV | 350 | 196.85±124.20 | 14.93–673.27 | 159.59 | |
| NA | 18 | | | | |
| Lymph node metastasis | | | | | 0.118 |
| Yes | 459 | 197.63±123.92 | 15.54–673.27 | 161.57 | |
| No | 565 | 190.54±131.51 | 15.96–686.28 | 150.21 | |
| NA | 58 | | | | |
| Pathologic types | | | | | 0.086 |
| LUSC | 371 | 201.22±130.39 | 15.54–686.28 | 157.12 | |
| LUAD | 705 | 188.34±125.66 | 14.93–681.09 | 155.91 | |
| NA | 6 | | | | |
| Differentiation | | | | | 0.137 |
| High | 124 | 171.14±109.59 | 31.78–498.61 | 137.50 | |
| Middle | 550 | 196.88±129.22 | 15.96–681.09 | 164.41 | |
| Low | 378 | 197.34±130.44 | 14.93–686.28 | 156.26 | |
| NA | 30 | | | | |
| Smoking history | | | | | 0.170 |
| Yes | 576 | 196.41±126.28 | 15.96–686.28 | 157.69 | |
| No | 499 | 188.39±127.84 | 14.93–681.09 | 155.01 | |
| NA | 7 | | | | |
| Family history | | | | | 0.143 |
| Yes | 166 | 181.69±127.28 | 16.66–673.49 | 133.43 | |
| No | 904 | 194.63±126.87 | 14.93–686.28 | 160.56 | |
| NA | 12 | | | | |

[a]Lung cancer versus healthy group.
[b]SCLC versus NSCLC. (SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer.)

was significantly higher than that in NSCLC (Fig. 1A, $P < 0.001$).

## The expression of HSP90β in tumor tissues of lung cancer was higher than that in normal tissues

IHC was conducted in tumor tissues and adjacent normal tissues of 279 out of 1162 patients, including 142 LUAD, 110 LUSC, and 27 SCLC cases, to validate the expression of HSP90β in tissues (Table S3). Representative images of IHC for the three types of lung cancer are shown in Fig. 1B. The expression of HSP90β in tumor tissues was significantly higher than that in normal tissues in the three types of lung cancer (Fig. 1C and Table 2, $P < 0.001$). In clinical practice, plasma is good for biomarker discovery without receiving surgery. Therefore, a prediction model was conducted using the plasma expression data.

## The recursive partitioning decision tree could distinguish between high- and low-risk groups

The risk prediction model evaluated the prognosis of 671 patients with lung cancer (Table S2). A total of 229 patients with bipolar prognosis were selected, including 83 high-risk patients (survival time $< 30$ months) and 146 low-risk patients (survival time $> 60$ months). Survival analysis showed significantly different prognosis among the two groups, with the high-risk group indicating worse prognosis (Fig. S1). In the training group ($n = 150$), the recursive partitioning decision tree was constructed with the accuracy of 97.3% (146/150, Fig. 2). When the tree model was applied to the test group of the remaining 79 patients, the accuracy was 64.6% (51/79).

The risk prediction model was then used in the remaining 442 patients. The results indicated 147 high-risk patients and 295 low-risk patients. The prediction accuracy of the remaining 442 patients was 65.84%, higher than that in HSP90β alone, with the accuracy of 55.43%. Combined with the 229 patients with bipolar prognosis (total of 671 patients), the risk prediction model identified 238 high-risk patients and 433 low-risk patients. The prediction accuracy of all the patients was 72.73%, higher than that in HSP90β alone, with the accuracy of 57.08%. The survival analysis of all the patients is shown in Fig. 3A. This analysis using Kaplan–Meier plot and log-rank test revealed significant differences between the two

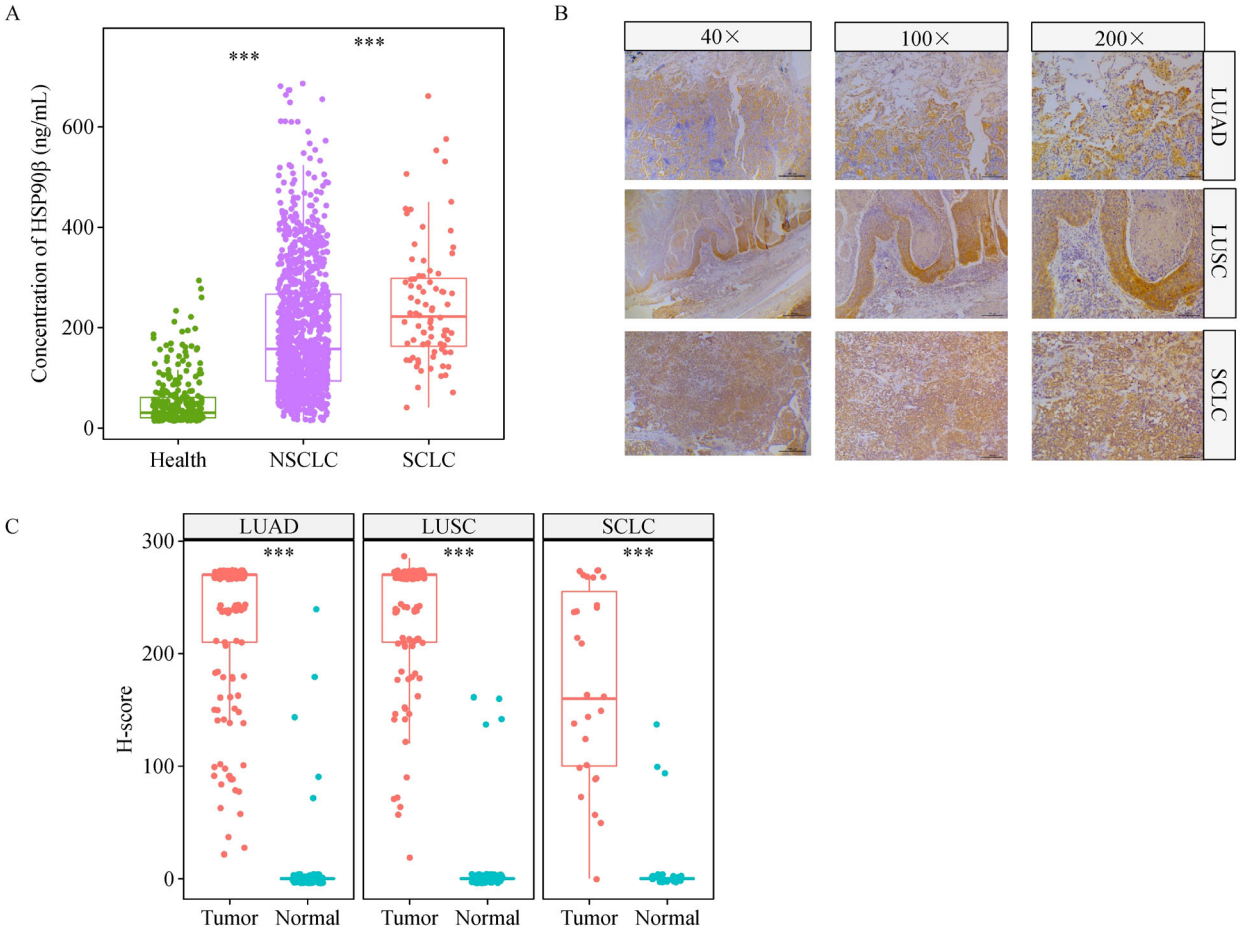


**Fig. 1** (A) Plasma protein concentration of HSP90β in patients with lung cancer (NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer). (B) Representative images of IHC for three types of lung cancer. (C) Expression of HSP90β in three types of lung cancer tumor and normal tissues.

**Table 2** Statistical result of HSP90β expression in tissues of three lung cancer types

| Pathologic type | Number ($N = 279$) | H-score | | | | *P* value |
|---|---|---|---|---|---|---|
| | | Negative (0) | Low (1–50) | Medium (51–150) | High (151–300) | |
| LUAD | 142 | | | | | <0.001 |
| Tumor | | 0 | 3 | 22 | 117 | |
| Normal | | 137 | 0 | 3 | 2 | |
| LUSC | 110 | | | | | |
| Tumor | | 0 | 1 | 12 | 97 | <0.001 |
| Normal | | 106 | 0 | 2 | 2 | |
| SCLC | 27 | | | | | |
| Tumor | | 1 | 1 | 10 | 15 | <0.001 |
| Normal | | 24 | 0 | 3 | 0 | |

groups, with the high-risk group showing worse prognosis. Univariate analysis and multivariate Cox proportional hazard regression models of all the 671 patients showed that the prediction model could be an independent risk factor for the OS of patients with lung cancer (Fig. 3B). Therefore, the risk prediction model is simple and suitable for clinical doctors to predict patient survival rapidly, and it could be used as a stratified tool for clinical research. The risk prediction model may also help clinicians determine decision-making and clinical research design.

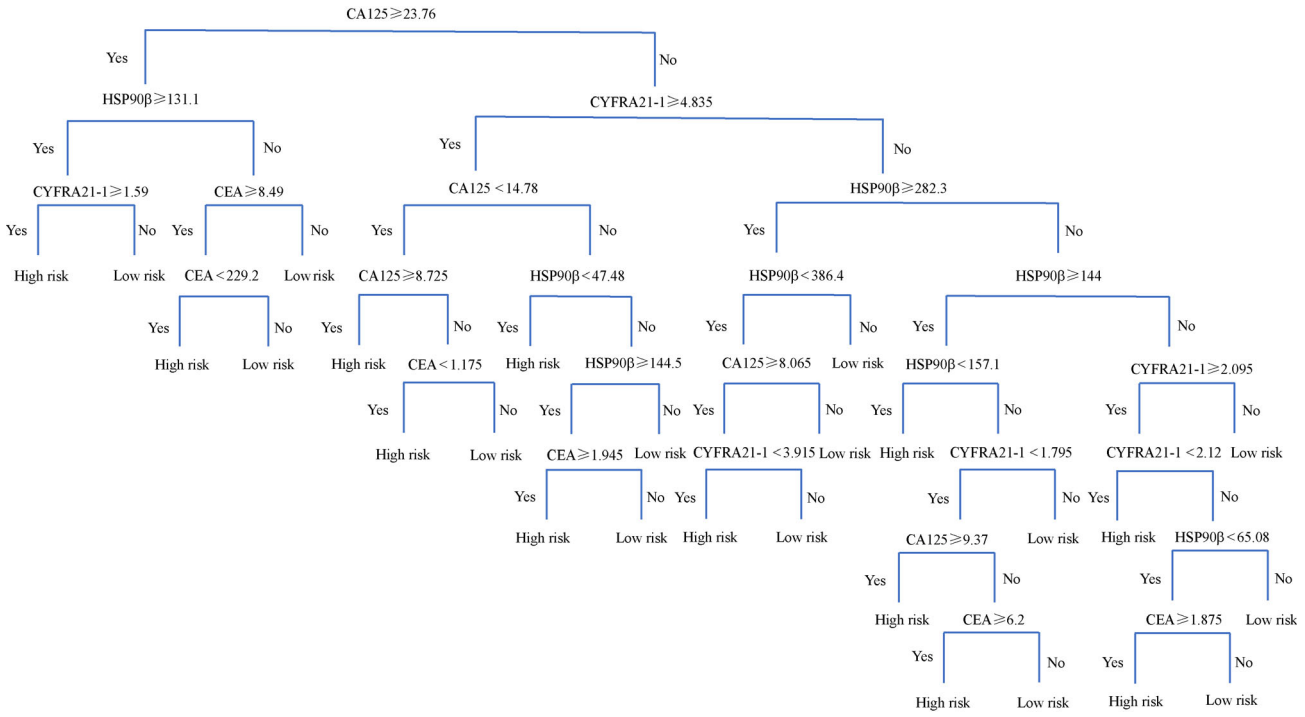


**Fig. 2** Recursive partitioning decision tree model constructed using 150 patients with lung cancer. CA125, U/mL; CYFRA21-1, CEA, HSP90β, ng/mL.

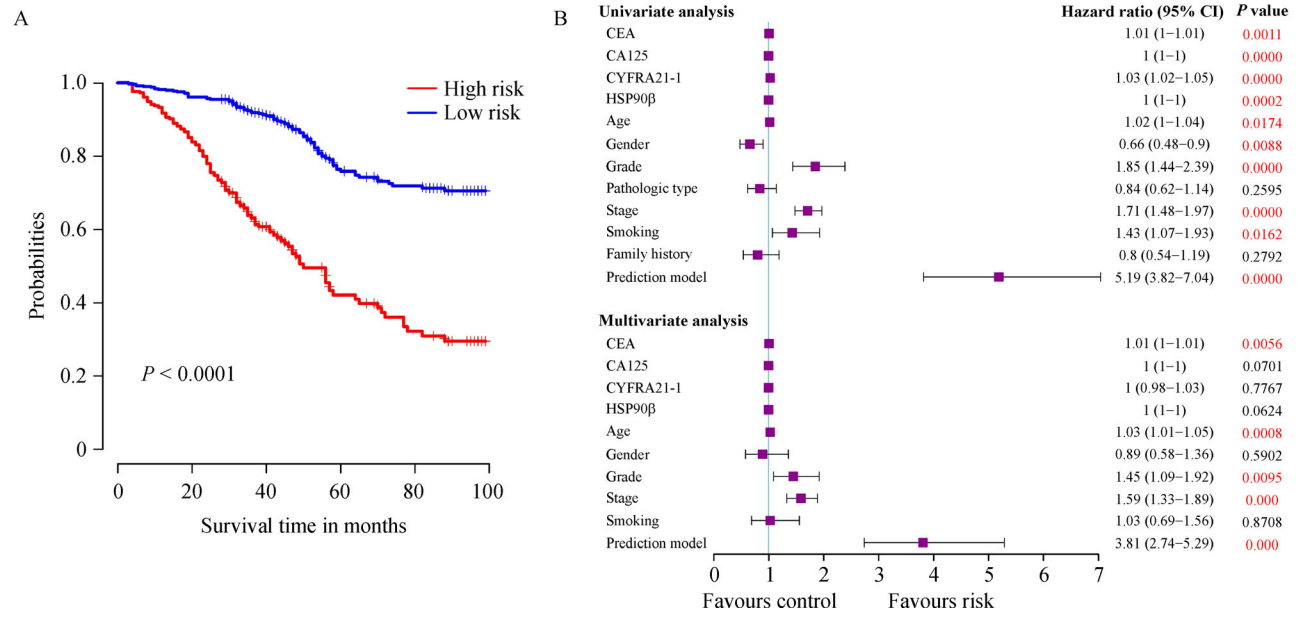


**Fig. 3** (A) Overall survival analysis of all patients (log-rank test) based on high or low risk. (B) Forest plot illustrating the hazard ratio (95% CI) for overall survival calculated using univariate and multivariable Cox proportional hazard regression models. CI, confidence interval.

## Discussion

Lung cancer is one of the most common malignant tumors and the deadliest cancer in the world; the 5-year relative survival rate for lung cancer is 17% [1]. In the present study, the protein level of HSP90β in lung cancer plasma was higher than that in health population. HSP90β constitutively was expressed to a higher level than

HSP90α in most tissues, and it was important for long-term cellular adaptation, differentiation, and evolution [32]. Thus, the HSP90β in plasma may be better and more accurate in the diagnosis and prognosis of lung cancer than HSP90α.

Some studies demonstrated that HSP90 is overexpressed in NSCLC, and it had significant association with prognosis [33]. In the previous study, survival analysis revealed that the prognosis of patients with lung cancer with high HSP90β protein levels was poor [29,30]. Beside lung cancer, HSP90β caused poor survival among patients with $HER2^{-}/ER^{+}$ breast cancer subtype through increased risk of distant metastasis [34]. In salivary gland tumors, HSP90β was increasingly expressed and positively correlated with malignant tumors [35]. HSP90 is important in maintaining cell morphology; migration; and the activation of matrix metalloproteinase 2, a key player in matrix degradation and cancer cell invasion [36–38]. HSP90β may be a critical molecular chaperone in tumor recurrence and malignant transformation.

CEA, CA125, and CYFRA21-1 were commonly used as auxiliary biomarkers for diagnosing lung cancer. Their sensitivities were 76% (CYFRA21-1), 55% (CA125), and 52% (CEA). All of these tumor biomarkers showed a clear relationship with tumor stage and histology and thus enabled an enhanced histological diagnosis [26]. The serum level of CEA carries prognostic and predictive information of risk of recurrence and death in NSCLC [39]. CYFRA21-1 or CA125 tends to imply a negative prognosis. CYFRA21-1 and CA125 imply the worst prognosis [40]. One study showed that patients with elevated CEA, CA125, and CYFRA21-1 presented the worst prognosis [28]. In our study, the patients with high HSP90β, CEA, CA125, or CYFRA21-1 protein levels had poor prognosis. Thus, the risk prediction model was established by using the decision tree algorithm on the basis of HSP90β, CEA, CA125, and CYFRA21-1 protein concentration. Decision tree has been used to predict many disease outcomes [41–43]. The decision tree prediction model in the present study could accurately predict the risk of 671 patients with lung cancer. The training group showed higher accuracy than the test group, while the remaining large group of patients displayed similar accuracy with the test group. Both groups presented higher accuracy than only one marker, HSP90β. The sample size and the bipolar prognosis of the patients for the construction of the model may lead to the discrepancy between the training group and the test group or the remaining group. Overall, the combination detection of HSP90β, CEA, CA125, and CYFRA21-1 had the advantage for the diagnosis and prognosis of lung cancer, and it provided reference for the treatment of patients with tumor after operation.

In conclusion, HSP90β displayed high levels in the plasma and tumor tissues of patients with lung cancer. In addition, comprehensive analysis of HSP90β, CEA, CA125, and CYFRA21-1 protein levels in peripheral blood of patients with lung cancer could more accurately assess the prognosis. Therefore, the joint detection of these four markers in the peripheral blood of patients with lung cancer and the prediction model could be used as an auxiliary method for the diagnosis and prognosis of lung cancer.

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## Compliance with ethics guidelines

Xiang Wang, Minghui Wang, Lin Feng, Jie Song, Xin Dong, Ting Xiao, and Shujun Cheng declare that they have no conflicts of interest. This manuscript does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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