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Elevated plasma HSP90α as a prognostic marker in EGFR-mutant non-small cell lung cancer

XIAOYU TAN[1*], JIAYING TANG[1*], YAMEI TANG[1*], HAIGUANG WANG[1], SHANLI MO[1], XIAOQIAN HUANG[1] and HAIJIE GAN[2]

[1]Department of Experimental Research, Guangxi Medical University Cancer Hospital, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China; [2]Department of Respiratory Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China

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Abstract. The heat shock protein 90α (HSP90α), as a molecular chaperone, plays an important role in the development and progression of various malignant tumors. The aim of the present study was to assess the plasma level of heat shock protein 90α (HSP90α) in patients with non-small cell lung cancer (NSCLC) harboring different epidermal growth factor receptor (*EGFR*) mutations and its association with clinical characteristics and *EGFR* gene mutations. Plasma HSP90α levels, clinicopathological data and prognostic information were collected and analyzed from 1,347 patients with a pathological diagnosis of lung cancer to evaluate their association with EGFR gene mutations. The results demonstrated that patients with elevated plasma HSP90α levels are more likely to have SCLC, advanced tumor stages and poorer prognosis compared to patients with lower HSP90α levels. Among patients with NSCLC harboring EGFR mutations, those with higher plasma HSP90α levels are more frequently associated with L858R wild-type and exon 20 mutations compared to other EGFR mutation subtypes. Furthermore, using the optimal cutoff value of 62.1 ng/ml for plasma HSP90α levels, the results revealed that patients with lower plasma HSP90α levels have a better prognosis compared to those with higher HSP90α levels. In conclusion, elevated plasma HSP90α expression is associated with poor overall survival in patients with NSCLC and could serve as a prognostic indicator independent of EGFR mutation status.

Correspondence to: Professor Haijie Gan, Department of Respiratory Oncology, Guangxi Medical University Cancer Hospital, 71 Hedi Road, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China
E-mail: haijie_gan@outlook.com

[*]Contributed equally

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Introduction

Lung cancer is one of the most common malignancies worldwide, with an age-standardized incidence rate of 23.6 per 100,000 individuals (1). The introduction of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) has transformed the treatment of non-small cell lung cancer (NSCLC), enabling numerous patients with EGFR mutations to benefit from targeted therapy (2). In China, the EGFR mutation rate among patients with lung adenocarcinoma is ~40% (3). Nonetheless, 20-30% of patients exhibit primary resistance to EGFR-TKIs (4). Studies have reported marked heterogeneity in treatment outcomes among patients with EGFR-mutated NSCLC who initially respond to EGFR-TKIs, with the duration of clinical benefit ranging from a few weeks to several years (5). Furthermore, biomarkers in peripheral blood are widely recognized as convenient and accessible predictors of cancer prognosis and treatment response (6).

Heat shock protein 90α (HSP90α) is a highly conserved molecular chaperone that is effectively upregulated in response to trauma, infection and tumor-related stimuli. Newly synthesized HSP90α can be secreted into the extracellular environment or translocated into the nucleus, where it contributes to immune memory formation and tumor development (7,8). Notably, HSP90α serves a crucial role in regulating DNA damage responses, cell cycle progression, gene expression and carcinogenesis (8). HSP90α stabilizes mutant EGFR proteins through multiple molecular mechanisms. For instance, Ahsan *et al* (9) reported that it facilitates the folding, stabilization and protection of mutant EGFR from degradation via its ATP-dependent chaperone cycle. In glioblastoma cells, HSP90α promotes EGFR phosphorylation and enhances cell migration and invasion (10). In addition, HSP90α inhibits the ubiquitination and proteasomal degradation of mutant EGFR, thereby prolonging its half-life (11). Under stress conditions, such as radiation or chemotherapy, the expression of HSP90α increases, further stabilizing mutant EGFR and enabling tumor cells to adapt to external stress and survive (12). Other molecular chaperones form complexes with HSP90AA1 to jointly regulate the stability of EGFR and its associated signaling pathways (13). By stabilizing mutant

EGFR, HSP90α contributes to tumor cell resistance to TKIs. Furthermore, in patients harboring EGFR T790M mutations, HSP90α has been reported to diminish the therapeutic efficacy of TKIs (14).

Elevated plasma HSP90α levels have been reported to be specific to malignant tumors, with higher levels observed in patients with metastatic liver, lung and breast cancers compared with those without metastasis (15-18). As a result, plasma-free HSP90α is increasingly being recognized as a potential tumor biomarker (17,19). Therefore, the present retrospective study aimed to assess the pre-treatment plasma HSP90α expression in patients with lung cancer and evaluate its prognostic value in patients with NSCLC with different gene mutations.

Materials and methods

Patient information. A total of 1,347 patients with pathologically confirmed lung cancer, treated at the Departments of Respiratory Oncology and Thoracic Oncology Surgery at the Guangxi Medical University Cancer Hospital (Nanning, China) between June 2018 and June 2021, were included in the present study (Table I). The cohort consisted of 840 men and 507 women, aged 25-87 years (mean ± standard deviation, 58.78±10.18 years). Diagnoses included 110 cases of small cell lung cancer, 892 adenocarcinomas, 184 squamous cell carcinomas and 170 unclassified cases. Clinical staging (8th edition of the TNM classification) (20) identified 259 patients at stage I, 315 at stage II, 179 at stage III and 594 with stage IV lung cancer. Among these, 862 patients had distant metastases, whilst 485 did not. Genetic testing was performed on 499 patients, with 240 harboring several EGFR mutations and 259 testing negative for EGFR mutations. All selected patients were newly diagnosed and had no history of other malignancies, severe liver or kidney dysfunction, or endocrine or metabolic disorders. The overall mean plasma HSP90α level was 60.4±54.0 ng/ml, with a mean of 49.4±34.8 ng/ml in surviving patients and 92.0±80.4 ng/ml in non-surviving patients.

The present retrospective study received ethical approval from the ethics committee of Guangxi Medical University Cancer Hospital (approval no. KY2023803).

Determination of HSP90α concentration. On the second day after admission, 2 ml fasting venous blood was collected from all patients using EDTA anticoagulant tubes. Samples were centrifuged at 4˚C at 1,000 x g for 10 min to separate plasma, which was then stored at -80˚C. After all specimens were thawed simultaneously, plasma HSP90α concentrations were measured using an ELISA kit (Yantai Progi Biotechnology Development Co., Ltd) according to the manufacturer's instructions. The detection process utilized a double antibody sandwich ELISA method, which consisted of sample addition followed by incubation at 37˚C for 30-40 min, plate washing by shaking or immersing in washing solution for 1-2 min repeated 4-6 times, binding of the biotinylated antibodies, addition of the Streptavidin-Biotin Complex and 3,3',5,5'-tetramethylbenzidine color development with under the avoidance of light at 37˚C for 10-20 min. Absorbance was measured at 450 nm. To minimize batch effects, ELISA kits from different lots were not mixed, except for the stop solution. Disposable consumables were used throughout the experiment to prevent cross-contamination. Sample addition time was completed within 10 min to reduce pre-incubation variability. During data analysis, batch correction algorithms such as ComBat (sva R package; version 3.20.0; https://bioconductor.org/packages/sva/) were applied.

EGFR gene detection. Genomic DNA from blood and tissue samples was extracted using a DNA extraction kit (cat. no. 69504; Qiagen China Co., Ltd.). After DNA isolation, a micro-volume UV spectrophotometer was used to determine the DNA concentration. The DNA concentration should be >2 ng/μl and it is recommended that the optical density at 260 nm (OD_{260})/OD_{280} ratio is between 1.5 and 2.3. Prior to testing, the DNA should be diluted to 2 ng/μl using 1X TE buffer (pH 8.0). The loading concentration can be adjusted appropriately based on the quality of the sample. The mutation status was assessed using a human EGFR/anaplastic lymphoma kinase/ROS proto-oncogene 1 gene mutation detection kit based on real-time PCR (cat. no. 8.0223601X036G; Medical Device Filing No. 20150082; Amoy Diagnostics Co., Ltd). DNA testing was conducted using the ADx-ARMS® technology to detect gene mutations in the sample DNA. The FAM (6-carboxyfluorescein) and ROX (6-carboxy-X-rhodamine) signals respectively indicate different mutation sites. ADx-ARMS® technology utilizes mutation-specific primers designed for the targeted mutation sites. During PCR amplification, if the 3' terminal base of the primer is fully complementary to the mutant template, the primer is extended and the mutant template is amplified. By contrast, if the primer does not fully match the wild-type template, the extension is blocked and amplification of the wild-type template is inhibited. This enables the specific detection of gene mutations. This method is used for DNA mutation detection. Enzyme Mix B is added to sample DNA, positive and negative controls, mixed thoroughly, and then loaded into LET Reaction Strip B for PCR amplification. Each step requires thorough mixing by vortexing and quick centrifugation, with amplification conditions set strictly according to the recommended protocol. The PCR amplification program consists of four stages: Stage 1 includes a single cycle with reactions carried out at 42˚C for 5 min, followed by 95˚C for 5 min. Stage 2 involves 10 cycles, each comprising denaturation at 95˚C for 25 sec, annealing at 64˚C for 20 sec and extension at 72˚C for 20 sec. Stage 3 consists of 36 cycles, including denaturation of 93˚C for 25 sec, annealing at 60˚C for 35 sec (during which fluorescence signals from FAM, VIC and ROX are collected) and extension at 72˚C for 20 sec. Stage 4 is the final extension, performed at 40˚C for 30 sec. To determine the gene mutation status, fluorescence reference correction was disabled and each reaction tube was analyzed sequentially by selecting a single fluorescence channel. The threshold was set at the inflection point of the positive control amplification curve and cycle threshold (Ct) values were obtained from the positive control, negative control and sample tubes. Mutation determination was based on the Ct values from tubes FAM/ROX signals and the control tube in the LET B strip. The ΔCt value is calculated as the difference between the mutation Ct and control Ct, and results are classified into negative, positive zone A or positive zone B. If multiple tubes show positivity, the one with the

Table I. Clinicopathological characteristics of 1,347 patients with lung cancer.

	Prognostic outcome		
Clinicopathological characteristic	Survival (n=999)	Death (n=348)	Total (n=1,347)
Age, years			
Mean ± standard deviation	58.3±10.2	60.3±10.1	58.8±10.2
Median (min, max)	58.0 (25.0, 87.0)	61.0 (25.0, 87.0)	59.0 (25.0, 87.0)
Sex			
Male	585 (58.6)	255 (73.3)	840 (62.4)
Female	414 (41.4)	93 (26.7)	507 (37.6)
Pathological classification			
Small cell lung cancer	63 (6.3)	47 (13.5)	110 (8.2)
Squamous cell lung carcinoma	133 (13.3)	51 (14.7)	184 (13.7)
Adenocarcinoma	693 (69.4)	199 (57.2)	892 (66.2)
Other	110 (11.0)	51 (14.7)	161 (12.0)
T stage			
T1	208 (20.8)	51 (14.7)	259 (19.2)
T2	241 (24.1)	74 (21.3)	315 (23.4)
T3	130 (13.0)	49 (14.1)	179 (13.3)
T4	420 (42.0)	174 (50.0)	594 (44.1)
N stage			
N0	254 (25.4)	20 (5.7)	274 (20.3)
N1	174 (17.4)	80 (23.0)	254 (18.9)
N2	273 (27.3)	97 (27.9)	370 (27.5)
N3	298 (29.8)	151 (43.4)	449 (33.3)
M stage			
M0	419 (41.9)	66 (19.0)	485 (36.0)
M1	580 (58.1)	282 (81.0)	862 (64.0)
Plasma HSP90α, ng/ml			
Mean ± standard deviation	49.4±34.8	92.0±80.4	60.4±54.0
Median (min, max)	43.3 (7.98, 401)	62.7 (15.1, 584)	46.6 (7.98, 584)

Values are expressed as n (%) unless otherwise specified. T, tumor; N, node; M, metastasis; HSP90α, heat shock protein 90α.

lowest Ct is considered truly positive; others are evaluated by comparing ΔCt with the cross-signal threshold: ΔCt below the threshold confirms true positivity; otherwise, it's a cross-signal and considered negative.

Selection of the optimal cutoff value. The surv_cutpoint function from the survminer package (version 0.4.9; https://cran.r-project.org/package=survminer) was used to determine the optimal cutoff value by dichotomizing the dataset into two groups. Kaplan-Meier survival analysis was then performed to compare these groups, with the cutoff yielding the smallest Log-rank P-value selected as optimal. This method was based on maximally selected rank statistics calculated using the maxstat package (version 0.7-25; https://cran.r-project.org/package=maxstat).

Statistical analysis. Each experiment was performed in triplicate to ensure the reliability of the results. Quantitative data are presented as the mean ± standard deviation and categorical data are expressed as n (%). For comparisons involving two independent groups, the Wilcoxon rank-sum test (also known as the Mann-Whitney U-test) was used, while the Wilcoxon signed-rank test was applied for paired data. To compare more than two groups, the Kruskal-Wallis test was conducted. When the Kruskal-Wallis test showed a significant result, post-hoc analysis was performed using Dunn's test. Patients were grouped based on the cutoff value, and Kaplan-Meier survival curves for overall survival (OS) were generated using R 4.0.3 software (The R Foundation). Cox regression analysis was performed to evaluate the prognostic significance of different EGFR mutation types and plasma HSP90α expression levels. P<0.05 was considered to indicate a statistically significant difference.

Results

HSP90α expression is associated with clinicopathological features in patients with lung cancer. The association between the expression level of HSP90α and several clinicopathological characteristics were compared among 1,347 patients

Table II. Association between plasma heat shock protein 90α and clinicopathological characteristic.

Clinicopathological characteristic	Plasma HSP90α level	P-value
Sex		1.72×10^{-5}
Male	62.0±50.3	
Female	57.8±59.4	
Pathological classification		1.837×10^{-5}
Small cell lung cancer	90.1±91.2	
Squamous cell lung carcinoma	57.7±39.4	
Adenocarcinoma	56.6±48.1	
Other	64.5±58.8	
T stage		1.477×10^{-5}
T1	50.4±37.1	
T2	58.9±49.4	
T3	59.0±43.5	
T4	66.1±63.8	
N stage		3.735×10^{-9}
N0	48.8±37.9	
N1	63.9±62.7	
N2	58.7±52.9	
N3	67.0±56.7	
M stage		8.819×10^{-12}
M0	50.2±36.5	
M1	66.2±60.9	

Data are presented as the mean ± standard deviation. The Wilcoxon test was used to compare two groups, and the Kruskal-Wallis statistical method was used to compare multiple groups. HSP90α, heat shock protein 90α; T, tumor; N, node; M, metastasis.

Table III. Association between heat shock protein 90α and epidermal growth factor receptor gene mutation status.

EGFR mutation status	Plasma HSP90α level	P-value
EGFR mutation	61.1±57.6	-
19 Exon deletion		0.826
WT	61.8±50.6	
MUT	60.6±62.9	
21 Exon L858R mutation		0.041[a]
WT	62.9±59.3	
MUT	55.9±52.2	
20 Exon mutation		0.014[a]
WT	59.1±56.8	
MUT	85.9±62.9	
T790M mutation		0.249
WT	60.9±59.1	
MUT	63.7±38.4	
Compound mutation status		0.209
Single genetic mutation	64.8±63.1	
Multiple genetic mutations	46.3±19.3	

[a]$P<0.05$. Data are presented as the mean ± standard deviation. The Mann-Whitney U-test was used to analyze the differences between the groups. EGFR, epidermal growth factor receptor; HSP90α, heat shock protein 90α; WT, wild type; MUT, mutant.

with lung cancer. The results revealed that HSP90α expression was significantly associated with the sex, pathological type and tumor (T)-node (N)-metastasis (M) stage ($P<0.05$; Table II) (20). Fig. 1 presents the differences in HSP90α expression across pathological conditions, clinical stages, T, N and M stages, as well as prognostic status ($P<0.05$).

Plasma HSP90α levels vary according to EGFR mutation type in NSCLC. Subsequently, the association between plasma HSP90α expression levels and EGFR gene status was assessed in 240 patients with NSCLC harboring EGFR mutations. The median plasma HSP90α level in this cohort was 46.8 ng/ml, with a range of 12.9-584 ng/ml. Notably, patients with exon 19 deletion showed no significant difference in plasma HSP90α expression compared with wild type patients. Conversely, compared with wild type patients, patients with the exon 21 L858R mutation had significantly reduced expression levels, whilst those with exon 20 mutations exhibited significantly higher levels. Furthermore, although patients with the T790M mutation showed increased plasma HSP90α expression, the difference was not statistically significant compared with wild-type patients. No significant difference was also observed between patients with multiple EGFR mutation subtypes and those with wild-type EGFR (Table III and Fig. 2). Taken together, these results suggest that different EGFR mutations may not exert additive or synergistic effects on plasma HSP90α expression, indicating that HSP90α levels are more likely influenced by specific mutation types rather than the number of mutations. This may reflect differences in downstream signaling activation or varying cellular stress responses associated with distinct EGFR alterations (21-23).

Determining the optimal cutoff value of HSP90α for prognosis. The optimal cutoff value was determined to be 62.1 ng/ml by dichotomizing the dataset into two groups according to prognostic OS status using the surv_cutpoint function in the survminer package. Kaplan-Meier curve analysis was then used to assess the association between HSP90α expression levels and prognosis in 1,347 patients with lung cancer. Among the overall patient cohort, those in the low-risk group with an HSP90α level of <62.1 ng/ml had an improved prognosis compared with those in the high-risk group (Fig. 3A).

High plasma HSP90α expression is associated with poor prognosis in patients with lung cancer with an EGFR gene mutation. To assess the association between plasma HSP90α expression and survival outcomes in patients with EGFR-mutant lung cancer, OS data from the EGFR-mutant cohort was used to generate Kaplan-Meier cumulative hazard curves. The results revealed that high plasma HSP90α expression was significantly associated with shorter OS ($P<0.01$; Fig. 3B). To determine whether the prognostic value

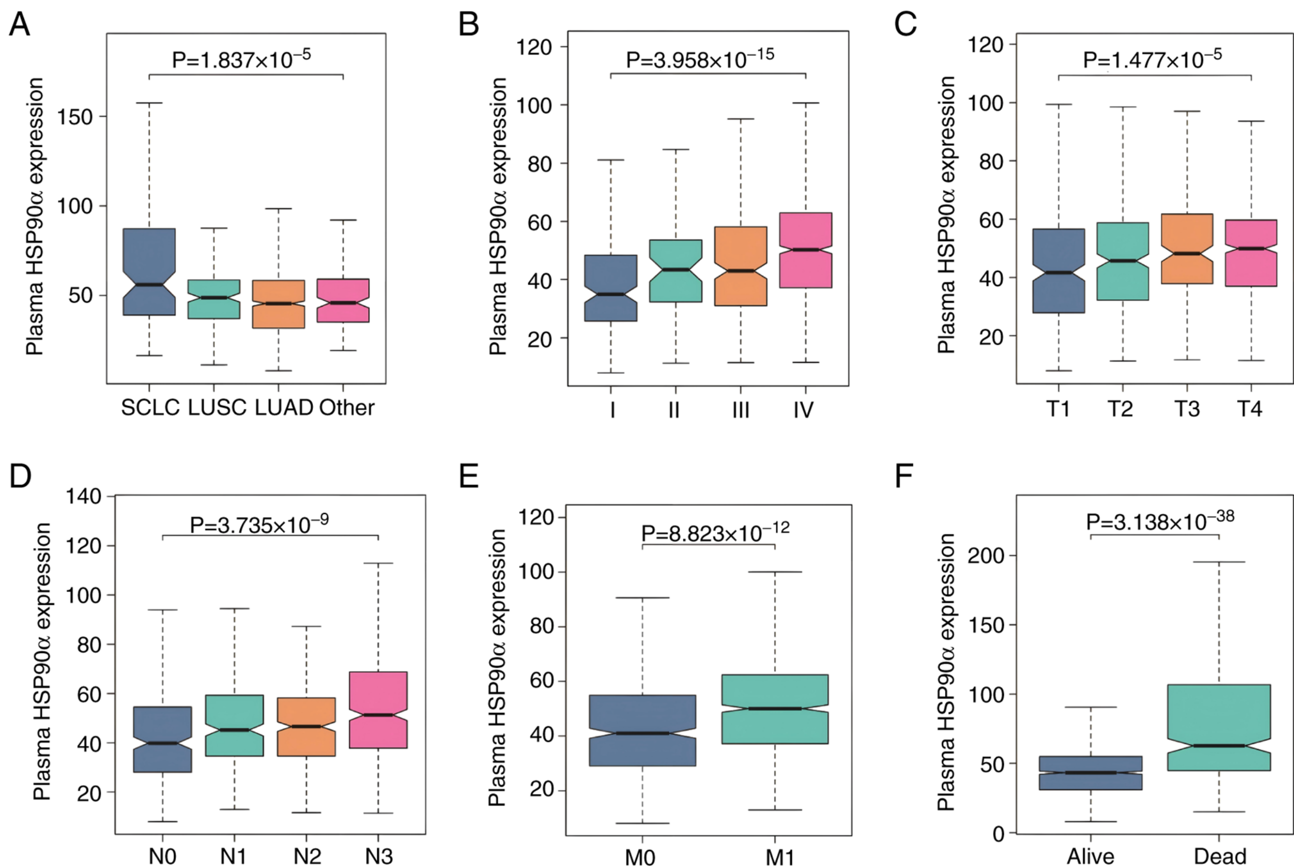

Figure 1. Box plots of plasma HSP90α expression according to different clinical characteristics. (A) Pathological classification revealing that plasma HSP90α levels were significantly higher in SCLC compared with LUSC, LUAD and other histological types. (B) Plasma HSP90α expression was significantly higher in patients with stage IV lung cancer than in those with earlier stages. Higher plasma HSP90α levels were demonstrated in patients with advanced (C) T, (D) N and (E) M stages. (F) Patients with lung cancer who died had significantly higher plasma HSP90α expression than survivors. Group differences were assessed using the Mann-Whitney U-test for two groups or the Kruskal-Wallis test for comparisons involving >2 groups. HSP90α, heat shock protein 90α; SCLC, small cell lung cancer; LUSC, lung squamous cell cancer; LUAD, lung adenocarcinoma; T, tumor; N, node; M, metastasis.

of plasma HSP90α expression depends on EGFR mutation status, univariate and multivariate Cox regression analyses were performed in 240 patients with EGFR-mutant NSCLC. Univariate analysis revealed that elevated HSP90α expression was significantly associated with worse OS (P<0.001; Table IV). Furthermore, multivariate analysis confirmed that high HSP90α expression (P<0.001) and M1 stage (P=0.004) independently predicted poor OS after adjustment for mutation-related factors identified in the univariate analysis (Table IV). These results suggest that HSP90α may serve as a prognostic indicator independent of EGFR mutation subtype.

Discussion

Growing evidence indicates that gene mutations are associated with the efficacy of EGFR-TKIs (24). A previous study analyzed data from 58 patients with EGFR-mutated metastatic NSCLC who received first-line EGFR-TKIs and reported that concomitant mutations are common and associated with a reduced objective response rate (ORR) and shorter OS (25). Moreover, studies have reported that EGFR-TKIs are less effective in patients with rare complex mutations, particularly those involving primary T790M or exon 20 insertions (26-28). Patients with complex mutations that include primary T790M or exon 20 insertions often exhibit resistance to first-line EGFR-TKIs and have poor ORR and progression-free survival PFS (29).

HSP90α is an evolutionarily conserved molecular chaperone (7) that serves an essential role in cellular function. It is induced by heat shock or stress and is secreted into the extracellular space by several cancer cells (30). Both *in vitro* and *in vivo* studies have demonstrated that HSP90α can promote cancer cell proliferation, metastasis, invasion and epithelial-mesenchymal transition (31,32), suggesting that it may be a promising target for cancer therapy. Furthermore, several studies have highlighted the diagnostic and prognostic value of plasma HSP90α in cancer (15,16). Large clinical trials have reported that plasma HSP90α has superior diagnostic performance in liver and lung cancers compared with α-fetoprotein or cytokeratin-19 (17,33). In the present study, patients with elevated HSP90α levels (≥62.1 ng/ml) had a significantly higher risk of poor prognosis than those with lower levels (P<0.0001), suggesting a positive association between elevated HSP90α and poor outcomes in lung cancer. Additionally, HSP90α expression revealed a decreasing trend in patients with the EGFR L858R mutation compared with those with wild-type EGFR (P=0.027). Nonetheless, the relationship between HSP90α expression and specific EGFR mutations remains unclear.

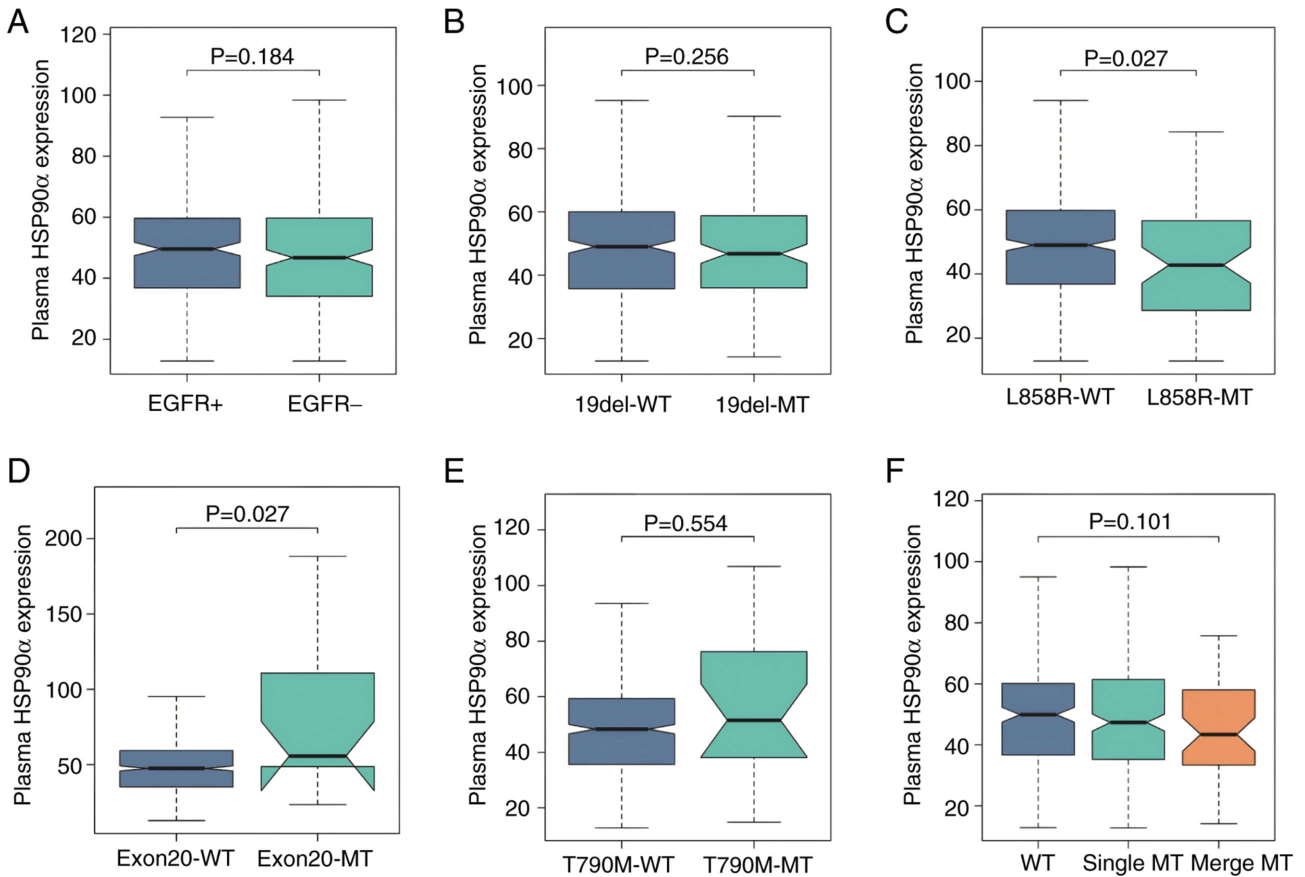

Figure 2. Box plots of plasma HSP90α expression according to different EGFR mutation types. (A) EGFR mutation status did not significantly affect plasma HSP90α levels. (B) Plasma HSP90α expression in patients with exon 19 deletion was comparable with that in wild type patients. (C) Plasma HSP90α levels were significantly decreased in patients with the exon 21 L858R mutation. (D) Patients with exon 20 mutations demonstrated significantly higher plasma HSP90α expression. (E) Plasma HSP90α expression was not significantly elevated in patients with T790M mutations. (F) There was no significant difference in plasma HSP90α levels between patients with multiple EGFR mutations and those with wild-type EGFR. Group differences were assessed using the Mann-Whitney U-test (two independent groups) or Kruskal-Wallis test for comparisons involving >2 groups. HSP90α, heat shock protein 90α; EGFR, epidermal growth factor receptor; WT, wild type; MT, mutant.

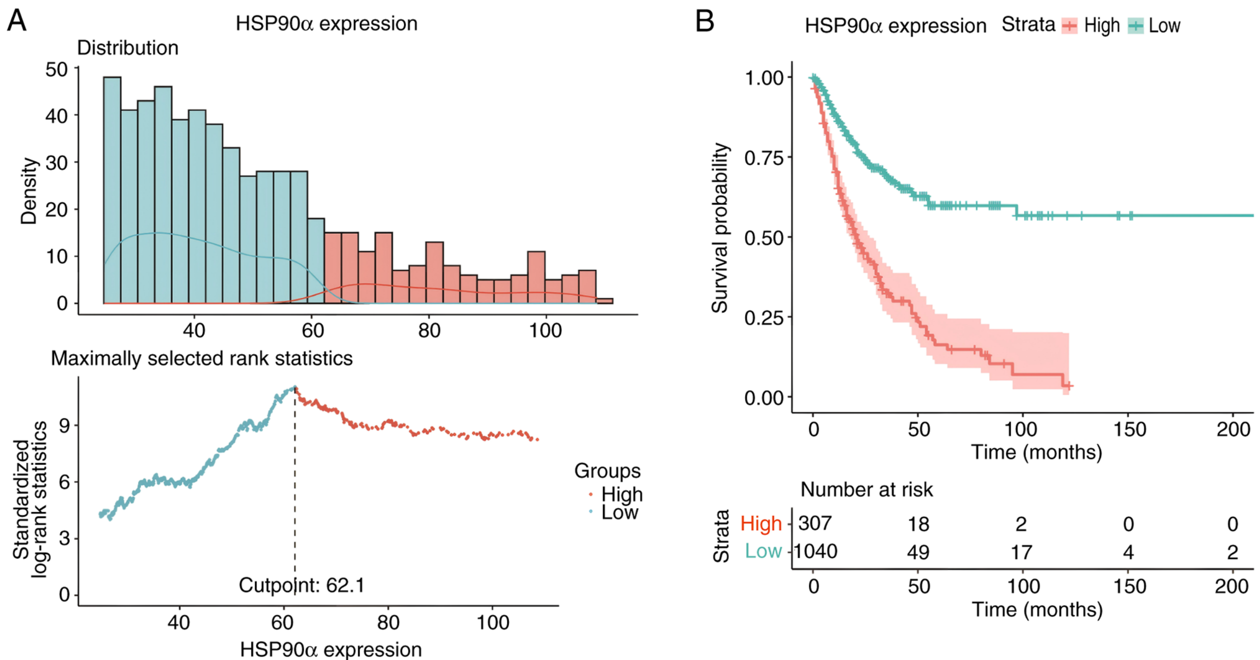

Figure 3. Selection of the optimal cutoff value for plasma HSP90α expression. (A) Density distribution of plasma HSP90α levels. Using the Log-rank test, the optimal cutoff value was determined to be 62.1 ng/ml. (B) Based on the cutoff, patients with lung cancer were stratified into risk groups, and overall survival curves were plotted. The high-risk group demonstrated a significantly worse prognosis (Log-rank P<0.01). HSP90α, heat shock protein 90α.

Table IV. Cox survival analysis of 240 patients with lung cancer with epidermal growth factor receptor gene mutations.

	Univariate Cox regression			Multivariate Cox regression		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
HSP90α (≥62.1 ng/ml)	2.741	1.935-3.881	<0.001	2.830	1.667-4.807	<0.001
Age	0.997	0.970-1.024	0.838	-	-	-
Sex (Female)	0.600	0.352-1.023	0.060	-	-	-
Pathological type						
Small cell lung cancer (yes)	1.633	0.223-11.970	0.630	-	-	-
Squamous cell lung cancer (yes)	1.089×10^{-7}	0.000-Inf	0.996	-	-	-
Lung adenocarcinoma (yes)	0.622	0.194-1.991	0.424	-	-	-
Stage						
II	6.264×10^{-8}	0.000-Inf	0.995	-	-	-
III	0.261	0.016-4.184	0.343	-	-	-
IV	2.026	0.279-14.690	0.485	-	-	-
T stage						
T2	0.715	0.331-1.548	0.385	-	-	-
T3	0.666	0.249-1.784	0.419	-	-	-
T4	0.965	0.492-1.892	0.918	-	-	-
N stage						
N1	2.726	0.788-9.437	0.113	-	-	-
N2	2.628	0.784-8.816	0.118	-	-	-
N3	3.126	0.931-10.493	0.065	-	-	-
M stage (M1)	7.726	1.887-31.630	0.004	8.506	2.013-35.94	0.004
Mutation						
EGFR	0.873	0.617-1.236	0.443	-	-	-
T790M	0.784	0.364-1.686	0.533	-	-	-
19del	0.740	0.499-1.097	0.134	-	-	-
L858R	1.241	0.744-2.069	0.408	-	-	-
Exon20	1.347	0.591-3.073	0.479	-	-	-
Merge	0.960	0.491-1.878	0.906	-	-	-

HR, hazard ratio; Inf., infinite.

Certain studies have reported that HSP90α can predict the efficacy of chemotherapy in non-small cell lung cancer, and that combining HSP90 inhibitors with EGFR-TKIs can enhance the effects of treatment on cell proliferation and apoptosis (34-37). Meanwhile, other studies have reported an association between high HSP90α expression and EGFR mutations (9,38). However, no studies have explored the relationship between specific EGFR mutation types and HSP90α expression, or the differences in the prognostic value of HSP90α across mutation subtypes, to the best of our knowledge. As gene testing increasingly guides clinical decision-making in lung cancer (39), evaluating the prognostic value of HSP90α within EGFR mutation subgroups is also of clinical relevance. The analysis in the present study demonstrated that high HSP90α expression was associated with several pathological types, clinical stages, TNM stages and prognostic outcomes in lung cancer. Although certain reports have indicated that plasma HSP90α levels, when combined with factors such as sex, clinical stage and metastasis, can help predict EGFR mutations (40-42), the findings in the present study suggest that this predictive value is limited to specific mutation types, such as L858R and exon 20 insertions. Previous studies have also reported that HSP90, a molecular chaperone protein, stabilizes several proteins involved in tumor progression, including EGFR (9,43,44). Moreover, HSP90 inhibitors, such as 17-deoxy-camptothecin, have been reported to overcome resistance to EGFR-TKIs by downregulating the expression of EGFR and MET (45,46).

HSP90α is an ATP-dependent chaperone protein that functions as a homodimer (HSP90αα), and its activity depends on dimerization (47). The structure contains three core domains: i) N-terminal domain (NTD), which is the ATP binding site and ATPase active center, driving conformational rearrangement cycle. This region is the target of most inhibitors (such as geldanamycin); middle domain, which is the client protein binding region, linked with the NTD through the charged connection region, regulating the chaperone cycle; and iii) C-terminal domain, which contains the EEVD motif, mediates binding with co-chaperone proteins containing tetratricopeptide repeat domains (such as heat shock organizing

protein/stress-inducible protein 1 and protein phosphatase 5) and regulates client protein recognition and stability (47). HSP90α drives conformational changes through the cycle of ATP binding, hydrolysis and ADP release, achieving folding and activation of client proteins. Disturbance of this cycle leads to abnormal accumulation of oncogenic proteins (such as human epidermal growth factor receptor 2 and Akt), promoting tumorigenesis (48). Although the α/β sequence homology is reported to be ~86%, the client protein spectrum is notably different (for example, HSP90β prefers Twist1) (49-51). Gene knockout models have reported that the functions are not interchangeable (52).

Although certain studies have reported the prognostic value of HSP90 mRNA or protein in several cancer tissues (53-55), data on plasma HSP90α in patients with cancer remain limited (17,56). Zhao *et al* (57) reported no prognostic value of HSP90α in patients with esophageal squamous cell carcinoma and no significant association between baseline levels and clinical characteristics. However, the present study demonstrated an association between HSP90α expression and prognosis in lung cancer. The findings suggest that plasma HSP90α has meaningful prognostic value, with high expression associated with worse overall survival. It was also demonstrated that HSP90α levels were higher in patients with small cell lung cancer compared with those with non-small cell lung cancer, consistent with the findings of Wang *et al* (40).

Although the results of the present study differ somewhat from those of previous studies, the present study included a broader range of EGFR mutation types and a larger number of participants, thereby providing greater statistical power. No difference was demonstrated in HSP90α levels between patients with exon 19 deletion mutations and those with wild-type EGFR, whereas HSP90α was significantly elevated in patients with exon 20 mutations. This may be because exon 19 deletions are typically sensitive mutations that do not substantially affect HSP90α expression or stability (58-60). By contrast, exon 20 insertion mutations confer drug resistance and involve distinct conformational changes, potentially increasing reliance on HSP90α for maintaining protein stability, thereby leading to a marked rise in HSP90α levels (22,61). Insufficient sample size may also contribute to the findings, particularly in the exon 20 group, where the small number of cases could limit statistical power and hinder the detection of true differences. Furthermore, the retrospective design of the present study may introduce selection bias. The absence of healthy controls is another limitation that could explain discrepancies with previous studies. Lastly, the present study was unable to adjust for confounding factors such as medication use and surgery. Therefore, the results require validation in larger, prospectively designed studies.

Furthermore, all data presented in the present study were obtained through routine clinical testing. However, a key limitation of the present work is the lack of cellular and molecular experiments, as we lack the infrastructure required to conduct in vitro studies. To address this limitation, future studies could incorporate *in vitro* and *in vivo* experiments using cell line models expressing specific EGFR mutations (such as exon 19 deletions and exon 20 insertions) to assess HSP90α expression levels, protein stability and HSP90 inhibition. Xenograft mouse models could also be used to evaluate the therapeutic efficacy of HSP90 inhibitors in EGFR mutant tumors.

In summary, elevated plasma HSP90α expression is associated with worse OS in patients with NSCLC and may serve as a prognostic indicator independent of EGFR mutation type. The results of the present study suggest that elevated HSP90α levels may serve as an indicator of specific EGFR mutations associated with poor prognosis, such as exon 20 insertions. In the future, when the EGFR mutation status is unclear, HSP90α could potentially act as an indirect biomarker to help identify drug-resistant mutations. Moreover, in cases where patients exhibit a poor response to EGFR-TKI therapy accompanied by elevated HSP90α levels, a change in treatment strategy may be warranted. Finally, for EGFR-mutant tumors that are highly dependent on HSP90 for protein stability, HSP90 inhibitors may offer a promising therapeutic option.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HG contributed to the study design. XT, JT, YT, SM, HW and XH contributed to data acquisition. XT performed data analysis and interpretation. XT re-analyzed the results. XT and JT drafted the manuscript. HG revised the manuscript. HG, JT, YT and XT confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Guangxi Medical University Cancer Hospital Ethics Committee reviewed and approved the present study (approval no. KY2023803). All patients provided written informed consent to participate.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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