

Prognostic value of the mRNA expression of members of the HSP90 family in non-small cell lung cancer

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Abstract. The aim of the present study was to investigate the potential prognostic value of members of the heat shock protein (HSP)90 family in non-small cell lung cancer (NSCLC) patients. The mRNA expression profiles of 1,926 NSCLC patients, which was available from the Kaplan-Meier plotter database, were included in the study. High expression of HSP90AA1 mRNA was significantly associated with a poorer rate of overall survival (OS) for all NSCLC patients [hazard ratio (HR), 1.21; 95% confidence interval (CI): 1.06-1.37; $P=0.004$], as well as for patients with adenocarcinoma (ADE; HR, 1.3; 95% CI: 1.02-1.65; $P=0.034$), but no significant correlation was identified for squamous cell carcinoma (SCC) patients (HR, 1.08; 95% CI: 0.85-1.38; $P=0.51$). High expression of HSP90AB1 and HSP90B1 mRNA was significantly associated with poorer rates of OS in lung SCC and ADE patients combined, as well as in lung ADE patients alone. By contrast, high expression of tumor necrosis factor receptor-associated protein 1 (TRAP1) mRNA was significantly associated with improved OS rates in all NSCLC patients combined (HR, 0.88; 95% CI: 0.77-0.99; $P=0.041$), as well as ADE patients. In stratified survival analysis, a high expression of HSP90AA1, HSP90AB1 and HSP90B1 predicted poor prognosis in stage I NSLCC patients, suggesting that these genes may serve as stage-independent prognostic indicators. As an elevated expression of HSP90AA1, HSP90AB1, HSP90B1 and TRAP1 was associated with poorer OS outcomes in patients with NSCLC, these HSP90 members may be potential prognostic biomarkers and drug targets for the treatment of NSCLC.

Introduction

Lung cancer was reported to be the most common cancer type in males in 2016, and is associated with a poor prognosis (1). Non-small cell lung cancer (NSCLC) accounts for 80-85% of all cases of lung cancer, with squamous cell carcinoma (SCC) and adenocarcinoma (ADE) being the most common histological subtypes (2,3). Despite advances in the early detection and therapeutic techniques, the overall 5-year survival rate is low. Consequently, further investigations into the mechanisms of initiation and progression, and the development of prognostic biomarkers and drug targets are required in order to improve the prognosis for NSCLC and allow for personalized therapy.

Heat shock protein 90 (HSP90) is one of the most widespread heat-associated proteins. HSP90 forms flexible homodimers and its basic structure comprises three parts: The N-terminal domain, the middle domain and the C-terminal domain. HSP90 is overexpressed in various cancer types, including pancreatic, ovarian, breast, lung and endometrial cancer, as well as oropharyngeal SCC and multiple myeloma (4-7). High expression of HSP90 was indicated to be a marker of poor prognosis in lung cancer, esophageal cancer, bladder cancer, melanoma and leukemia (8-11). Combination therapy with HSP90 inhibitors and conventional photon radiation delays tumor growth more effectively than radiotherapy alone (12). The HSP90 inhibitor NVP-AUY922 induces cell death in the lung and is currently undergoing clinical trials in lung cancer patients (13). However, the prognostic value of HSP90 in NSCLC has remained to be determined.

In the present study, the Kaplan-Meier (KM) plotter database (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>) was employed to assess the correlation between HSP90 mRNA expression and overall survival (OS). At present, the KM plotter database comprises gene expression information and clinical outcome parameters of various types of cancer (14-18). The KM plotter database may be used to analyze individual genes that may correlate with the OS of NSCLC patients. To date, the KM plotter database has been used to identify and validate numerous genes involved in NSCLC (15-18). In the present study, this database was used to evaluate the prognostic value of individual HSP90 protein members in patients with NSCLC.

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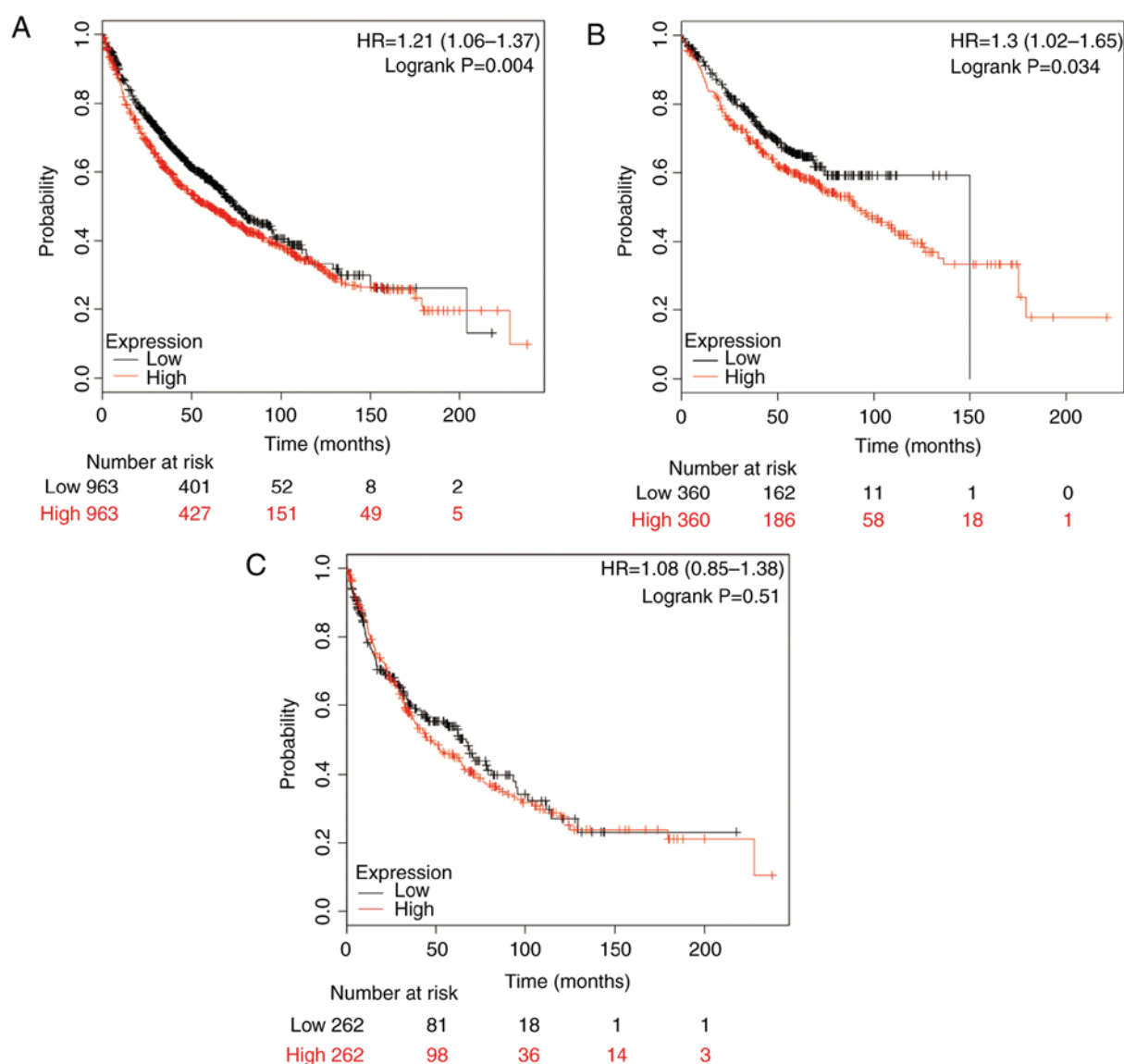


Figure 1. Kaplan-Meier analysis of the prognostic value of HSP90AA1 mRNA expression in the dataset [Affymetrix ID, 211969_at (HSP90AA1)]. Survival curves were plotted for (A) all non-small cell lung cancer patients (n=1,926), (B) those with adenocarcinoma (n=720) and (C) those with squamous cell carcinoma (n=524). Cut-off value, median expression level. HSP, heat shock protein; HR, hazard ratio (with 95% confidence interval).

Materials and methods

The information on the NSCLC patients contained in the KM plotter database was extracted from the Gene Expression Omnibus (GEO), the Cancer Biomedical Informatics Grid and The Cancer Genome Atlas database. The following NSCLC datasets were obtained from the GEO database: GSE14814, GSE19188, GSE29013, GSE30219, GSE31210, GSE3141, GSE31908, GSE37745, GSE43580, GSE4573, GSE50081 and GSE8894 (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>) (19). The KM plotter database was used to analyze the association of the mRNA expression of individual HSP90 members with relapse-free survival. WinStat 2013 software was used as an analysis tool. Data regarding age, sex, smoking history, histology, stage, success of surgery, radiotherapy and applied chemotherapy were recorded for all patients. In general, five HSP90 subfamily members [HSP90AA1, HSP90AA2, HSP90AB1, HSP90B1 and tumor necrosis factor

receptor-associated protein 1 (TRAP1)] were included in the KM plotter analysis to acquire KM survival plots, in which the number of patients at risk for certain time-points is compared between subgroups with different gene expression status. The hazard ratio (HR) and 95% confidence intervals (CI) and log-rank P-values were determined. A total of 1,926 lung cancer patients were included in the study. When the P-value was <0.05, the difference was regarded as statistically significant.

The Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (<https://david.ncifcrf.gov/>) was used to perform gene ontology (GO) functional annotation analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of various HSP90 members. Gene-gene interaction networks were generated with GeneMANIA (<http://genemania.org/>). Protein-protein interaction networks were constructed with the Search Tool for the Retrieval of Interacting Genes and Proteins (STRING; <https://string-db.org/>).

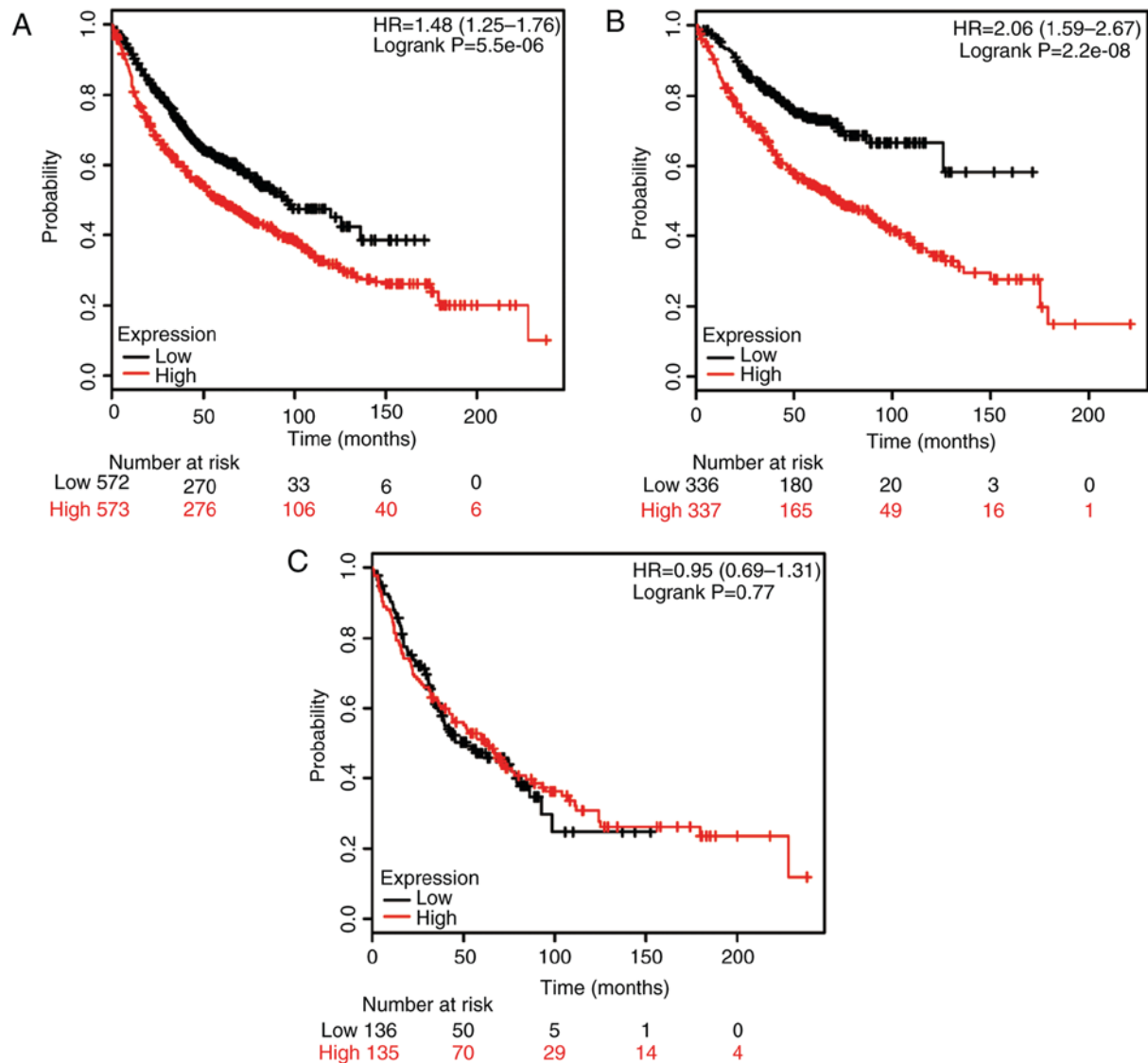


Figure 2. Kaplan-Meier analysis of the prognostic value of HSP90AB1 mRNA expression in the dataset [Affymetrix ID, 1557910_at (HSP90AB1)]. Survival curves were plotted for (A) all non-small cell lung cancer patients (n=1,926), (B) those with adenocarcinoma (n=720) and (C) those with squamous cell carcinoma (n=524). Cut-off value, median expression level. HSP, heat shock protein; HR, hazard ratio (with 95% confidence interval).

Results

Prognostic value of HSP90 members in NSCLC. The HSP90 family comprises five sub-members (HSP90AA1, HSP90AA2, HSP90AB1, HSP90B1 and TRAP1). Of these, only HSP90AA2 was not included in the KM plot database, possibly due to its low levels of expression.

First, the prognostic value of the mRNA expression of HSP90AA1 was evaluated in the dataset. The valid gene Affymetrix ID was 211969_at (HSP90AA1). Survival curves were generated to draw preliminary conclusions regarding the influence of HSP90AA1 on the prognosis of patients with NSCLC (n=1,926; Fig. 1A), ADE (n=720; Fig. 1B) and SCC (n=524; Fig. 1C). A high expression of HSP90AA1 mRNA was significantly associated with a worse OS for all NSCLC cases (HR, 1.21, 95% CI: 1.06-1.37; P=0.004; Fig. 1A). High expression of HSP90AA1 mRNA was also significantly associated with poor rates of OS in ADE patients (HR, 1.30; 95% CI: 1.02-1.65; P=0.034; Fig. 1B), while the association in

SCC patients was not significant (HR, 1.08; 95% CI, 0.85-1.38; P=0.51; Fig. 1C).

Next, the potential prognostic role of HSP90AB1 mRNA expression was evaluated using the database. The valid gene Affymetrix ID was 1557910_at (HSP90AB1). High expression of HSP90AB1 mRNA was significantly associated with poor rates of OS for all NSCLC patients (HR, 1.48; 95% CI: 1.25-1.76; P=5.5x10⁻⁶; Fig. 2A). High expression of HSP90AB1 mRNA was also associated with poor rates of OS in ADE patients (HR, 2.06; 95% CI: 1.59-2.67; P=2.2x10⁻⁸; Fig. 2B), but the correlation with OS rates in SCC patients was not significant (HR, 0.95; 95% CI: 0.69-1.31; P=0.77; Fig. 2C).

Furthermore, the potential prognostic value of HSP90B1 mRNA expression was evaluated using the database (Fig. 3). The valid gene Affymetrix ID was 216449_x_at (HSP90B1). High expression of HSP90B1 mRNA was significantly associated with poor rates of OS for all NSCLC patients (HR, 1.20; 95% CI: 1.06-1.37; P=0.0044; Fig. 3A), as well as for ADE patients (HR, 1.55; 95% CI: 1.22-1.97; P=0.00031; Fig. 3B);

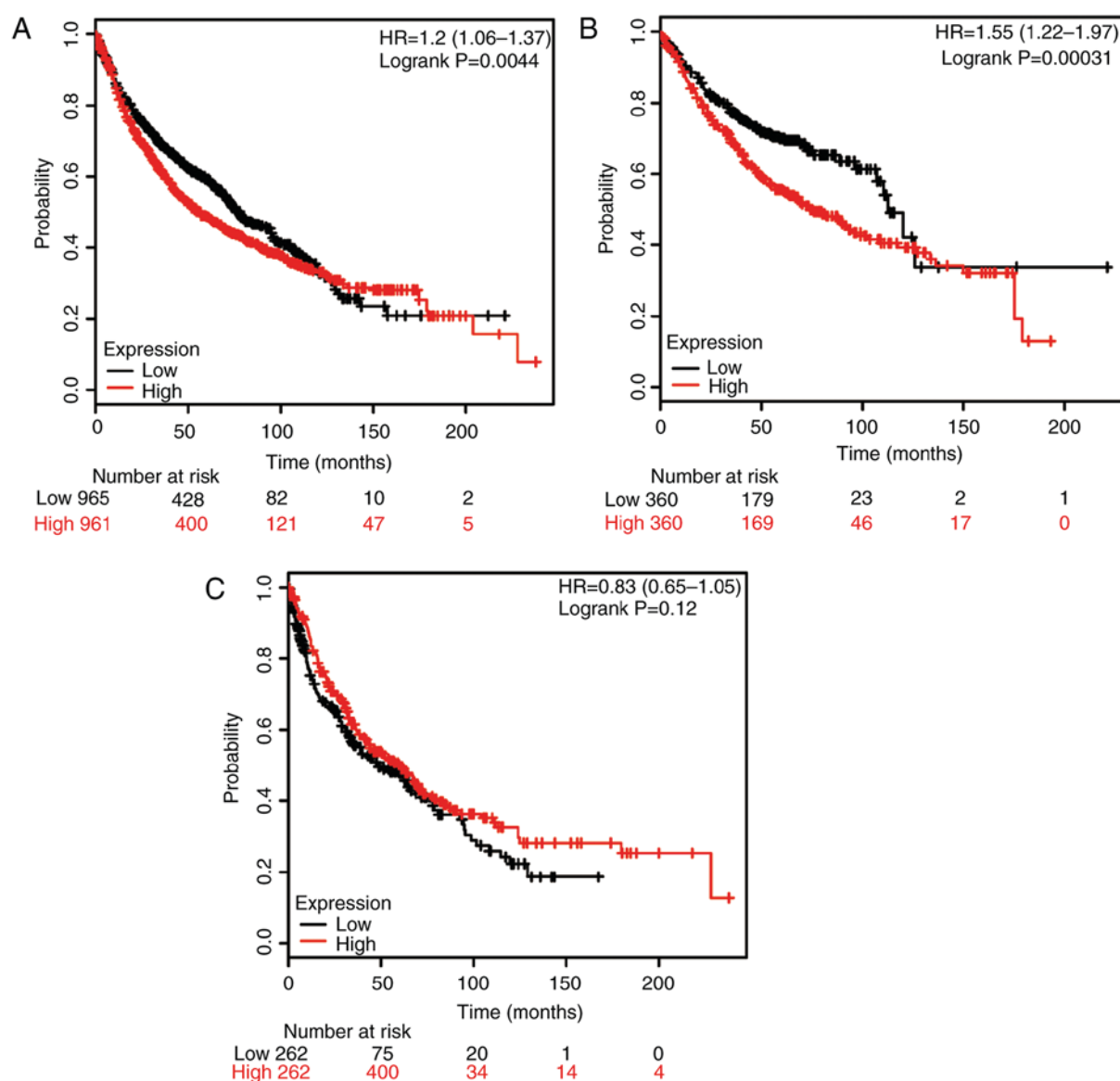


Figure 3. Kaplan-Meier analysis of the prognostic value of HSP90B1 mRNA expression in the dataset [Affymetrix ID, 216449_x_at (HSP90B1)]. Survival curves were plotted for (A) all non-small cell lung cancer patients (n=1,926), (B) those with adenocarcinoma (n=720) and (C) those with squamous cell carcinoma (n=524). Cut-off value, median expression level. HSP, heat shock protein; HR, hazard ratio (with 95% confidence interval).

however, no significant association was obtained for SCC patients (HR, 0.83; 95% CI: 0.65-1.05; P=0.12; Fig. 3C).

Finally, the potential prognostic role of TRAP1 mRNA expression was evaluated using the database. The valid gene Affymetrix ID was 201391_at (TRAP1). High expression of TRAP1 mRNA was significantly associated with higher OS rates for all patients (HR, 0.88; 95% CI: 0.77-0.99; P=0.041; Fig. 4A), as well as for ADE patients (HR, 0.72; 95% CI: 0.57-0.92; P=0.0072; Fig. 4B); however, no significant association was identified for SCC patients (HR, 1.02; 95% CI: 0.80-1.29; P=0.89; Fig. 4C).

Next, to assess whether the prognostic value of the mRNA expression status of individual HSP90 members depends on other clinicopathological factors, the influence of high vs. low expression on OS was determined for NSCLC patients stratified based on the following parameters: The patients' smoking history, clinical stage of the disease and chemotherapy status. As presented in Table I, high expression of HSP90AA1,

HSP90B1 and TRAP1 mRNA had no significant prognostic value in patients with or without a history of smoking. However, a high expression of HSP90AB1 was identified in patients with a smoking history and HSP90B1 in those that did not smoke. Table II reveals that there was only a significant prognostic value for HSP90AA1 and HSP90AB1 in stage I patients, and for HSP90B1 in stage I and II patients, but not in patients at stage III. No significant prognostic values were observed in all stages of TRAP1 in NSCLC. No significant association with OS was identified for any of the HSP90 members in patients that did or did not undergo chemotherapy (Table III).

GO functional annotation analysis and KEGG pathway analysis of HSP90 members. DAVID was used to investigate the biological functions of the HSP90 members (HSP90AA1, HSP90B1, HSP90AB1 and TRAP1). GO enrichment analysis in the categories cellular component and biological process was performed (Fig. 5A and B). The results revealed that cell

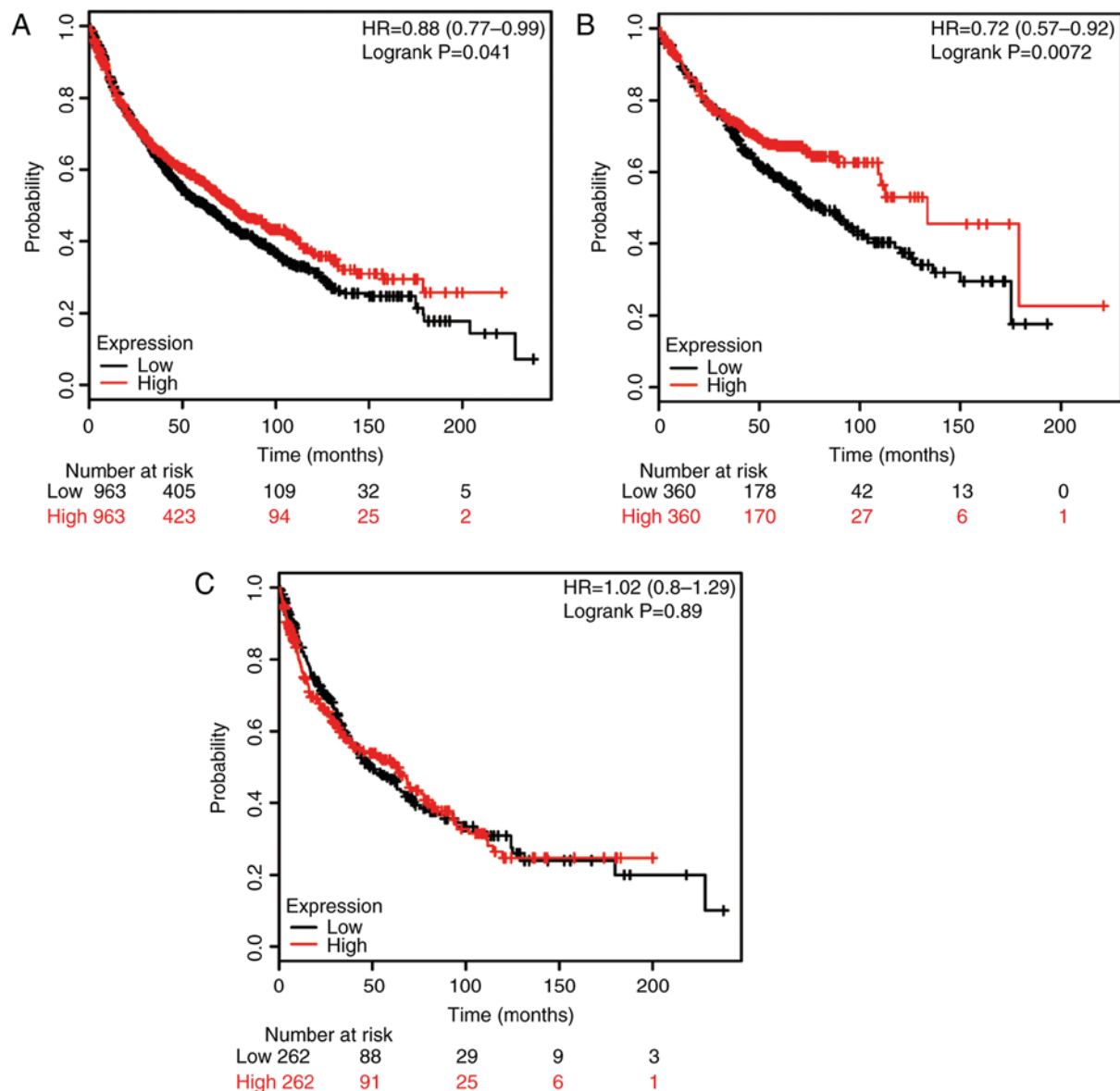


Figure 4. Kaplan-Meier analysis of the prognostic value of TRAP1 mRNA expression in the dataset [Affymetrix ID, 201391_at (TRAP1)]. Survival curves were plotted for (A) all non-small cell lung cancer patients (n=1,045), (B) those with adenocarcinoma (n=673) and (C) those with squamous cell carcinoma (n=217). Cut-off value, median expression level. TRAP1, tumor necrosis factor receptor-associated protein 1; HR, hazard ratio (with 95% confidence interval).

size was positively regulated and the response to stress was enriched. Enrichment of certain genes interacting with HSP90 family members in certain KEGG pathways was also identified (Fig. 5C) and included the phosphatidylinositol 3'kinase (PI3K)-Akt signaling pathway in cancer.

Gene-gene interaction networks were constructed with GeneMANIA (Fig. 6A) and protein-protein interaction networks were constructed using STRING (Fig. 6B). It was indicated that the HSP90 family has complex interactions, and that sub-members of the HSP90 family are co-expressed with each other.

Discussion

NSCLC is a highly malignant type of cancer with a poor 5-year survival rate (3,20). HSP90 has been reported to have a role in numerous types of cancer, and overexpression of this protein

has been proposed to enhance carcinogenesis and affect the prognosis of patients (21-25). HSP90 α , the protein encoded by HSP90AA1, is considered to have an important regulatory role in tumor invasion and migration (26,27). The present study indicated an influence of the HSP90 expression status on the OS rate of NSCLC patients. An association between HSP90 and the clinical features of NSCLC was also observed.

HSP90 α is secreted extracellularly and has an important role in wound healing and inflammation. These two processes are frequently hijacked by cancer, leading to malignant cell motility, metastasis and extravasion (28). HSP90 α modulates growth and lung metastasis by affecting the proliferation, migration and invasion of isolated primary carcinoma cells (29). One study indicated that the level of HSP90 α in the plasma of patients with the advanced stages of malignant tumors was relatively high (30). The expression of HSP90 α has been reported to correlate with the degree of malignancy

Table I. Influence of HSP90 mRNA expression on the prognosis of non-small cell lung cancer patients with a history of smoking (n=820) and those who never smoked (n=205).

Gene/smoking history	HR	95% CI	P-value
HSP90AA1			
Never smoked	0.81	0.46-1.42	0.46
Smoked	1.14	0.93-1.40	0.22
HSP90AB1			
Never smoked	1.89	0.84-4.33	0.12
Smoked	1.59	1.05-2.42	0.029
HSP90B1			
Never smoked	3.51	1.86-6.62	3.40x10 ⁻⁵
Smoked	1.11	0.90-1.37	0.31
TRAP1			
Never smoked	0.73	0.42-1.28	0.27
Smoked	0.92	0.75-1.13	0.43

TRAP1, tumor necrosis factor receptor-associated protein 1; HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

Table II. Influence of HSP90 mRNA expression on the prognosis of non-small cell lung cancer patients with the clinical stage of I (n=577), II (n=244) and III (n=70).

Gene/clinical stage	HR	95% CI	P-value
HSP90AA1			
I	1.56	1.29-1.88	2.70x10 ⁻⁶
II	1.18	0.82-1.71	0.38
III	1.02	0.58-1.79	0.94
HSP90AB1			
I	3.28	2.29-4.7	7.40x10 ⁻¹²
II	1.66	1.04-2.66	0.033
III	1.34	0.64-2.79	0.44
HSP90B1			
I	1.86	1.40-2.47	1.5x10 ⁻⁵
II	1.1	0.76-1.59	0.61
III	0.92	0.54-1.59	0.78
TRAP1			
I	0.83	0.63-1.09	0.17
II	1.14	0.79-1.65	0.48
III	0.68	0.39-1.17	0.16

TRAP1, tumor necrosis factor receptor-associated protein 1; HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

of esophageal SCC and the prognosis of affected patients (8). Furthermore, the present study suggested that a high expression of HSP90AA1 mRNA was associated with a poor prognosis in ADE patients, but not in SCC patients. Overall, it may be concluded that high expression of HSP90 α mRNA correlates with poorer rates of OS in all NSCLC patients combined.

Table III. Influence of HSP90 mRNA expression on the prognosis of non-small cell lung cancer patients with chemotherapy (n=176) and without chemotherapy (n=310).

Genes/chemotherapy	HR	95% CI	P-value
HSP90AA1			
No	1.17	0.84-1.64	0.36
Yes	0.91	0.60-1.39	0.67
HSP90AB1			
No	0.79	0.16-3.98	0.78
Yes	0.73	0.23-2.33	0.59
HSP90B1			
No	0.86	0.62-1.20	0.38
Yes	0.87	0.58-1.32	0.52
TRAP1			
No	1.16	0.83-1.62	0.39
Yes	1.27	0.84-1.89	0.25

TRAP1, tumor necrosis factor receptor-associated protein 1; HSP, heat shock protein; HR, hazard ratio; CI, confidence interval.

In previous study, the expression rate of HSP90AB1 in lung ADE tissues was determined to be obviously higher than that in lung SCC tissues, and its overexpression was associated with poor prognosis for lung ADE patients (31). In the present study, high expression of HSP90AB1 mRNA was significantly associated with a poorer OS rate in ADE patients but not in SCC patients.

HSP90B1 is essential for mediating the contribution of tumor-associated macrophages to inflammatory colon tumorigenesis in mice (32). Overexpression of HSP90 β leads to significantly increased proliferation, migration, invasion and tube formation of human endothelial cells. HSP90 β was reported to promote endothelial cell-dependent tumor angiogenesis by increasing vascular endothelial growth factor receptor expression (33). Serum HSP90B1 levels were reported to be significantly elevated in dogs with mammary tumors compared with those in healthy controls (34). Another study demonstrated that HSP90B1 is an oncogenic chaperone in hepatocyte carcinogenesis (35). Overexpression of HSP90B1 may be associated with the poor survival rate of hepatocellular carcinoma patients (36). In patients with early-stage liver cancer, a considerable increase in HSP90B1 is associated with tumor metastasis and recurrence (37). Analysis of survival rates demonstrated that HSP expression was an independent unfavorable prognostic factor for gall bladder cancer (38). Furthermore, HSP90B1 overexpression was indicated to be an accurate predictive factor for shorter OS and disease-free survival in NSCLC patients (39).

High TRAP1 expression has previously been reported to be an adverse prognostic factor for patients with NSCLC (40,41). Conversely, the present results indicated that TRAP1 mRNA expression is correlated with an improved prognosis in all NSCLC patients combined, as well as ADE patients alone. The significance of TRAP1 in NSCLC therefore requires further investigation.

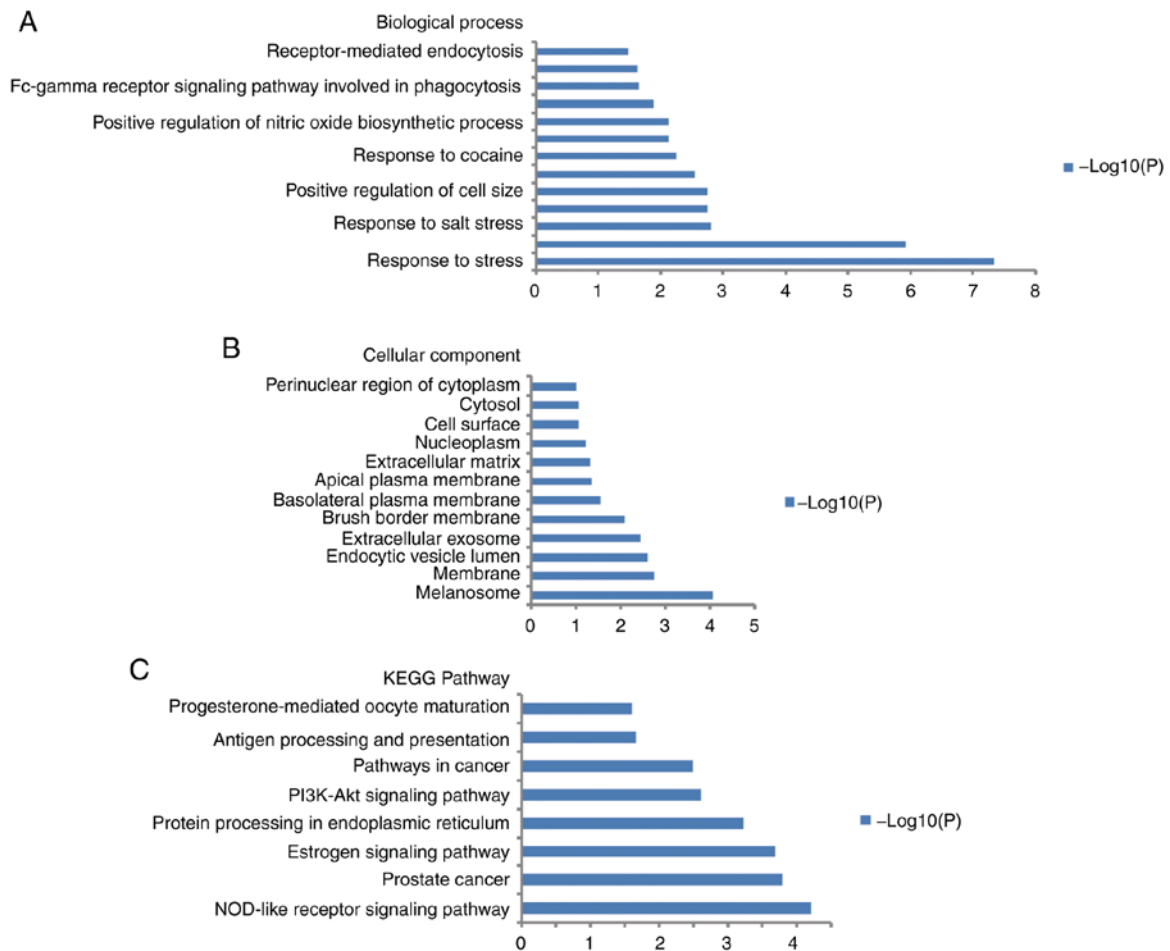


Figure 5. Analysis of GO terms and KEGG performed using the Database for the Annotation, Visualization and Integrated Discovery. (A) GO terms in the category biological processes. (B) GO terms in the category cellular component. (C) KEGG pathway analysis. GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PI3K, phosphoinositide-3 kinase.

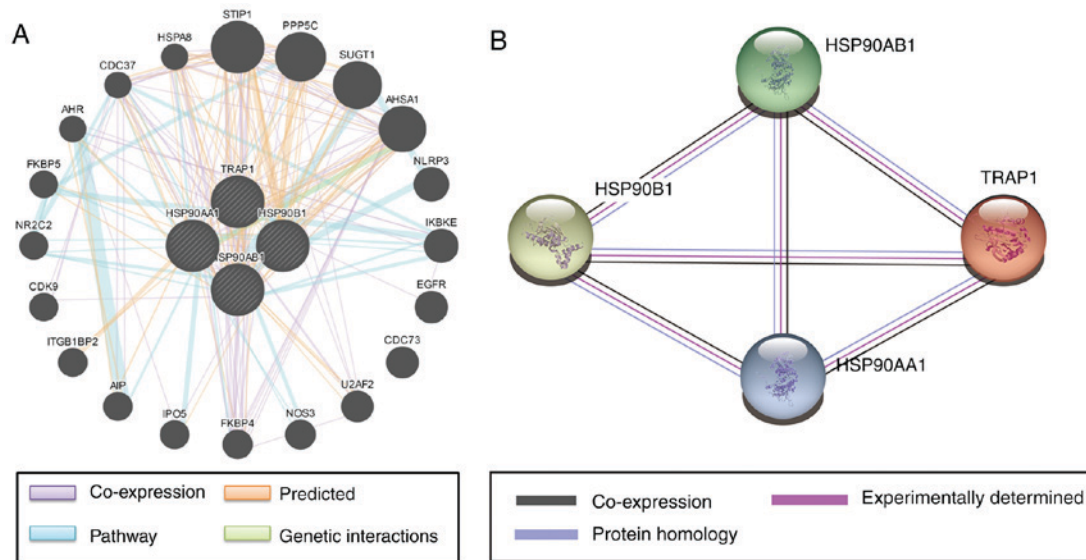


Figure 6. (A) Gene-gene interaction networks among selected genes constructed by GeneMANIA. (B) Protein-protein interaction networks among selected proteins constructed with the Search Tool for the Retrieval of Interacting Genes and Proteins. The size of the circles indicates the degree of interaction. HSP, heat shock protein; TRAP1, tumor necrosis factor receptor-associated protein 1; STIP1, stress induced phosphoprotein 1; PPP5C, protein phosphatase 5 catalytic; SUGT1, suppressor of G2 allele of sKPI; AHSA1, activator of HSP90 ATPase activity 1; NLRP3, NLR family pyrin domain containing 3; IKKBE, inhibitor of nuclear factor kappa B kinase subunit epsilon; EGFR, epidermal growth factor receptor; CDC73, cell division cycle 73; U2AF2, U2 small nuclear RNA auxiliary factor 2; NOS3, nitric oxide synthase 3; FKBP4, FK506 binding protein 4; IPO5, importin 5; AIP, aryl hydrocarbon receptor interacting protein; ITGB1BP2, integrin subunit beta 1 binding protein 2; CDK9, cyclin dependent kinase 9; NR2C2, nuclear receptor subfamily 2 group C member 2; FKBP5, FK506 binding protein 5; AHR, aryl hydrocarbon receptor; CDC37, cell division cycle 37; HSPA8, heat shock protein family A member 8.

Nicotine, a major addictive component of tobacco smoke, has been reported to induce cell cycle progression, angiogenesis and metastasis during cancer (42,43). However, the potential role of nicotine in HSP90 activation in NSCLC patients has not been previously addressed, to the best of our knowledge. Nicotine is known to induce apoptosis in human cells, possibly through increased HSP90 α expression (44). In the present study, a high expression of HSP90AB1 mRNA was identified to be a significant prognostic factor in NSCLC patients with a smoking history, but not in those who never smoked. However, high expression of HSP90B1 mRNA was identified to be a significant prognostic factor in NSCLC patients who never smoked, but not in those with a smoking history. Further research is required to confirm these results and identify the possible underlying mechanisms.

Previous studies have proposed that HSP90 may be a crucial drug target for the treatment of NSCLC. For instance, inhibition of the mitochondrial HSP90 network may be an effective treatment for highly refractory tumors (45), and topical application of the HSP90 inhibitor 17AAG is effective in preventing epidermal hyperplasia and SCC (46).

The current study has some limitations that need clarification. A stratified analysis for all NSCLC patients combined was performed, but included patients with SCC, which demonstrated no significance. Therefore, a stratified survival analysis for each type of NSCLC should be performed separately with a large sample size in the future.

In conclusion, the results of the present study suggest that HSP90AA1, HSP90AB1 and HSP90B1 may be potential biomarkers for the prognosis of NSCLC. However, the present results were obtained using bioinformatics methods and further research is required to explore the underlying molecular mechanisms in the future.

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Authors' contributions

RW designed the study and performed bioinformatics analysis. KL analyzed the data and wrote the manuscript. MK, WQ and JL analyzed the data. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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