Overexpression of HSP10 correlates with HSP60 and Mcl-1 levels and predicts poor prognosis in non-small cell lung cancer patients

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Abstract.
BACKGROUND: HSP60 and its partner HSP10 are members of heat shock proteins (HSPs) family, which help mitochondrial protein to fold correctly. Mcl-1, a member of the Bcl-2 family, plays a crucial role in regulation of cell apoptosis. Aberrant expression of HSP10, HSP60 and Mcl-1 is involved in the development of many tumors.
OBJECTIVE: To examine the association between expression of HSP10, HSP60 and Mcl-1 and clinicopathological features of non-small cell lung cancer (NSCLC).
METHODS: Tissue microarrays including 53 non-cancerous lung tissues (Non-CLT) and 354 surgically resected NSCLC were stained with anti-HSP10, anti-HSP60 and anti-Mcl-1 antibodies respectively by immunohistochemistry.
RESULTS: Higher expression of HSP10, HSP60 and Mcl-1 was found in NSCLC compared with Non-CLT. Both individual and combined HSP10 and HSP60 expression in patients with clinical stage III was higher than that in stage I ∼ II. Expression of HSP10 showed a positive correlation with HSP60 and Mcl-1. Overall survival time of NSCLC patients was remarkably shorter with elevated expression of HSP10, HSP60 and Mcl-1 alone and in combination. Moreover overexpression of HSP10 and Mcl-1 was poor independent prognostic factor for lung adenocarcinoma patients.
CONCLUSIONS: High expression of HSP10, HSP60 and Mcl-1 might act as novel biomarker of poor prognosis for NSCLC patients.

Keywords: HSP10, HSP60, Mcl-1, NSCLC, prognosis

1. Introduction

Lung cancer, particularly non-small cell lung cancer (NSCLC) is the most frequently diagnosed cancer and the leading cause of cancer-related death worldwide [1]. Approximately 80%–85% of diagnosed lung cancer patients are NSCLC, among which adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are the most two common histological subtypes [2].

Early detection of NSCLC is of critical importance for a favorable outcome after surgical resection, since the five-year survival rate of patients with stage IB could reach 68% [3]. Unfortunately, most of the patients are in advanced stages at the time of diagnosis [4]. Although recent years have seen great progress in treatment for patients with late stage lung cancer, the survival rate is still very low [3,4]. Therefore, it is of great necessity and urgency to discover and identify new biomarker(s) for the prediction, diagnosis, prognosis judgment and targeted therapy of NSCLC, in turn to lead more effective treatment and reduction of mortality.

Heat shock proteins (HSPs), also known as stress proteins, are highly conserved and maintain cellular home-
ostasis under various stress conditions, such as hyper-
thermia and hypoxia [5]. The 10 kDa heat shock protein
(HSP10), predominantly located in mitochondria, is a
co-chaperone protein of the 60 kDa heat shock protein
(HSP60). They interact to fold and transport proteins
to the mitochondria, in order to maintain the stability
of the mitochondrial protein [6]. Aberrant expression
of HSP10 and HSP60 is involved in the progression
of several major kinds of tumors, including colorectal
adenocarcinoma and glioblastoma [7–9]. In addition,
HSP10 and HSP60 are also involved in cell apoptosis. It
is well known that the activation of Bak/Bax is consid-
ered to be the most critical step of mitochondrial path-
way which leads to cell apoptosis [10]. Low expression
of HSP10 can promote testosterone-induced apopto-
sis in mouse ovarian granulosa cells by upregulating
pro-apoptotic factor Bax [11]. HSP60 can also form
complexes with Bax and Bak to play an anti-apoptotic
function [12,13].

Myeloid Cell Leukemia 1 (Mcl-1) is known as a
member of B-cell Lymphoma 2 (Bcl-2) family that
strictly controls the intrinsic apoptotic pathways [14].
Accumulating evidences suggest that Mcl-1 plays cru-
icial roles in suppressing mitochondria-dependent apop-
tosis [15] through regulation of the balance between
the long and short variants of the Mcl-1 gene to regu-
late mitochondrial fusion and fission machinery [16].
Moreover, elevated Mcl-1 has also been found in dif-
ferent types of cancer, such as breast cancer, acute
myeloid leukemia, hepatocellular carcinoma and lung
cancer [17–20].

It still remains unclear whether the expression of
HSP10, HSP60 and Mcl-1 is related to clinicopatho-
logical features of NSCLC patients. In this study, we
conducted the present retrospective study by using
NSCLC tissue microarrays (TMAs) and immunohis-
tochemistry (IHC) to evaluate the potential correlation
between HSP10, HSP60 and Mcl-1, and to investigate
whether there is a relationship between their expres-
sion, clinicopathological parameters and the prognostic
impact in NSCLC.

2. Materials and methods

2.1. Ethical statement

All protocols related to the research were approved by
the Institutional Human Experiment and Ethics
Committee of the Second Xiangya Hospital of Central
South University (approval No. S039/2011). Informed
consent was obtained from all subjects before surgery.

2.2. Clinical data

A total of 53 non-cancerous lung tissues (Non-CLT)
and 354 NSCLC tissues resected from 2003 to 2013
in the Thoracic Surgery Department of the Second Xi-
angya Hospital were obtained from the Department
of pathology, the Second Xiangya Hospital of Central
South University. No one received radiation ther-
apy or chemotherapy. All patients obtained the exact
histological diagnosis which was confirmed by well-
experienced pathologists. The clinical stage of each case
was confirmed according to the Eighth Edition Lung
Cancer Stage Classification [21]. Clinicopathological
data (Supplemental Table 1) were extracted from the
clinical record. In this study, we constructed NSCLC
TMAs by using the TMA technology as previously
described [22,23].

2.3. Antibody selections

Staining was done using primary antibodies as
follows: HSP10 (Catalog: SC-376313; Santa Cruz
Biotechnology, CA; 1:1000 dilution); HSP60 (Catalog:
#12165, Cell Signaling Technology; 1:1500 dilution);
Mcl-1 (Catalog: #94296, Cell Signaling Technology;
1:300 dilution).

2.4. Immunohistochemistry and scores

The staining for HSP10, HSP60 and Mcl-1 in TMAs
of NSCLC and non-CLT was carried out as described
previously and the staining conditions of each antibody
was adjusted in the light of previous laboratory experi-
ence [24,25].

Immunostaining was scored independently by QW
and SF. The score was based on intensity and distri-
bution of staining described as follows: the total score
is percentage score plus intensity score. Specifically,
the intensity of staining signal was scored as 0 (neg-
ative), 1 (mild expression), 2 (moderate expression)
and 3 (strong expression). According to the percent-
age of positive cells, the percentage score included 0
(0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–
100%). The total score ranges from 0 to 7. According
to NSCLC patients’ overall survival (OS) using the log-
rank test, the best cutoff levels for the three proteins
are 5, 4 and 4, respectively. The total scores more than 5,
4 and 4 were considered to be high expression of these
three proteins, others were considered low expression,
respectively. Agreement between the two pathologists
is 95%, and borderline and equivocal cases are solved
through remained and discussion.
Fig. 1. Expression of HSP10, HSP60 and Mcl-1 in lung ADC, lung SCC and Non-CLT were detected by IHC. Strong positive staining of HSP10 (A), HSP60 (D) and Mcl-1 (G) was found in cell cytoplasm of lung SCC cells. Strong positive staining of HSP10 (B), HSP60 (E) and Mcl-1 (H) was also showed in cell cytoplasm of lung ADC cells. Negative staining of HSP10 (C), HSP60 (F) and Mcl-1 proteins was found in Non-CLT (200 ×, IHC, DAB staining).

2.5. Statistical analysis

Statistical analysis was performed by using SPSS 24.0 software. The difference expression of proteins between NSCLC and Non-CLT was analyzed by Chi square test, which was also used for analyzing the relationship between HSP10, HSP60 and Mcl-1 and the clinicopathological features of NSCLC. Spearman’s rank correlation coefficient was performed on the correlation between HSP10, HSP60 and Mcl-1. Kaplan-Meier analysis was performed for survival rate, and comparisons of survival rate curve were analyzed by log-rank test. Cox proportional hazards regression model was carried out to assess independent prognostic factors. Statistical significance was set at $P < 0.05$ (Two-sided).

3. Results

3.1. Expression of HSP10, HSP60 and Mcl-1 increased significantly in NSCLC

The expression and subcellular localization of HSP10, HSP60 and Mcl-1 was detected in NSCLC and Non-CLT tissues by IHC. These three proteins were stained in cytoplasm of lung SCC and ADC cells (Fig. 1A, B, D, E, G and H), but were absent from Non-CLT (Fig. 1C, F and I). The high expression percentage of HSP10 and HSP60 was 58.4% (94/161) and 65.2% (105/161) in lung SCC, 59.1% (114/193) and 79.8% (154/193) in lung ADC, and 11.3% (6/53) and 26.4% (14/53) in Non-CLT, respectively. For Mcl-1, the
Table 1

Analysis of the association between expression of HSP10, HSP60 and Mcl-1 proteins and clinicopathological features of NSCLC (n = 354)

| Clinicopathological features (n) | HSP10 | HSP60 | Mcl-1 | HSP10/HSP60 # | HSP10/HSP60/Mcl-1 # |
|---------------------------------|-------|-------|-------|---------------|----------------------|
|                                 | High (%) | Low (%) | P-value | High (%) | Low (%) | P-value | P⁺ (%) | N− (%) | P-value | P⁺ (%) | N− (%) | P-value |
| Age (years)                     |        |        |         |        |        |         |        |        |         |        |        |         |
| ≤ 50 (n = 97)                   | 60 (61.9) | 37 (38.1) |       | 68 (70.1) | 29 (29.9) | 0.545 | 63 (64.9) | 34 (35.1) | 1.000 | 50 (51.5) | 47 (48.5) | 1.000 |
| > 50 (n = 257)                  | 149 (57.6) | 109 (42.4) | 0.080 | 191 (74.3) | 66 (25.7) |        | 166 (66.6) | 91 (34.5) | 1.000 | 133 (51.8) | 124 (48.2) | 1.000 |
| Gender                          |        |        |         |        |        |         |        |        |         |        |        |         |
| Male (n = 271)                  | 158 (58.3) | 113 (41.7) |       | 198 (73.1) | 73 (26.9) | 0.800 | 176 (64.9) | 95 (35.1) | 0.896 | 139 (51.3) | 122 (48.7) | 1.000 |
| Female (n = 83)                 | 30 (60.2) | 33 (39.8) | 0.080 | 61 (73.5) | 22 (26.5) | 0.000 | 53 (63.9) | 30 (36.1) | 0.000 | 46 (53.0) | 39 (47.0) | 0.000 |
| Clinical stages                 |        |        |         |        |        |         |        |        |         |        |        |         |
| Stage I-II (n = 151)            | 78 (51.7) | 73 (48.3) | 0.022 | 101 (66.9) | 50 (33.1) |        | 90 (59.6) | 61 (40.4) | 0.000 | 67 (44.4) | 84 (55.6) | 0.018 |
| Stage III (n = 203)             | 130 (64.0) | 73 (36.0) | 0.022* | 158 (77.8) | 45 (22.2) |        | 139 (68.5) | 64 (31.5) | 0.000 | 116 (57.1) | 87 (42.9) | 0.018* |
| LN status                       |        |        |         |        |        |         |        |        |         |        |        |         |
| LNM (n = 213)                   | 133 (62.4) | 80 (37.6) |       | 166 (77.9) | 47 (22.1) | 0.000* | 140 (65.7) | 73 (34.3) | 0.000 | 118 (55.4) | 95 (44.6) | 0.000 |
| No LNM (n = 141)                | 75 (53.2) | 66 (46.8) | 0.000* | 93 (66.0) | 48 (34.0) | 0.014* | 89 (63.1) | 52 (36.9) | 0.000 | 65 (46.1) | 76 (53.9) | 0.000 |
| Histological type               |        |        |         |        |        |         |        |        |         |        |        |         |
| SCC (n = 161)                   | 94 (58.4) | 67 (41.6) |       | 105 (65.2) | 56 (34.8) | 0.000* | 90 (55.9) | 71 (44.1) | 0.000 | 76 (47.2) | 85 (52.8) | 0.000 |
| ADC (n = 193)                   | 114 (59.1) | 79 (40.9) | 0.914 | 154 (79.8) | 39 (20.2) | 0.000* | 139 (72.0) | 54 (28.0) | 0.000 | 107 (55.4) | 86 (44.6) | 0.000 |
| Pathological grade              |        |        |         |        |        |         |        |        |         |        |        |         |
| Well (n = 6)                    | 2 (33.3) | 4 (66.7) |       | 2 (33.3) | 4 (66.7) | 0.000* | 6 (100.0) | 0 (0.0) | 0.000 | 1 (16.7) | 5 (83.3) | 0.000 |
| Moderate (n = 146)              | 84 (57.5) | 62 (42.5) |       | 105 (71.9) | 41 (28.1) | 0.000* | 94 (64.4) | 52 (35.6) | 0.000 | 71 (48.6) | 75 (51.4) | 0.000 |
| Poor (n = 202)                  | 122 (60.4) | 80 (39.6) | 0.384 | 152 (75.2) | 50 (24.8) | 0.000* | 122 (63.9) | 57 (36.1) | 0.000 | 111 (55.0) | 91 (45.0) | 0.000 |
| Survival status                 |        |        |         |        |        |         |        |        |         |        |        |         |
| Alive (n = 194)                 | 99 (51.0) | 95 (49.0) |       | 131 (67.5) | 63 (32.5) | 0.000* | 110 (56.7) | 84 (43.3) | 0.000 | 84 (43.3) | 110 (56.7) | 0.000 |
| Dead (n = 160)                  | 109 (68.1) | 51 (31.9) | 0.000* | 128 (80.0) | 32 (20.0) | 0.000* | 119 (74.4) | 41 (25.6) | 0.000* | 99 (61.9) | 61 (47.9) | 0.000* |

*Chi-square test, statistically significant difference (P < 0.05). Abbreviations: LNM, lymph node metastasis; SCC, squamous cell carcinoma; HSP10/HSP60#, common high expression of HSP10 and HSP60; P⁺, other combination of these two proteins; HSP10/HSP60/Mcl-1 P⁺, common high expression of these three proteins; P⁻, other combination of expression of these three proteins.
3.3. Correlation between HSP10, HSP60 and Mcl-1 in NSCLC

We analyzed the correlation between HSP10, HSP60 and Mcl-1 expression in NSCLC. As data shown in Table 2, the overexpression of HSP10 was positively correlated with expression of HSP60 and Mcl-1 in NSCLC. Moreover, we used Kaplan-Meier analysis to estimate the association between expression of the three proteins and patients’ survival rate with lung SCC and ADC alone or in different combination. As shown in Fig. 3, the survival rates of lung SCC patients with low HSP10 expression (P = 0.017, Fig. 3A), HSP60 (P = 0.012, Fig. 3B) or Mcl-1 (P = 0.025, Fig. 3C) were higher than those with high expression. Lung ADC patients with low expression of HSP10 (P = 0.007, Fig. 3D) and Mcl-1 (P = 0.026, Fig. 3F) had longer survival time than those with high expression of these two proteins, there was no significant difference in survival regardless of HSP60 expression level in ADC patients (P = 0.233, Fig. 3E). In combined condition, lung SCC and ADC patients with high HSP10 and HSP60 owned a lower survival rate than that of others (P = 0.003, Fig. 4A; P = 0.012, Fig. 4C). Similarly, both lung SCC and ADC patients who had combined high expression of HSP10, HSP60 and Mcl-1 had obviously lower OS rates than patients with any other patterns (P = 0.001, Fig. 4B; P = 0.003, Fig. 4D).

Furthermore, we analyzed the correlation between survival rate and clinicopathological features. As the data shown in Tables 3 and 4, for lung SCC and ADC patients, the survival rate of patients in clinical stage III was lower than that of patients in clinical stage I ∼ II (all P < 0.001), and patients without LNM had a longer survival time than that of patients with LNM (P = 0.005; P = 0.002). Similar trend also reflected in pathological grade, lung SCC patients with higher pathological grade owned a lower the survival rate (P = 0.004).

We further investigated whether the high HSP10, HSP60 and/or Mcl-1 expression could act as independent prognostic marker for NSCLC patients via Cox proportional risk regression analysis. Table 3 showed that pathological grade (P = 0.013) and clinical stage (P = 0.049) might be independent prognostic factors for lung SCC patients. Table 4 showed that expression of HSP10 (P = 0.022) and Mcl-1 (P = 0.018) and LNM (P = 0.044) were independent prognostic factors for patients with lung ADC.

### 3.4. Status of expression of HSP10, HSP60 and Mcl-1 on patients’ prognosis

Table 2

|       | HSP10 | HSP60 | Mcl-1 |
|-------|-------|-------|-------|
| HSP10 |       |       |       |
| Spearman’s correlation coefficient | 1     | 0.399 | 0.137 |
| Sig. (2-tailed) |   | 0.000* |     |
| HSP60 | 0.399 | 1     | 0.219 |
| Spearman’s correlation coefficient |       |    |     |
| Sig. (2-tailed) | 0.000* |     |
| Mcl-1 | 0.137 | 0.219 | 1     |
| Spearman’s correlation coefficient |       |     |     |
| Sig. (2-tailed) | 0.010* | 0.000* |

*Spearman’s rank correlation test, statistically significant difference (P < 0.05).
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Fig. 3. Kaplan-Meier curves for overall survival of lung SCC and ADC patients with expression of HSP10, HSP60 and Mcl-1. (A) Lung SCC patients with high expression of HSP10 showed worse overall survival rates compared to patients with low HSP10 expression ($P = 0.017$, two sided). (B) Lung SCC patients with high HSP60 expression showed worse overall survival rates compared to patients with low HSP60 expression ($P = 0.012$, two sided). (C) Lung SCC patients with high Mcl-1 expression had worse overall survival rates than patients with low one ($P = 0.025$, two sided). (D) Lung ADC patients with high expression of HSP10 had worse overall survival rates than that with low one ($P = 0.007$, two sided). (E) High expression of HSP60 had not significantly correlation with overall survival rates of lung ADC patients ($P > 0.05$, two sided). (F) Lung ADC patients with low Mcl-1 expression survived longer than those with high one ($P = 0.026$, two sided).

Fig. 4. Kaplan-Meier curves for overall survival of lung SCC and ADC patients with combined expression of HSP10/HSP60 and HSP10/HSP60/Mcl-1. (A) Kaplan-Meier curves showed lung SCC patients with combined high expression of HSP60 and HSP10 had worse overall survival rates than these with others ($P = 0.003$, two sided). (B) Lung SCC patients with high HSP10, HSP60 and Mcl-1 expression owned poor prognosis compared with that with other expression patterns of these three proteins ($P = 0.001$, two sided). (C) Lung ADC patients with common high expression of HSP60 and HSP10 proteins showed worse overall survival rates compared with others ($P = 0.012$, two sided). (D) Patients with combined high expression of HSP10, HSP60 and Mcl-1 all have higher survival rates than others ($P = 0.003$, two sided).
### Table 3
Summary of univariate/multivariate analysis for overall survival in patients with lung SCC (n = 161)

| Variables | Univariate analysis | Multivariate analysis |
|-----------|---------------------|-----------------------|
|           | Average survival time (SE) | 95% CI | P-value | Exp (B) | 95.0% CI | P-value |
| HSP10     | High expression       | 48.766 (4.511)       | 39.925–57.607 | 0.017*  | 1.463 | 0.840–2.550 | 0.179  |
|           | Low expression        | 77.114 (8.269)       | 60.905–93.322 |          |       |         |       |
| HSP60     | High expression       | 51.516 (4.992)       | 41.733–61.300 | 0.012*  | 1.609 | 0.873–2.976 | 0.128  |
|           | Low expression        | 80.509 (8.967)       | 62.934–98.087 |          |       |         |       |
| Mcl-1     | High expression       | 56.467 (6.553)       | 43.623–69.310 | 0.025*  | 1.333 | 0.784–2.256 | 0.290  |
|           | Low expression        | 69.234 (6.155)       | 57.197–81.306 |          |       |         |       |
| Clinical stages |               |               |               |       |       |         |       |
|           | Stage I–II           | 77.678 (5.901)       | 66.111–89.244 | 0.000*  | 1.895 | 1.004–3.578 | 0.049* |
|           | Stage III            | 53.732 (5.906)       | 42.157–65.307 |          |       |         |       |
| LN status | LNM                  | 46.512 (4.476)       | 37.739–55.258 | 0.005*  | 1.709 | 0.958–3.051 | 0.070  |
|           | No LNM               | 80.439 (8.389)       | 63.996–96.881 |          |       |         |       |
| Pathological grade |           |               |               |       |       |         |       |
|           | Well/moderated       | 68.856 (5.510)       | 58.763–78.950 | 0.004*  | 1.970 | 1.152–3.372 | 0.013* |
|           | Poor                 | 56.314 (6.201)       | 44.160–68.469 |          |       |         |       |
| Age       | ≤ 50                 | 51.443 (5.738)       | 40.196–62.690 | 0.776   | 1.005 | 0.575–1.756 | 0.986  |
|           | > 50                 | 65.619 (6.273)       | 53.324–77.913 |          |       |         |       |
| Gender    | Female               | 64.358 (6.912)       | 50.836–77.159 | 0.063   | 0.318 | 0.075–1.342 | 0.119  |
|           | Male                 | 62.314 (5.533)       | 51.470–73.159 |          |       |         |       |

Abbreviations: CI, confidence interval; Exp(B), odds ratio; SE, standard error; LNM, lymph node metastasis; SCC, squamous cell carcinoma. * P < 0.05.

### Table 4
Summary of univariate/multivariate analysis for overall survival in patients with lung ADC (n = 193)

| Variables | Univariate analysis | Multivariate analysis |
|-----------|---------------------|-----------------------|
|           | Average survival time (SE) | 95% CI | P-value | Exp (B) | 95.0% CI | P-value |
| HSP10     | High expression       | 44.716 (4.333)       | 36.224–53.208 | 0.007*  | 1.763 | 1.086–2.862 | 0.022* |
|           | Low expression        | 59.130 (4.821)       | 49.680–68.580 |          |       |         |       |
| HSP60     | High expression       | 49.987 (3.847)       | 42.447–57.526 | 0.233   | 0.795 | 0.433–1.457 | 0.457  |
|           | Low expression        | 57.787 (6.878)       | 44.306–71.268 |          |       |         |       |
| Mcl-1     | High expression       | 45.140 (3.484)       | 38.311–51.970 | 0.026*  | 1.845 | 1.109–2.256 | 0.018* |
|           | Low expression        | 67.201 (6.419)       | 54.619–79.782 |          |       |         |       |
| Clinical stages |           |               |               |       |       |         |       |
|           | Stage I–II           | 59.815 (4.579)       | 50.839–68.790 | 0.000*  | 1.547 | 0.933–2.567 | 0.091  |
|           | Stage III            | 43.371 (4.264)       | 35.013–51.728 |          |       |         |       |
| LN status | LNM                  | 45.793 (4.258)       | 37.447–54.139 | 0.002*  | 1.741 | 1.016–2.984 | 0.044* |
|           | No LNM               | 59.878 (8.331)       | 50.408–69.348 |          |       |         |       |
| Pathological grade |           |               |               |       |       |         |       |
|           | Well/moderated       | 53.915 (4.572)       | 44.954–62.876 | 0.113   | 1.199 | 0.804–1.790 | 0.374  |
|           | Poor                 | 48.897 (4.585)       | 39.910–57.883 |          |       |         |       |
| Age       | ≤ 50                 | 41.866 (4.407)       | 33.229–50.502 | 0.510   | 1.073 | 0.674–1.709 | 0.766  |
|           | > 50                 | 53.961 (4.113)       | 45.900–62.021 |          |       |         |       |
| Gender    | Female               | 52.183 (4.935)       | 42.510–61.855 | 0.457   | 0.864 | 0.555–1.344 | 0.516  |
|           | Male                 | 50.635 (4.274)       | 42.257–59.012 |          |       |         |       |

Abbreviations: CI, confidence interval; Exp(B), odds ratio; SE, standard error; LNM, lymph node metastasis; ADC, adenocarcinoma. * P < 0.05.
4. Discussion

HSP10 is a co-chaperone protein of HSP60 in mitochondria where it is involved in folding and transporting proteins into mitochondria, and maintaining the stability of mitochondrial proteins [6]. In addition, HSP10 plays a crucial role in cancer progression and development [26], as indicated by its increased expression in many kinds of malignant tumors, including astrocytoma, colorectal cancer, and liver cancer [7,27,28]. It has suggested that HSP10 might be an independent prognostic factor for poor prognosis of nasopharyngeal carcinoma and invasive ductal breast carcinoma [29,30]. In this study, we found higher HSP10, HSP60 and Mcl-1 expression in NSCLC compared with Non-CLT. Patients in clinical stage III had higher HSP10 and/or HSP60 than that in stage I ~ II. On protein level, there existed a positive correlation between HSP10, HSP60 and Mcl-1. Moreover, OS time in NSCLC patients was remarkably shorter in cases with elevated expression of HSP10, HSP60 and Mcl-1 alone and in combination. High HSP10 and Mcl-1 could be poor independent prognostic factors for lung ADC patients.

Our results indicated that the expression of HSP10 was significantly increased in NSCLC, which is consistent with the previous discoveries. In addition, increased expression of HSP10 in patients with advanced clinical stage provides further evidence for its role in tumorigenesis and progression of NSCLC. The synergistic effects of HSP10 and HSP60 may occur not only in maintaining the stability of mitochondrial protein, but also in the process of carcinogenesis. Indeed, we found that both HSP10 and HSP60 were highly expressed in NSCLC compared with Non-CLT, and the percentage of patients with the combined high expression of HSP10 and HSP60 in clinical stage III was significantly higher than that in clinical stage I ~ II. Patients with high co-expression of HSP10 and HSP60 also suffered a lower OS rate than those with other phenotypes of these two proteins. Since HSP10 and HSP60 are localized head to head and separated by a bidirectional promoter on chromosome 2 [31], the region between these two genes might act as a two-way promoter whose transcriptional activity could be triggered by the same stimulus. This could explain why there is a positive correlation between HSP10 and HSP60 expression. Moreover, co-expression of HSP10 and HSP60 might have a synergistic effect against apoptosis in NSCLC cells. As reported, combined expression of HSP10 and HSP60 can protect cardiac myocytes from apoptosis coaxed by simulated ischemia and reoxygenation [32]. Mutation in the HSP10 mobile loop region impairs its ability to bind with HSP60 and therefore abolish the protective effect of HSP10 on cardiomyocytes [33]. In addition, HSP10, HSP60 and pro-caspase 3 can form a complex, while highly expressed HSP10 and HSP60 can inhibit the dissociation of pro-caspase 3 thus antagonize stress or other factors induced apoptosis [34].

Our data showed that both HSP10 and HSP60 expression were positively correlated with Mcl-1 expression. Patients with combined overexpression of HSP10, HSP60 and Mcl-1 owned lower OS rate than those with other phenotypes. All these results strongly indicate that HSP10 and HSP60 might be involved in the anti-apoptosis pathway of Mcl-1 protein. A plausible explanation is that the expression of HSP10, HSP60 and Mcl-1 could be regulated by same transcription regulator, such as signal transducer and activator of transcription 3 (STAT3), which expresses highly in NSCLC and plays an inhibitory role in the process of tumor cell apoptosis [35,36]. Previous studies found that the inactivation of STAT3 can downregulate the transcriptional level of MCL-1 gene [36,37]. Since the bidirectional promoter between HSP10 and HSP60 contains STAT3-responsive element, STAT3 can also associate with this binding site to induce the up-regulation of HSP10 and HSP60 levels [38]. Another likely explanation is that both HSP60 and Mcl-1 are involved in the same signal pathway. On one hand, as a highly unstable protein, Mcl-1 is very sensitive to activity of mechanistic target of rapamycin complex 1 (mTORC1)-dependent translation [39,40]. On the other hand, HSP60 gene knockdown can activate AMPK which inhibit mTORC1 and its downstream targets, thereby reducing protein synthesis [9,41]. As mentioned above, it indicates that HSP60 might affect Mcl-1 expression level through mTORC1 pathway. The regulatory network of Mcl-1 is very complicated and the underlying mechanisms of relationship between HSP10, HSP60 and Mcl-1 still need further study in the future.

In summary, the expression of HSP10, HSP60 and Mcl-1 in NSCLC patients increased significantly. HSP10 and HSP60 may play a synergistic role in the development of NSCLC and participate in the anti-apoptosis pathway of mitochondria in NSCLC. HSP10 and HSP60 might be regarded as novel biomarkers of poor prognosis for NSCLC patients.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Supplementary data

Supplementary Table 1
Clinicopathological features of patients with NSCLC and non-cancerous control lung tissues

| Patients characteristics | No. of patients (%) |
|--------------------------|---------------------|
| NSCLC                    |                     |
| Age (years)              |                     |
| ≤ 50                     | 97 (27.4)           |
| > 50                     | 257 (72.6)          |
| Gender                   |                     |
| Male                     | 271 (76.6)          |
| Female                   | 83 (23.4)           |
| Clinical stages          |                     |
| Stage I                  | 76 (21.5)           |
| Stage II                 | 75 (21.2)           |
| Stage III                | 203 (57.3)          |
| Lymph node status        |                     |
| N0                       | 141 (39.8)          |
| N1/N2/N3                 | 213 (60.2)          |
| Histological type        |                     |
| SCC                      | 161 (45.5)          |
| ADC                      | 193 (54.5)          |
| Pathological grade       |                     |
| Well                     | 6 (1.7)             |
| Moderate                 | 146 (41.2)          |
| Poor                     | 202 (57.1)          |
| Non-cancerous control lung tissues |   |
| Age (years)              |                     |
| ≤ 50                     | 22 (41.5)           |
| > 50                     | 31 (58.5)           |
| Gender                   |                     |
| Male                     | 27 (50.9)           |
| Female                   | 26 (49.1)           |