Hsp90α Is Suitable for Therapy Monitoring in Multiple Cancers

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Abstract

Background: Heat shock protein 90α (Hsp90α) is associated with the occurrence and development of cancer. Previous studies have shown that plasma Hsp90α protein has high sensitivity and specificity in the diagnosis of liver cancer and lung cancer. Although the study of the Hsp90α protein in cancer screening is more common, the application of the Hsp90α protein in diagnosis and monitoring of cancer is rare.

Methods: In this study, 370 cancer patients were enrolled from Nanyang Second People's Hospital (Henan, China). The pre- and post-treatment protein expression of Hsp90α were inspected and the data were analyzed.

Results: In our research, Hsp90α expression was lower in the T1 phase of lung cancer, esophageal cancer, and gastric cancer patients with prior treatment and higher in breast cancer patients. Apart from colorectal cancer, Hsp90α expression of different cancer types in the T4 phase was highest compared to T1 to T3 phase, and the expression Hsp90α protein increased with cancer progression. These results substantiated that Hsp90α protein expression was consistent with cancer progression overall, although the expression difference between tumor stages was not obvious. Hsp90α was detected in 54 lung cancer patients pre- and post-treatment, and the coincidence rate between the progression of disease and expression change in the Hsp90α protein was 79.63%. For further verification, some patients of them underwent long-term monitoring, and the coincidence rate was 75%.

Conclusions: Hsp90α was a potential biomarker for therapeutic monitoring of cancer prognosis, including lung cancer, esophageal cancer, gastric cancer and breast cancer.

Introduction

Heat shock proteins (HSPs) cover a large family of proteins, and the major groups are classified as heat shock protein 27 (HSP27), heat shock protein 40 (HSP40), heat shock protein 60 (HSP60), heat shock protein 70 (HSP70), heat shock protein 90 (HSP90), and large HSPs based on their molecular weights.[1] HSPs play a significant role in cellular proliferation, differentiation, and carcinogenesis, and high expression of these proteins is reported in breast, prostate, colorectal, lung, ovarian, gastric, oral and esophageal cancers.[2, 3] In humans, heat shock protein 90α (Hsp90α) is encoded by the *HSP90AA1* gene, which is located on chromosome 14q32.33.[4–6] Hsp90α is a highly conserved and abundant protein, constituting approximately 1% of the total intracellular protein.[7, 8] In the cytoplasm, Hsp90α has over 200 interacting proteins, and many of these proteins correlate with signaling pathways of tumorigenesis.[9–11]

Numerous studies have verified that Hsp90α has differences in expression between cancer patients and healthy people. Based on its expression, there are many potential clinical applications of Hsp90α as an effective tumor biomarker.[10, 12] Han et al. found that plasma Hsp90α protein levels correlated well with squamous cell associated antigen (SCC-Ag) levels in cervical cancer patients and that the combination of

Hsp90α and SCC-Ag could be a useful diagnostic biomarker.[13] In a study on a total of 409 hepatocellular carcinoma (HCC) patients, the combination of Hsp90α, alpha-fetoprotein (AFP) and thymidine kinase 1 (TK1) improved the diagnostic sensitivity (89.24%) of HCC.[14] Hsp90α conferred an advantage in the diagnosis of early colorectal cancer (CRC).[15] In a study on a total of 105 esophageal squamous cell carcinoma (ESCC) patients, the expression of Hsp90α and cyclin B1 protein was associated with tumor malignancy and prognosis of ESCC patients.[16]

In China, research by Chen et al. showed that extracellular Hsp90α (eHsp90α) further promoted cellular epithelial-mesenchymal transition, migration, and invasion in PDE cells.[17] A large-scale (1647 participants) clinical trial by Luo et al., receiver operating characteristic (ROC) curve analysis, showed that plasma Hsp90α can discriminate liver cancer with a sensitivity of 92.7% and specificity of 91.3% from non-liver cancer controls, and Hsp90α can be used as a biomarker for the diagnosis of liver cancer and can be used to evaluate the therapeutic efficacy of liver cancer patients undergoing surgery or interventional therapy.[18–20] Increased Hsp90β in malignant pleural effusion (MPE) was correlated with the malignant biological behavior of lung cancer patients.[21] Therefore, the Hsp90α protein has been studied in many cancer screening studies and therapeutic efficacy evaluations.

Although the Hsp90α protein has been reported to have good screening effect and clinical application value, the actual effect still needs to be confirmed, especially between multiple cancers. In previous studies, research involving drug therapy evaluation of Hsp90α was also limited; therefore, in this study, these queries were explored. A total of 370 cancer patients from Nanyang Second People's Hospital were enrolled to inspect the pre- and post-treatment protein expression of Hsp90α, and the data were analyzed to confirm the clinical application value of the Hsp90α protein.

Methods

Patients

A total of 370 cancer patients, including 124 lung cancer patients, 22 liver cancer patients, 58 breast cancer patients, 63 esophageal cancer patients, 74 gastric cancer patients and 29 colorectal cancer patients, including 236 males and 134 females, at Nanyang Second People's Hospital (Henan, China) from January 2018 to June 2020 were enrolled (Table 1). All the participating patients signed a consent form to participate in this research. Permission to use peripheral blood was obtained from Nanyang Second People's Hospital Ethics Committees. In the fasting state, 1 mL of peripheral blood was collected in an EDTA-K_2 anticoagulant tube, which was gently reversed 3 times and centrifuged for 10 min at 3000 r/min. Then, the supernatant plasma was divided into a 1.5 mL centrifuge tube to detect the Hsp90α protein.

Table 1
Participant information (prior to treatment)

	Male (n)	Female (n)	All (n)	Age (mean ± SD)	T1, Hsp90α (ng/ml)	T2, Hsp90α (ng/ml)	T3, Hsp90α (ng/ml)	T4, Hsp90α (ng/ml)
Lung cancer	97	27	124	63.85 ± 10.32	70.44 ± 21.05	71.86 ± 42.90	100.70 ± 71.03	121.21 ± 135.04
Liver cancer	14	8	22	56.45 ± 11.34		79.13 ± 59.82	189.22 ± 155.33	228.67 ± 167.87
Breast cancer	0	58	58	54.65 ± 11.32	100.11 ± 73.73	83.10 ± 87.50	159.64 ± 188.23	294.63 ± 250.82
Esophageal cancer	44	19	63	67.90 ± 9.50	59.36 ± 29.73	63.61 ± 31.33	86.23 ± 50.59	88.61 ± 46.41
Gastric cancer	63	11	74	64.48 ± 10.18	61.85 ± 47.30	67.06 ± 38.35	92.05 ± 67.72	108.89 ± 55.12
Colorectal cancer	18	11	29	61.55 ± 13.04		57.97 ± 25.85	135.29 ± 128.07	80.22 ± 17.76

Hsp90α protein detection

For the detection of Hsp90α protein, a Hsp90α protein quantitative detection kit (96T) purchased from Yantai Projee Biotechnology Development Co., Ltd was been used. The technique details were described in Supplementary Materials.

Statistical analysis.

Data were analyzed by GraphPad Prism 5 statistical software (GraphPad Software, Inc., San Diego, CA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Hsp90α had discrepant screening effectiveness in different cancers

Yongzhang Luo et al. found that Hsp90α had a high sensitivity and specificity for early liver cancer.[18] To confirm the clinical application value of Hsp90α protein, the Hsp90α expression of six types of cancer patients with prior treatment, including 124 lung cancer patients, 22 liver cancer patients, 58 breast cancer patients, 63 esophageal cancer patients, 74 gastric cancer patients, and 29 colorectal cancer patients, was researched (*Table 1*). By analyzing the mean expression of the Hsp90α protein in the T1, T2, T3 and T4 cancer phases of these six cancers, we found that the expression level in the T1 phase was lower in lung cancer, esophageal cancer, and gastric cancer and higher in the T4 phase in lung cancer, liver cancer, breast cancer, esophageal cancer and gastric cancer (*Figure 1 A-F* and *Table 1*).

Except for colorectal cancer, the expression of the Hsp90α protein increased following cancer progression. Meanwhile, Hsp90α expression in the T2 and T3 phases was obviously different ($p<0.05$) in lung cancer, liver cancer and esophageal cancer patients (*Figure 1A*, *1B* and *1D*), and there were no obvious differences between the T3 and T4 phases. For the reference range for the Hsp90α protein, normal values were 0-82.06 ng/mL (Hsp90α protein quantitative detection kit). According to this standard, the coincidence rates of Hsp90α expression on lung cancer, liver cancer, breast cancer, esophageal cancer, gastric cancer and colorectal cancer patients were 42.74% (53/124), 50% (11/22), 50% (29/58), 23.81% (15/63), 35.14% (26/74), and 37.93% (11/29), respectively, and esophageal cancer had the minimum coincidence rate.

As there were no T1 phase cancer patients in all six cancers, the mean expression of Hsp90α in the T2, T3 and T4 phases in these six cancers was analyzed, and there were no obvious differences in the T2 phase (*Figure2A*), while there were significant differences in the T3 phase between liver cancer, lung cancer, esophageal cancer and gastric cancer; esophageal cancer had the lowest Hsp90α expression (86.23 ± 50.59 ng/mL), while liver cancer had highest Hsp90α expression (228.67 ± 167.87 ng/mL) (*Figure2B* and *Table 1*). These results indicated that Hsp90α protein expression was mostly consistent with cancer progression, even though there were a few inconsistencies, such as in colorectal cancer. There were no obvious differences in the Hsp90α protein expression in the T2 and T4 phases, but in the T3 phase, the overall protein expression of Hsp90α was discrepant in multiple cancers (*Figure2A-C*).

Hsp90α was more suitable for cancer treatment monitoring

To explore the ability to use the Hsp90α protein to monitor the therapeutic effect, Hsp90α was detected before and after therapy, and retrospective data from 54 lung cancer patients (44 males and 10 females) were analyzed (*Figure3A* and *3B*). Hsp90α was detected in twenty-nine lung cancer patients prior to treatment, and Hsp90α detected after treatment was accompanied with disease remission; a total of 79.31% (23/29) of patients had reduced Hsp90α expression after treatment. To observe the dynamic changes in Hsp90α expression, the trend change is shown in *Figure3A*, the total of 29 patients, except patient 3, patient 11, patient 18, patient 20, patient 28 and patient 29, showed a downward trend in Hsp90α expression when the disease was in remission after therapy.

Similarly, Hsp90α was detected in twenty-five lung cancer patients before treatment, and Hsp90α that was detected after treatment was accompanied by disease progression; meanwhile, 80% (20/25) of patients had increased Hsp90α expression, and the overall coincidence rate was 79.63% (43/54). As shown in *Figure3B*, the total of 20 lung cancer patients, apart from patient 4, patient 15, patient 23, patient 24 and patient 25, had elevated Hsp90α expression. These results demonstrated that dynamic changes in Hsp90 expression were highly consistent with the diagnostic efficacy.

Long-term monitoring effect analysis on typical samples

Next, the long-term monitoring effect of Hsp90α protein was analyzed. In the retrospective analysis of two patients, the Hsp90α protein was detected 10 times during their therapy. Patient A was a 63-year-old

male with tumor stage T4 and had passed away. This patient originally had an Hsp90α protein level of 61.58 ng/mL, which was stable after treatment; eventually, the disease progressed until his death. The 10 Hsp90α protein tests in the whole process of progression reached 70% coincidence rate (7/10) (*Figure3C*). Patient B was a 58-year-old male with tumor stage T3 and had passed away. In patient B, the Hsp90α protein was 31.91 ng/mL initially, which was similar to that before treatment, and was stable after treatment; eventually, the disease progressed until his death. The coincidence rate in this patient was 80% (8/10) (*Figure3C*). Therefore, the retrospective study results also showed that the Hsp90α protein has an excellent ability to monitor the therapeutic effect and can be used in long-term monitoring during the course of cancer therapy.

Discussion

As a tumor-related protein, Hsp90α has attracted much attention and research in recent years.[9, 10] Plasma Hsp90α could distinguish liver cancer with a sensitivity of 92.7% and specificity of 91.3% from non-liver cancer controls.[18–20] In this study, 370 cancer patients, including 124 lung cancer patients, 22 liver cancer patients, 58 breast cancer patients, 63 esophageal cancer patients, 74 gastric cancer patients and 29 colorectal cancer patients, were enrolled (Table 1). Before treatment, Hsp90α expression in these patients was detected, and Hsp90α expression was not exactly consistent with cancer stage, although most of the expression levels were low in the T1 phase and high in the T4 phase (Fig. 1). There were no significant differences in the T1 or T4 phase; nevertheless, Hsp90α expression in the T2 and T3 stages had significant differences in lung cancer, liver cancer and esophageal cancer patients (Fig. 1A, 1B and 1D). These results suggested that Hsp90α protein was not effective at distinguishing T1, T2, T3 and T4 cancer stages.

The mean expression of Hsp90α in the T2, T3 and T4 phases was compared in these six cancers, and there were no significant differences in the T2 or T4 phase (Fig. 2A and 2C). The T3 phase had some significant differences (Fig. 2B), while esophageal cancer had the lowest Hsp90α expression in the T3 phase (86.23 ± 50.59 ng/mL). Overall, although Hsp90α protein expression was consistent with cancer progression, Hsp90α was not the best biomarker for distinguishing cancer stage. As the reference range for normal Hsp90α protein values is 0-82.06 ng/mL (Hsp90α protein quantitative detection kit), the pretreatment detection values of patients with lung cancer, liver cancer, breast cancer, esophageal cancer, gastric cancer and colorectal cancer were 42.74% (53/124), 50% (11/22), 50% (29/58), 23.81% (15/63), 35.14% (26/74), and 37.93% (11/29), respectively, which were lower than those in other reports.[18–20]

Study on our retrospective monitoring data, after 54 lung cancer patients (44 males and 10 females) were analyzed pre- and post-treatment Hsp90α expression changes, 29 lung cancer patients with remission after treatment had a 79.31% (23/29) coincidence rate on Hsp90α expression, and 25 patients with disease progression after treatment had an 80% (20/25) coincidence rate on Hsp90α expression, which showed that Hsp90α protein had very good ability to monitor the treatment effect (Fig. 3A and 3B). Analysis data from two patients who had been monitored 10 times also confirmed the outstanding

performance of the ability to use Hsp90α to monitor the therapeutic effect (Fig. 3C). From our research, Hsp90α protein revealed limited screening effect and an excellent monitoring effect.

The main reason for this maybe that, the Hsp90α protein expression in different patients with the same cancer or different cancers was quite different, as a result, there not good enough to distinguish the cancer stages by Hsp90α protein expression differences. However, using the Hsp90α protein to monitor the therapeutic effect was based on one patient, and his own Hsp90α protein expression was compared before and after treatment; therefore, intergroup error could be reduced to obtain more accurate analysis results. In conclusion, Hsp90α expression can be used to monitor the therapeutic effect of cancer.

Declarations

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Authors' contributions: (I) Conception and design: M Fu, F Du, C Xu, X Wang and XY Zhao; (II) Administrative support: M Fu and C Xu; (III) Provision of study materials: M Fu, F Du and Z Wei; (IV) Collection and assembly of data: Z Wei, X Wang; (V) Data analysis and interpretation: M Fu, F Du, C Xu, X Wang and XY Zhao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Ethics approval and consent to participate: All experimental protocols in this study was approved by the ethics committee of Nanyang Second People's Hospital. All patients provided written informed consent for this study. All methods in this study carried out in accordance with the ***Declaration of Helsinki***.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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Figures

Figure 1

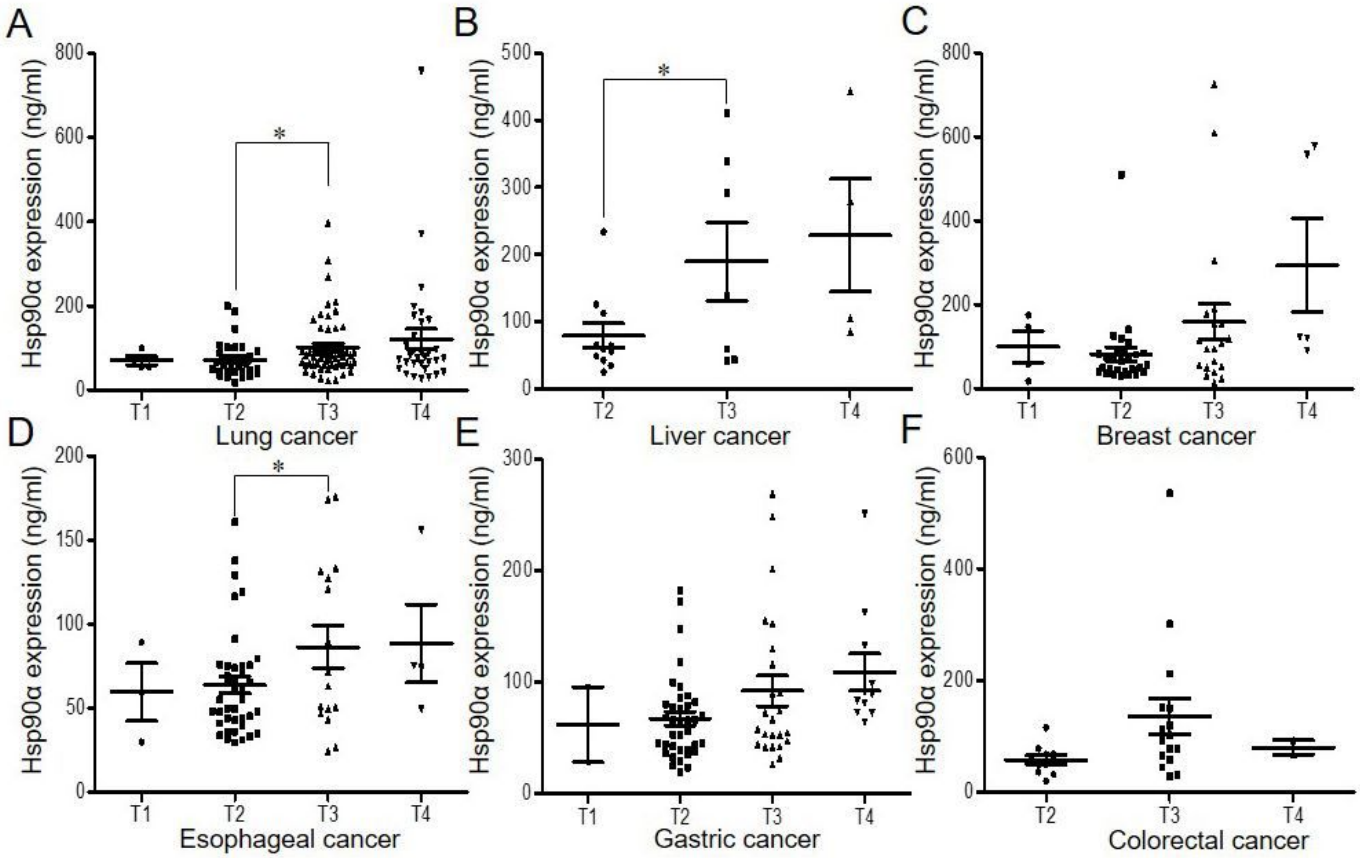

Figure 1

The mean expression of Hsp90α in six cancers. (A) The mean expression of Hsp90α in the T1, T2, T3 and T4 phases of lung cancer (n=124). (B) The mean expression of Hsp90α in the T2, T3 and T4 phases of liver cancer (n=22). (C) The mean expression of Hsp90α in the T1, T2, T3 and T4 phases of breast cancer (n=58). (D) The mean expression of Hsp90α in the T1, T2, T3 and T4 phases of esophageal cancer (n=63). (E) The mean expression of Hsp90α in the T1, T2, T3 and T4 phases of gastric cancer (n=74). (F) The mean expression of Hsp90α in the T2, T3 and T4 phases of colorectal cancer (n=29).

Figure 2

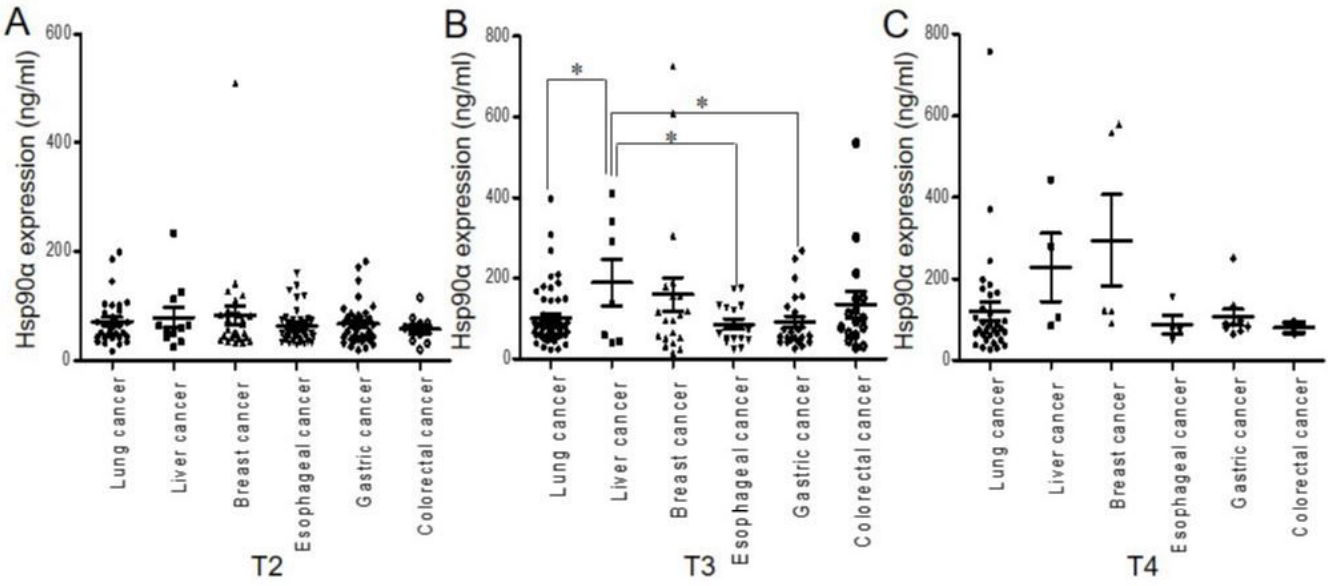

Figure 2

The mean expression of Hsp90α in the T2, T3 and T4 phases. (A) The mean expression of Hsp90α in the T2 phase of six cancers. (B) The mean expression of Hsp90α in the T3 phase of six cancers. (C) The mean expression of Hsp90α in the T4 phase of six cancers.

Figure 3

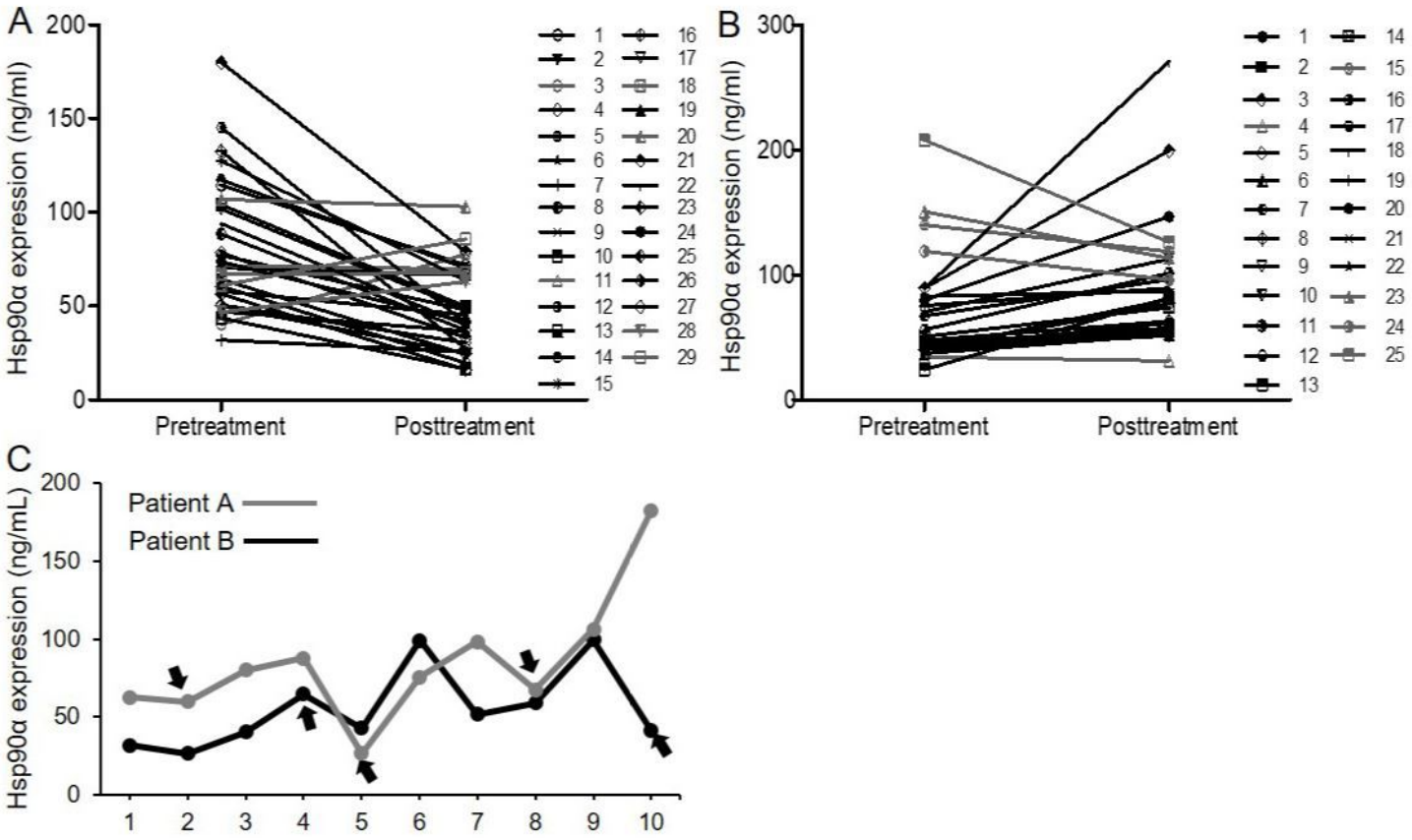

Figure 3

The mean expression of Hsp90α for efficacy monitoring. (A) The mean pre- and posttreatment expression of Hsp90α in 29 lung cancer patients for remission detection. (B) The mean pre- and posttreatment expression of Hsp90α in 25 lung cancer patients for progression detection. (C) Ten test results of Hsp90α expression in patient A and patient B. The black arrows indicate incidences in which the test results of Hsp90α did not match the clinical diagnosis.

Supplementary Files

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