Heat-shock protein 90α is a potential prognostic and predictive biomarker in hepatocellular carcinoma: a large-scale and multicenter study

Ke Su1 · Yanlin Liu1 · Pan Wang2 · Kun He3 · Fei Wang4 · Hao Chi5 · Mingyue Rao1 · Xueting Li6 · Lianbin Wen7 · Yaqiong Song8 · Jianwen Zhang1 · Tao Gu1 · Ke Xu1 · Qi Li1 · Jiali Chen1 · Zhenying Wu1 · Han Li1 · Weihong Huang5 · Lan Chen9 · Jian Tong10 · Hongyan Li11 · Xunjie Feng5 · Siyu Chen5 · Binbin Yang5 · Hongping Jin5 · Yue Yang5 · Hanlin Liu5 · Chao Yang5 · Ming Wu5 · Fangyu Xiong12 · Keyi Peng5 · Lechuan Zhu5 · Yaoyang Xu5 · Xue Tang5 · Zunyuan Tan5 · Xiaotong Luo5 · Hanyue Zheng5 · Yuxin Zhang5 · Lu Guo13 · Yunwei Han1,14,15

Received: 8 May 2022 / Accepted: 9 July 2022 / Published online: 16 August 2022
© The Author(s) 2022

Abstract

Background Although the diagnostic value of plasma heat-shock protein 90α (HSP90α) in hepatocellular carcinoma (HCC) has been previously reported, the causal effect of the plasma HSP90α levels on HCC prognosis remains largely unclear. To this extent, we sought to assess whether the plasma HSP90α acts as a prognostic factor for HCC patients.

Methods A total of 2150 HCC patients were included in this retrospective study between August 2016 and July 2021. Plasma HSP90α levels were tested within a week before treatment and their association with prognosis was assessed.

Results An optimal cutoff value of 143.5 for the HSP90α based on the overall survival (OS) was determined using the X-tile software. HCC patients with HSP90α < 143.5 ng/mL (low HSP90α) before and after propensity score matching (PSM) indicated longer median OS (mOS) relative to those with HSP90α ≥ 143.5 ng/mL (high HSP90α) (37.0 vs. 9.0 months, \( p < 0.001 \); 19.2 vs. 9.6 months, \( p < 0.001 \); respectively). In addition, the high HSP90α plasma level is an independent poor prognostic factor for OS in HCC patients. In our subgroup analysis, including the supportive care group, surgery group, transarterial chemoembolization (TACE) group, adjuvant TACE group, an immune checkpoint inhibitor (ICI) plus targeted therapy group, and TACE plus ICI group, the high HSP90α group demonstrated better OS compared to the low HSP90α group. Moreover, in the supportive care, TACE, ICI plus targeted therapy, TACE plus ICI groups, and high HSP90α levels were also an independent poor prognostic factors for OS.

Conclusions Our study confirmed that the plasma HSP90α level can be used as a prognostic biomarker for HCC.

Keywords Heat-shock protein 90α · Hepatocellular carcinoma · Biomarker · Overall survival · Primary liver cancer · Transarterial chemoembolization · Immune checkpoint inhibitor · Targeted therapy · Prognostic factor · Predictive biomarker

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and a common cause of cancer death [1]. The median overall survival (mOS) of HCC patients without effective treatment is only 4 months [2]. Nowadays, a plethora of therapeutic approaches has been investigated in HCC. Recently, the combination of the anti-program death ligand 1 (PD-L1) antibody, atezolizumab plus the anti-VEGF bevacizumab was approved by the Food and Drug Administration (FDA) and recommended by the National Comprehensive Cancer Network (NCCN)
guidelines as the first-line treatment for advanced HCC, which could extend OS to 19.2 months [3, 4]. Yusheng et al. reported that the median progression-free survival (mPFS) of advanced HCC patients receiving transarterial chemoembolization (TACE) plus camrelizumab was 9 months [5]. In addition, in a randomized controlled study of operable HCC, patients who received adjuvant TACE had a higher three-year OS rate compared to patients who underwent surgery alone (85.2% vs. 77.4%; \( p = 0.04 \)) [6]. Although the survival of HCC has been greatly prolonged, predicting treatment efficacy and response remains a challenging bottleneck.

In clinical practice, alpha-fetoprotein (AFP) is the most commonly used diagnostic and prognostic marker for HCC [7]. However, its reduced sensitivity of 52.1–62.5% underlies numerous limitations [8, 9]. Furthermore, AFP-negative tumors account for up to 30–40% of pathologically diagnosed HCC patients, which significantly hinders the application of AFP in the diagnosis and prognosis of HCC [10–12]. Therefore, there is an urgent need to identify new prognostic and predictive biomarkers to improve the management of HCC patients.

Heat-shock protein 90 (HSP90) is a highly conserved molecular chaperone through species and evolution. Interestingly, HSP90 has been reported to be secreted by a variety of cancer cell types [13, 14]. Previous studies had demonstrated that the HSP90 expression was associated with tumor proliferation and metastasis [15–17]. HSP90α is a subtype of HSP90, which has become a remarkable focus of current research due to its role in the regulation of signal transduction [18]. In a large multicenter study with 1,647 enrollees for the diagnosis of HCC, HSP90α displayed 92.7% and 91.3% diagnostic sensitivity and specificity, respectively [19]. Nevertheless, despite these promising results, there is still a shortfall of clinical studies, with large sample sizes, to determine the relationship between HSP90α level and HCC prognosis. Therefore, we initiated this multicenter study to assess whether plasma HSP90α could be used as a prognostic factor in HCC patients.

**Materials and methods**

**Patients**

A total of 2150 HCC patients were initially enrolled at three Chinese tertiary hospitals between August 2016 and July 2021. The inclusion criteria were as follows: (a) pathologically or clinically diagnosed HCC; (b) no prior anti-tumor therapy; (c) presence of measurable lesions according to Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1); and (d) plasma HSP90α test completed within a week before treatment. Patients with other malignant tumors or incomplete clinical data were excluded. The

**Data collection**

We retrospectively reviewed and recorded clinical data through individual patients’ files. Demographic information included sex and age. HCC etiology factors of interest included alcohol, hepatitis B virus (HBV), hepatitis C virus (HCV), and nonalcoholic fatty liver disease (NAFLD). The patient’s liver function was evaluated using the Child–Pugh score and albumin–bilirubin (ALBI). Laboratory data included the HSP90α plasma levels, AFP, alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin, albumin, leukocyte and platelet count, and creatinine. Tumor burden was interpreted by radiologists by computed tomography (CT) and magnetic resonance imaging (MRI), which included the maximum tumor diameter, number of tumors, portal vein tumor thrombus (PVTT), lymph node metastasis, and extrahepatic metastasis. The Barcelona Clinic Liver Cancer (BCLC) staging system was used to determine the tumor stage. All HCC treatments, hypertension, and diabetes were documented based on the patient’s medical record. OS was defined as the time from the start of the first treatment until death or the last follow-up.

**Statistical analysis**

For statistical analysis, the Chi-square (\( \chi^2 \)) test and McNemar analysis were used to analyze categorical variables. Mann–Whitney U and Wilcoxon matched-pairs signed-rank tests were used to analyze continuous variables. An optimal cutoff value of the HSP90α levels based on OS was determined using X-tile software (Yale University, New Haven, CT). A time-dependent receiver operating characteristic (ROC) curve was used to assess the ability of HSP90α to predict efficacy. The relationship between the HSP90α level and baseline characteristics was assessed by using a univariate and multivariate logistic regression model. Propensity score matching (PSM) was performed to determine the high and low HSP90α level groups with a similar baseline. Subsequently, the mOS was estimated and compared using Kaplan–Meier statistics and log-rank test, respectively. After identifying Factors affecting OS \( (p < 0.05) \) via univariate Cox analysis, we introduced them into multivariate models to determine independent prognostic factors for OS. All statistical analyses were carried out in SPSS (version 26.0) and R 3.3.2 software. Two-sided \( p < 0.05 \) were considered statistically significant.

**Ethics Committee of The Affiliated Hospital of Southwest Medical University approved this study with the affiliated approval number KY2020254. Due to the retrospective nature of the study, informed consent was waived.**
Results

Patient characteristics

A total of 2150 HCC patients were included in our retrospective study. The median HSP90α plasma concentration was 100.4 ng/mL (IQR 56.5–203.5). The percentages of male, child A, AFP < 200 ng/mL, and multiple tumors were 80.3%, 72.8%, 55.1%, and 72.9%, respectively. Most of the patients were presented with BCLC stage C (52.9%) and ALBI grade 2 (60.9%). In addition, the percentages of patients with only supportive care were 21.3%. Table 1 summarizes the baseline characteristics of all enrolled patients.

HSP90α levels and overall survival before and after PSM

An optimal cutoff value of 143.5 ng/mL for the HSP90α based on OS was determined using X-tile software (Yale University, New Haven, CT). Patients were sub-grouped into high HSP90α (HSP90α ≥ 143.5 ng/mL) and low HSP90α (HSP90α < 143.5 ng/mL) groups. Before PSM, no significant differences were noted between the two groups in terms of the HBV and HCV infection status. However, in the high HSP90α group the patients had older median age, more aggressive baseline BCLC stage, reduced liver function, and elevated tumor burden (p < 0.05), compared with the low HSP90α group (Table 1). In this study, the median follow-up was 24.4 months in all patients, 23.7 months in the high HSP90α group, and 24.7 months in the low HSP90α group. The mOS of this HCC patient cohort was 15.3 (95% CI 19.4–24.4) months (Fig. 1A). Patients in the high HSP90α group showed shorter mOS than patients in the low HSP90α group (9.0 vs. 37.0 months, HR = 2.663 (95% CI 2.357–3.009), p < 0.001; Fig. 1B).

After PSM, no significant differences were noted between the two groups for any covariate (Supplementary Table 1). The mOS of this HCC patient cohort was 21.9 (95% CI 19.4–24.4) months (Fig. 1A). Patients in the high HSP90α group showed shorter mOS than patients in the low HSP90α group (9.0 vs. 37.0 months, HR = 2.663 (95% CI 2.357–3.009), p < 0.001; Fig. 1B).

Factors associated with the OS

By utilizing univariate and multivariate analyses, we confirmed the HSP90α ≥ 143.5 ng/mL (p < 0.001), AFP ≥ 400 ng/mL (p = 0.043), child B plus C (p = 0.013), ALP ≥ 125 U/L (p < 0.001), tumor number ≥ 2 (p = 0.010), no any anti-tumor tumors (p < 0.001), and more advanced BCLC staging (p = 0.006), and these were independent risk prognostic factors for OS (Table 2). After PSM, HSP90α ≥ 143.5 ng/mL remained a negative independent prognostic marker for OS (Supplementary Table 2). In addition, the time-dependent ROC curves based on the HSP90α level demonstrated that the area under the curve (AUC) values for predicting OS at 1, 2, and 3 years was 0.718, 0.685, and 0.691, respectively (Fig. 2).

Subgroup analysis of different treatment modalities

The patients were divided into different subgroups according to various treatments. The specific subgroups are as follows: supportive care group (n = 457), surgery group (n = 275), TACE group (n = 780), adjuvant TACE group (n = 107), immune checkpoint inhibitor (ICI) plus targeted therapy group (n = 93), and TACE plus ICI group (n = 74). Following this subgrouping, we were willing to elucidate the relationship between HSP90α levels and baseline characteristics in different subgroups, demonstrating that patients with high HSP90α plasma levels were significantly associated with worse tumor burden and more aggressive BCLC staging (Supplementary Tables 3–8).

More importantly, in all the six subgroups, patients within the low HSP90α groups consistently demonstrated improved OS compared to the high HSP90α groups (Fig. 3). In univariate and multivariate Cox regression analyses of supportive care, TACE, ICI plus targeted therapy, and TACE plus ICI groups, high HSP90α ≥ 143.5 ng/mL was an independent poor prognostic factor for OS (p = 0.006, p < 0.001, p = 0.047, p = 0.027, respectively) (Supplementary Tables 9, 11, 13, 14). Notably, in the surgery and adjuvant TACE group, the HSP90α plasma level was not a significant prognostic factor for OS (Supplementary Tables 10, 12).

Relationship between HSP90α level and baseline characteristics

Through logistic regression analyses, we confirmed that the age, Child–Pugh class, ALBI grade, AFP, ALP, platelet, ALT, leukocyte, tumor diameter, and PVTT were independent influencing factors for the HSP90α expression (Supplementary Table 15). Moreover, to evaluate the significance of the HSP90α levels in a clinical setting, we further explored the relationship between HSP90α levels and baseline characteristics. The results revealed that the HSP90α level was not related to HBV infection. However, higher HSP90α was associated with older age ≥ 65 (p = 0.001), increased AFP ≥ 400 ng/mL (p < 0.001), male gender (p < 0.001), multiple HCC tumors (p < 0.001), more aggressive Child grade (p < 0.001) and ALBI score (p < 0.001), larger tumor diameter (p < 0.001), and more aggressive BCLC staging (p < 0.001) (Fig. 4).
| Variable                      | Total  | HSP90α < 143.5 ng/mL | HSP90α ≥ 143.5 ng/mL | p       |
|-------------------------------|--------|----------------------|----------------------|---------|
| Patients                     | 2150   | 1370                 | 780                  |         |
| Male sex                     | 1726 (80.3) | 1073 (78.3)           | 653 (83.7)           | 0.002   |
| Age ≥ 65 years                | 569 (26.5) | 399 (29.1)            | 170 (21.8)           | <0.001  |
| Etiology                      |        |                      |                      |         |
| HBV                          | 1183 (55.0) | 754 (55.0)            | 429 (55.0)           | 0.987   |
| HCV                          | 45 (2.1) | 31 (2.3)              | 14 (1.8)             | 0.466   |
| Alcohol                      | 885 (41.2) | 540 (39.4)            | 345 (44.2)           | 0.029   |
| NAFLD                        | 30 (1.4) | 19 (1.4)              | 11 (1.4)             | 0.965   |
| Other                         | 38 (1.8) | 24 (1.8)              | 14 (1.8)             | 0.942   |
| Diabetes mellitus            | 206 (9.6) | 145 (10.6)            | 61 (7.8)             | 0.036   |
| Hypertension                  | 329 (15.3) | 227 (16.6)            | 102 (13.1)           | 0.031   |
| Child–Pugh class             |        |                      |                      | <0.001  |
| A                             | 1565 (72.8) | 1100 (80.3)           | 465 (59.6)           |         |
| B                             | 553 (25.7) | 258 (18.8)            | 295 (37.8)           |         |
| C                             | 32 (1.5) | 12 (0.9)              | 20 (2.6)             |         |
| ALBI grade                    |        |                      |                      | <0.001  |
| 1                             | 644 (30.0) | 508 (37.1)            | 136 (17.4)           |         |
| 2                             | 1310 (60.9) | 776 (56.6)            | 534 (68.5)           |         |
| 3                             | 196 (9.1) | 86 (6.3)              | 110 (14.1)           |         |
| HSP90α, median (IQR, ng/mL)  | 100.4 (56.5–203.5) | 66.0 (45.8–95.5)      | 251.5 (189.9–336.5) |         |
| Creatinine, median (IQR, mg/dL) | 64.0 (54.5–73.6) | 64.9 (55.0–74.1)      | 62.2 (53.0–72.3)     | 0.010   |
| Serum AFP, ng/mL              |        |                      |                      | <0.001  |
| < 200                         | 1184 (55.1) | 887 (64.7)            | 297 (38.1)           |         |
| ≥ 200, < 400                  | 139 (6.5) | 91 (6.6)              | 48 (6.2)             |         |
| ≥ 400                         | 827 (38.5) | 392 (28.6)            | 435 (55.8)           |         |
| ALP levels ≥ 125 U/L          | 1164 (54.1) | 568 (41.5)            | 596 (76.4)           | <0.001  |
| Platelet count ≥ 100×10^9/L   | 1570 (73.0) | 944 (68.9)            | 626 (80.3)           | <0.001  |
| ALT levels ≥ 40 U/L           | 1116 (51.9) | 613 (44.7)            | 503 (64.5)           | <0.001  |
| Leukocyte ≥ 4×10^9/L          | 1791 (83.3) | 1087 (79.3)           | 704 (90.3)           | <0.001  |
| BCLC stage                    |        |                      |                      | <0.001  |
| 0/A                           | 486 (22.6) | 425 (31.0)            | 61 (7.8)             |         |
| B                             | 494 (23.0) | 384 (28.0)            | 110 (14.1)           |         |
| C                             | 1138 (52.9) | 549 (40.1)            | 589 (75.5)           |         |
| D                             | 32 (1.5) | 12 (0.9)              | 20 (2.6)             |         |
| Number of tumors ≥ 2          | 1568 (72.9) | 920 (67.2)            | 648 (83.1)           | <0.001  |
| Tumor diameter, cm            |        |                      |                      | <0.001  |
| < 3                           | 327 (15.2) | 286 (20.9)            | 41 (5.3)             |         |
| ≥ 3, < 5                      | 467 (21.7) | 380 (27.7)            | 87 (11.2)            |         |
| ≥ 5, < 10                     | 820 (38.1) | 519 (37.9)            | 301 (38.6)           |         |
| ≥ 10                          | 536 (24.9) | 185 (13.5)            | 351 (45)             |         |
| PVTT                          | 717 (33.3) | 284 (20.7)            | 433 (55.5)           | <0.001  |
| Lymph node metastasis         | 822 (38.2) | 386 (28.2)            | 436 (55.9)           | <0.001  |
| Extrahepatic metastases       | 414 (19.3) | 196 (14.3)            | 218 (27.9)           | <0.001  |
| Lung                          | 259 (12.0) | 106 (7.7)             | 153 (19.6)           |         |
| Bone                          | 107 (5.0) | 58 (4.2)              | 49 (6.3)             |         |
| Other                         | 166 (7.7) | 90 (6.6)              | 76 (9.7)             |         |
| Treatments                    |        |                      |                      |         |
| Supportive care               | 457 (21.3) | 232 (16.9)            | 225 (28.8)           | <0.001  |
| Liver resection               | 489 (22.7) | 405 (29.6)            | 84 (10.8)            | <0.001  |
| Radiotherapy                  | 52 (2.4) | 29 (2.1)              | 23 (2.9)             | 0.227   |
Discussion

HCC is cancer with an aggressive clinical course and high morbidity. Prognostic markers are widely used in clinical practice and have high clinical value as efficient treatment determinants [20]. HSP90α has been previously reported to have a high diagnostic value in patients with HCC [18, 19]. Our novel large-scale, the multicenter study provided robust data on the suitability of the HSP90α plasma level as a prognostic biomarker for HCC. Our results suggested that patients with HSP90α < 143.5 ng/mL had longer mOS compared to patients with HSP90α ≥ 143.5 ng/mL: (p < 0.001), implicating that HSP90α ≥ 143.5 ng/mL is an independent poor prognostic factor for OS.
AFP is the most widely used biomarker in HCC to date. Nevertheless, AFP-negative tumors account for about 30% of cases of HCC, with several studies revealing AFP’s inability to evaluate this subset of HCC tumors [10, 11, 21]. Therefore, to eradicate this clinical decision gap, new prognostic markers are urgently needed. HSP90α is a master regulator and molecular chaperone regulating key cell signaling networks [22]. The secretion of HSP90α in normal cells promotes tissue repair under stress, while the secretion in tumor cells can promote cancer cell proliferation and metastatic potential [17]. Previous studies have confirmed increased expression of HSP90α levels in several tumor types, including HCC [23, 24]. Furthermore, clinically, HSP90α can be used as a diagnostic biomarker for HCC, lung cancer, breast cancer, and gastric cancer [19, 24–26]. Despite its strong diagnostic value, few studies have elucidated the prognostic value of HSP90α in human cancer. Li et al. reported that lung cancer patients with high HSP90α levels had poorer OS and PFS compared to low HSP90α patients [27]. In addition, a study by Fu et al. found that HSP90α positively correlated with tumor volume after surgery or interventional therapy \( p < 0.05 \) [19]. However, the author did not explore the relationship between HSP90α levels with baseline characteristics and patient outcomes. Our study demonstrated that patients with high HSP90α levels had shorter OS and HSP90α was an independent factor for OS in HCC.

Although the protein kinase inhibitor, sorafenib had been used in HCC for many years, its efficacy as monotherapy is still poor, with mOS of only 6.5 months [28]. In recent years, the plethora of studies and drug development advancements.

### Table 2

Univariate and multivariate Cox regression analysis of overall survival before PSM

|                        | Univariable Cox regression | Multivariable Cox regression |
|------------------------|----------------------------|------------------------------|
|                        | HR 95% CI                  | HR 95% CI                    |
| Sex (male/female)      | 1.189 1.015–1.393          | 1.113 0.947–1.309            |
| Age (≥ 65/ < 65 years) | 0.979 0.852–1.124          | 0.763                        |
| HBV (positive/negative)| 0.944 0.836–1.066          | 0.354                        |
| HCV (positive/negative)| 0.714 0.442–1.154          | 0.169                        |
| Alcoholism (positive/negative) | 1.092 0.966–1.234 | 0.160                        |
| NAFLD (positive/negative) | 0.859 0.486–1.518     | 0.602                        |
| Diabetes mellitus (positive/negative) | 0.961 0.779–1.186 | 0.712                        |
| Hypertension (positive/negative) | 0.888 0.745–1.060 | 0.188                        |
| Child–Pugh class (B + C/A) | 2.059 1.812–2.340    | < 0.001                      |
| ALBI grade (2 + 3/1)   | 1.594 1.387–1.831          | < 0.001                      |
| HSP90α (≥ 143.5/ < 143.5 ng/mL) | 2.663 2.357–3.009 | < 0.001                      |
| AFP (≥ 400/ < 400 ng/mL) | 1.520 1.346–1.716       | < 0.001                      |
| ALP (≥ 125/ < 125 U/L) | 2.345 2.063–2.666          | < 0.001                      |
| Platelet (< 100,000/ ≥ 100,000/μL) | 1.092 0.952–1.252 | 0.211                        |
| ALT (≥ 40/ < 40 U/L)   | 1.338 1.184–1.512          | < 0.001                      |
| Leukocyte (< 4000/ ≥ 4000/μL) | 1.161 0.984–1.370   | 0.077                        |
| BCLC stage             |                            |                              |
| 0/A                    | 1.000                      | 1.000                        |
| B                      | 1.733 1.388–2.162          | < 0.001                      |
| C                      | 3.712 3.070–4.488          | < 0.001                      |
| D                      | 6.656 4.332–10.227         | < 0.001                      |
| Number of tumor (≥ 2/ < 2) | 2.030 1.736–2.374    | < 0.001                      |
| Tumor diameter (≥ 5/ < 5 cm) | 1.735 1.519–1.981 | < 0.001                      |
| PVTT (positive/negative) | 2.177 1.924–2.464    | < 0.001                      |
| Lymph node metastasis (yes/no) | 2.232 1.975–2.523 | < 0.001                      |
| Extrahepatic metastases (yes/no) | 1.947 1.691–2.241 | < 0.001                      |
| Anti-tumor therapy (no/yes) | 2.818 2.469–3.216   | < 0.001                      |

PSM propensity score matching, HBV hepatitis B virus, HCV hepatitis C virus, NAFLD nonalcoholic fatty liver disease, ALBI albumin–bilirubin, HSP90α heat-shock protein 90α, AFP alpha-fetoprotein, ALP alkaline phosphatase, ALT alanine aminotransferase, BCLC Barcelona Clinic Liver Cancer, PVTT portal vein tumor thrombus.
of ICIs have expanded our therapeutic arsenal for cancer. The combination of ICIs and targeted drugs has significantly improved the clinical outcomes of HCC patients [29–31]. In the same direction, the combination of TACE plus cam-relizumab increased the PFS of advanced HCC patients to 9 months [5]. Nevertheless, predicting the efficacy of HCC patients receiving ICIs remains a clinical challenge with a definite positive outcome in the quality of patient care. In our subgroup analysis (supportive care group, surgery group, TACE group, adjuvant TACE group, ICI plus targeted therapy group, and TACE plus ICI group), all the low HSP90α expressing patient groups demonstrated better OS than the high HSP90α ones. In the multivariate Cox analysis of the supportive care group, TACE group, TACE plus ICI group, and ICI plus targeted therapy group, the HSP90α ≥ 143.5 ng/mL cutoff was also an independent poor prognostic factor for OS. More importantly, in contrast to other more invasive diagnostic techniques, the liquid biopsy technique for the determination of plasma HSP90α levels is characterized by low invasiveness and high convenience. It is a promising, simple, and effective biomarker for assessing survival in HCC patients and discerning the patients who may benefit from specific treatment modalities. Furthermore, our study confirms that HSP90α is associated with prognosis; thus, the follow-up interval should be reduced for HCC patients with a high HSP90α expression. This approach can better predict disease progression and guide in deciding the next treatment strategy. In conclusion, assessing the HSP90α plasma levels is a robust approach to evaluating the treatment efficacy and response of HCC patients.

In our current study, we further explored the relationship between plasma HSP90α levels and baseline clinical characteristics. Strikingly, high HSP90α plasma levels were associated with multiple tumors co-occurrence, worse child grade and ALBI score, larger tumor diameter, and more aggressive BCLC staging. These results further implicate HSP90α as a prognostic factor in HCC. In accordance with our results, recent studies have also demonstrated that high HSP90α levels correlate with a more aggressive clinical stage [18, 19, 24]. Furthermore, our data showed that patients with AFP ≥ 400 ng/mL had higher HSP90α levels compared to patients with AFP < 400 ng/mL. Notably, a study by Xu et al. showed that the HSP90α level detected by immunohistochemistry in HCC tissues did not associate with serum AFP levels [32]. Nevertheless, we interpret these differences based on the fact that HSP90α plasma level determination is a more sensitive method compared to tissue expression via immunohistochemistry.

In addition, our study determined that the high ALP level before and after PSM is an independent negative prognostic factor for OS. Past studies had confirmed that patients with a high ALP expression had a shorter OS than those with a low ALP expression [33–35]. To our knowledge, this is the first comprehensive study with a large sample size to elucidate the association between plasma HSP90α levels and prognosis in HCC patients. As far as the cutoff value is concerned, the value of 143.5 ng/mL was determined as the optimal value by the X-tile software. Subsequently, in our subgroup analysis, we also confirmed that this cutoff value can also be applied as a prognostic and predictive value in different treatment groups. These data have robust clinical significance implicating that the HSP90α plasma level is an important factor to evaluate the therapeutic response of HCC patients in various therapeutic interventions. Despite the advantages of our study, there are still some limitations. First, selection bias cannot be eliminated due to the nature of retrospective studies. Nevertheless, the large sample of our cohort significantly increased the power and robustness of our study. Second, although our study confirmed that the HSP90α level can predict the response of HCC patients to immunotherapy, our results may be affected by the underlying heterogeneity of different ICIs. Future studies with larger cohort samples and classes of ICI should be designed to safely assess these interesting preliminary findings.
Conclusions

In conclusion, our study confirmed that the plasma HSP90α level can be used as a prognostic and predictive biomarker for HCC. Patients with HSP90α < 143.5 ng/mL had longer mOS compared to those with HSP90α ≥ 143.5 ng/mL. More importantly, HSP90α ≥ 143.5 ng/mL cutoff level was an independent poor prognostic factor for OS in HCC patients. Future prospective studies are required to expand

Fig. 3 Kaplan–Meier plots for overall survival in the supportive care group (A), surgery group (B), transcatheter arterial chemoembolization (TACE) group (C), adjuvant TACE group (D), immune checkpoint inhibitor (ICI) plus targeted therapy group (E), and TACE plus ICI group (F)
Fig. 4 Relationship between the HSP90α levels and baseline characteristics. The HSP90α level was not related to the status of HBV infection (C). Higher HSP90α was associated with age ≥ 65 years (A), male gender (B), worse Child–Pugh grade (D), ALBI score (E), later BCLC staging (F), multiple tumors (G), upregulated AFP (H), and larger tumor diameter (I). HSP90α heat-shock protein 90α, HBV hepatitis B virus, ALBI albumin–bilirubin, BCLC Barcelona clinic liver cancer, AFP alpha-fetoprotein

our knowledge on the causal relationship between HSP90α levels and the prognosis of HCC.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12072-022-10391-y.
Author contributions KS, YL, PW, KH, FW, MR, XL, LW, YS, JZ, TG, KX, QC, JL, ZW, HL, WH, LC, JT, HL, XF, SC, BY, HJ, YY, HL, CY, MW, FX, KP, LZ, YX, XT, ZT, XL, HZ, YZ, LG, and YH collected the data. YH and LG designed the research study. KS, YL, and PW wrote the manuscript and analyzed the data. HC and KS participated in the revision of the manuscript. All authors approved the final version of the manuscript.

Funding This work was supported by a grant from Project of Science and Technology Department of Sichuan Province (2020TD00036).

Data availability All data generated or analyzed during this study are included in this article and its supplementary materials. Further inquiries can be directed to the corresponding author (Lanpaoxiansheng@126.com).

Declarations

Conflict of interest Ke Su, Yanlin Liu, Pan Wang, Kun He, Fei Wang, Hao Chi, Mingyue Rao, Xueling Li, Lianbin Wen, Yanqiong Song, Jianwen Zhang, Tao Gu, Ke Xu, Qi Li, Jiali Chen, Zhenying Wu, Han Li, Weihong Huang, Lan Chen, Jian Tong, Hongyan Li, Xunjie Feng, Siyu Chen, Binbin Yang, Hongping Jin, Yue Yang, Hanlin Liu, Chao Yang, Ming Wu, Fangyu Xiong, Keyi Peng, Luechun Zhu, Yaoyang Xu, Xue Tang, Zunyang Tan, Xiaotong Luo, Hanyue Zheng, Yuxin Zhang, Lu Guo, Yunwei Han declare no conflict of interest regarding the content of this paper.

Animal research (ethics) This research did not involve animal experiments.

Consent to participate (ethics) This retrospective study was approved by the Ethics Committee of The Affiliated Hospital of Southwest Medical University (approval number KY2020254) and complied with the standards of the Declaration of Helsinki. Written informed consent was waived because of the retrospective study.

Plant reproducibility None.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jamal A, et al. Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clin. 2021;71(3):209–249

2. Schöning-Hekele M, Müller C, Kutilek M, Oesterreicher C, Ferenci P, Gangl AIG. Hepatocellular carcinoma in Central Europe: prognostic features and survival. Gut. 2001;48(1):103–109

3. Finn R, Qin S, Ikeda M, Galle P, Ducreux M, Kim T, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med. 2020;382(20):1894–1905

4. Cheng A, Qin S, Ikeda M, Galle P, Ducreux M, Kim T, et al. Updated efficacy and safety data from IMbrave150: atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. J Hepatol. 2022;76(4):862–873

5. Guo Y, Ren Y, Chen L, Sun T, Zhang W, Sun B, et al. Transarterial chemoembolization combined with camrelizumab for recurrent hepatocellular carcinoma. BMC Cancer. 2022;22(1):270

6. Wang Z, Ren Z, Chen Y, Hu J, Yang G, Yu L, et al. Adjuvant transarterial chemoembolization for HBV-related hepatocellular carcinoma after resection: a randomized controlled study. Clin Cancer Res. 2018;24(9):2074–2081

7. Forner A, Bruix J. Biomarkers for early diagnosis of hepatocellular carcinoma. Lancet Oncol. 2012;13(8):750–751

8. Wang N, Cao Y, Song W, He K, Li T, Wang J, et al. Serum peptide pattern that differentially diagnoses hepatitis B virus-related hepatocellular carcinoma from liver cirrhosis. J Gastroenterol Hepatol. 2014;29(7):1544–1550

9. Wang G, Lu X, Du Q, Zhang G, Wang D, Wang Q, et al. Diagnostic value of the γ-glutamyl transferase and alanine transaminase ratio, alpha-fetoprotein, and protein induced by vitamin K absence or antagonist II in hepatitis B virus-related hepatocellular carcinoma. Sci Rep. 2020;10(1):13519

10. Farinati F, Marino D, De Giorgio M, Baldan A, Cantarini M, Curcillo C, et al. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? Am J Gastroenterol. 2006;101(3):524–532

11. Giannini E, Marenco S, Borgonovo G, Savarino V, Farinati F, Del Poggio P, et al. Alpha-fetoprotein has no prognostic role in small hepatocellular carcinoma identified during surveillance in compensated cirrhosis. Hepatology. 2012;56(4):1371–1379

12. Agopian V, Harlander-Locke M, Markovic D, Zarrinpars A, Kaldas F, Cheng E, et al. Evaluation of patients with hepatocellular carcinomas that do not produce α-fetoprotein. JAMA Surg. 2017;152(1):55–64

13. Friedman J. Folding of newly translated proteins in vivo: the role of molecular chaperones. Ann Rev Biochem. 2001;70:603–647

14. Eustace B, Sakurai T, Stewart J, Yimlamai D, Unger C, Zehetmeier C, et al. Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. Nat Cell Biol. 2004;6(6):507–514

15. Du Y, Wu J, Luo L. Secreted heat shock protein 90α attenuated the effect of anticancer drugs in small-cell lung cancer cells through AKT/GSK3β/catenin signaling. Cancer Control. 2018. https://doi.org/10.1177/1073274818804489

16. Zhou X, Wen Y, Tian Y, He M, Ke X, Huang Z, et al. Heat shock protein 90α-dependent B-cell-2-associated transcription factor 1 promotes hepatocellular carcinoma proliferation by regulating MYC proto-oncogene c-MYC mRNA stability. Hepatology. 2019;69(4):1564–1581

17. Wu J, Liu T, Rios Z, Mei Q, Lin X, Cao S. Heat shock proteins and cancer. Trends Pharmacol Sci. 2017;38(3):226–236

18. Wei W, Liu M, Ning S, Wei J, Zhong J, Li J, et al. Diagnostic value of plasma HSP90α levels for detection of hepatocellular carcinoma. BMC Cancer. 2020. https://doi.org/10.1186/s12885-019-6489-0

19. Fu Y, Xu X, Huang D, Cui D, Liu L, Liu J, et al. Plasma heat shock protein 90alpha as a biomarker for the diagnosis of liver cancer: an official, large-scale, and multicenter clinical trial. EBioMedicine. 2017;24:56–63
20. Rich N, Murphy C, Yopp A, Tiro J, Marrero J, Singal AG, et al. Sex disparities in presentation and prognosis of 1110 patients with hepatocellular carcinoma. Aliment Pharmacol Ther. 2020;52(4):701–709
21. Luo P, Wu S, Yu Y, Ming X, Li S, Zuo X, et al. Current status and perspective biomarkers in AFP negative HCC: towards screening for and diagnosing hepatocellular carcinoma at an earlier stage. Pathol Oncol Res. 2020;26(2):599–603
22. El-Serag H, Marrero J, Rudolph L, Reddy KJG. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology. 2008;134(6):1752–1763
23. Zhou Y, Deng X, Zang N, Li H, Li G, Li C, et al. Transcriptomic and proteomic investigation of HSP90α as a potential biomarker for HCC. Med Sci Monit. 2015;21:4039–4049
24. Hou Q, Chen S, An Q, Li B, Fu Y, Luo Y. Extracellular Hsp90α promotes tumor lymphangiogenesis and lymph node metastasis in breast cancer. Int J Mol Sci. 2021;22(14):7747
25. Shi Y, Liu X, Lou J, Han X, Zhang L, Wang Q, et al. Plasma levels of heat shock protein 90 alpha associated with lung cancer development and treatment responses. Clin Cancer Res. 2014;20(23):6016–6022
26. Liang X, Li K, Li Z, Xie M, Tang Y, Du J, et al. Diagnostic and prognostic value of plasma heat shock protein 90alpha in gastric cancer. Int Immunopharmacol. 2021;90:107145
27. Li X, Tong X, Liu B, Li Z, Ding J, Li J, et al. Potential predictive value of plasma heat shock protein 90α in lung cancer. J Med Res. 2021. https://doi.org/10.1177/03000605211064393
28. Cheng A, Kang Y, Chen Z, Tsao C, Qin S, Kim J, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34
29. Finn R, Ikeda M, Zhu A, Sung M, Baron A, Kudo M, et al. Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. J Clin Oncol. 2020;38(26):2960–2970
30. Ren Z, Xu J, Bai Y, Xu A, Cang S, Du C, et al. Sintilimab plus bevacizumab biobis (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): a randomised, open-label, phase 2–3 study. Lancet Oncol. 2021;22(7):977–990
31. Xu J, Shen J, Gu S, Zhang Y, Wu L, Wu J, et al. Camrelizumab in combination with apatinib in patients with advanced hepatocellular carcinoma (RESOLVE): a nonrandomized, open-label, phase II trial. Clin Cancer Res. 2021;27(4):1003–1011
32. Xu Q, Tu J, Dou C, Zhang J, Yang L, Liu X, et al. HSP90 promotes cell glycolysis, proliferation and inhibits apoptosis by regulating PKM2 abundance via Thr-328 phosphorylation in hepatocellular carcinoma. Mol Cancer. 2017;16(1):178
33. Wu S, Lin Y, Ye H, Xiong Z, Li Z, Cheng NJ. Prognostic value of alkaline phosphatase, gamma-glutamyl transpeptidase and lactate dehydrogenase in hepatocellular carcinoma patients treated with liver resection. Int J Surg. 2016;36:143–151
34. Fang K, Kao W, Su C, Chen P, Lee P, Huang Y, et al. The prognosis of single large hepatocellular carcinoma was distinct from Barcelona clinic liver cancer stage A or B: the role of albumin-bilirubin grade liver cancer. Liver Cancer. 2018;7(4):335–358
35. Llovet J, Singal A, Villanueva A, Finn R, Kudo M, Galle P, et al. Prognostic and predictive factors in patients with advanced HCC and elevated alpha-fetoprotein treated with ramucirumab in two randomized phase III trials. Clin Cancer Res. 2022;28(11):2297–2305

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Ke Su1 · Yanlin Liu1 · Pan Wang2 · Kun He3 · Fei Wang4 · Hao Chi5 · Mingyue Rao1 · Xueting Li6 · Lianbin Wen7 · Yanqiong Song8 · Jianwen Zhang1 · Tao Gu1 · Ke Xu1 · Qi Li1 · Jiali Chen1 · Zhenying Wu1 · Han Li1 · Weihong Huang5 · Lan Chen9 · Jian Tong10 · Hongyan Li11 · Kunjie Feng5 · Siyu Chen5 · Binbin Yang5 · Hongping Jin5 · Yue Yang5 · Hanlin Liu5 · Chao Yang5 · Ming Wu5 · Fangyu Xiong12 · Keyi Peng5 · Lechuan Zhu5 · Yaoyang Xu5 · Xue Tang5 · Zunyuan Tan5 · Xiaotong Luo5 · Hanyue Zheng5 · Yuxin Zhang5 · Lu Guo13 · Yunwei Han1,14,15

1 Department of Oncology, The Affiliated Hospital of Southwest Medical University, 25 Taiping Street, Luzhou 646000, Sichuan, China
2 Clinical Skills Center, The Affiliated Hospital of Southwest Medical University, Luzhou 646000, China
3 Clinical Research Institute, The Affiliated Hospital of Southwest Medical University, Luzhou 646000, China
4 Department of General Surgery, Luzhou People’s Hospital, Luzhou 646199, China
5 Clinical Medical College, Southwest Medical University, Luzhou 646000, China
6 Department of Oncology, 363 Hospital, Chengdu 610041, China
7 Department of Geriatric Cardiology, Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital, Chengdu 610072, China
8 Department of Radiotherapy, Sichuan Cancer Hospital and Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, Chengdu 610042, China
9 Department of Oncology and Hematology, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou 646000, China
10 Department of Spinal Surgery, No.1 Orthopedics Hospital of Chengdu, Chengdu 610000, China
11 Department of Anesthesiology, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou 646000, China
12 Department of Medical Inspection Technology, Southwest Medical University, Luzhou 646000, China
13 Department of Ophthalmology, The Affiliated Hospital of Southwest Medical University, 25 Taiping Street, Luzhou 646000, Sichuan, China

© Springer
14 Nuclear Medicine and Molecular Imaging Key Laboratory of Sichuan Province, Luzhou 646000, China
15 Academician (Expert) Workstation of Sichuan Province, Luzhou 646000, China