

Clinical significance of heat shock protein 90 α in the diagnosis, efficacy and prognosis of breast cancer

Yingze Zhu

Harbin Medical University Cancer Hospital

Yingcui Chen

Harbin Medical University Cancer Hospital

Yuhan Tang

Harbin Medical University Cancer Hospital

Wenjing Xiong✉**Shangziyan Li**

Harbin Medical University Cancer Hospital

Ting Guo

Harbin Medical University Cancer Hospital

Yue Lu

Harbin Medical University Cancer Hospital

Hui Pang

panghui@hrbmu.edu.cn

Harbin Medical University Cancer Hospital

Research Article

Keywords: Breast cancer, HSP90 α , HSP90AA1, Biomarker, Diagnosis, Prognosis

Posted Date: August 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10104275/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Heat shock protein 90α (HSP90α) is implicated in tumor progression, yet its specific diagnostic, prognostic, and biological utility in breast cancer (BC) warrants comprehensive elucidation.

Methods

Plasma HSP90α, CEA, and CA15-3 levels were quantified in 797 BC patients and 764 benign controls. Diagnostic performance and prognostic associations were evaluated using ROC, Kaplan–Meier, and Cox regression analyses. The therapeutic monitoring value was assessed in 85 advanced BC patients undergoing chemotherapy. Complementary bioinformatics analyses investigated HSP90AA1 expression, DNA methylation, and immune infiltration. Functional assays in T-47D cells verified the biological impact of HSP90.

Results

Plasma HSP90α was significantly elevated in BC patients compared to controls ($p < 0.05$) and positively correlated with advanced clinical stage, nodal involvement, and distant metastasis. While HSP90α alone exhibited high specificity, its combination with CEA and CA15-3 maximized diagnostic accuracy. Longitudinally, plasma HSP90α levels significantly decreased in patients achieving clinical benefit from chemotherapy, confirming its value in monitoring treatment response. Multivariate analysis identified elevated plasma HSP90α as an independent risk factor for poor Overall Survival. At the tissue level, HSP90AA1 was upregulated and served as an independent prognostic factor. Mechanistically, HSP90AA1 expression negatively correlated with DNA methylation, enriched in cell cycle and RNA transport pathways, and significantly associated with immune cell infiltration. In vitro, HSP90 knockdown suppressed proliferation and migration, whereas overexpression promoted these malignant behaviors.

Conclusion

Circulating HSP90α serves as a robust biomarker for diagnosis, prognostic stratification, and chemotherapy monitoring in breast cancer. Integrated with its critical role in tumor proliferation and immune modulation, HSP90 represents a promising therapeutic target and clinical indicator.

1. INTRODUCTION

Breast cancer (BC) is one of the most common cancers among women worldwide, and early diagnosis and treatment are key to improving prognosis. There are various methods for diagnosing breast cancer, and in clinical practice, serum tumor markers offer advantages such as simplicity, convenience, and

minimal invasiveness, and are commonly used for the early diagnosis of malignant tumors. In breast cancer, Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen 153 (CA153) are frequently used to screen for early-stage disease. In recent years, with further research into tumor markers, a new marker, HSP90 α , has been identified for detecting early malignant tumors.

HSP90 α is a highly conserved and ubiquitous chaperone protein that is essential for cell survival, comprising 1–2% of the total protein content [1]. Under conditions of stress or high temperatures, it promotes protein folding, inhibits protein denaturation in cells, and maintains the functional activity of other proteins [2]. Under normal conditions, the levels of HSP90 α in plasma are extremely low. However, in many disease states, such as malignant tumors, rheumatoid arthritis, neurodegenerative diseases, cardiovascular diseases, and other pathological conditions, the expression levels of HSP90 α can be significantly elevated [3–6]. As research on HSP90 α has progressed, it has been found to have a close association with cancer. Acting as a tumor regulatory factor, HSP90 α is involved in promoting cell growth, cycle regulation, cell proliferation, and signal transduction processes. It can also be secreted outside the cell, playing a crucial role in tissue invasion. Moreover, HSP90 α contributes to drug resistance by establishing mechanisms for immune evasion and influences the onset, survival, and progression of tumors by regulating the biological functions of various protein kinases, transcription factors, and co-factors [7].

Current research has discovered that HSP90 α and its encoding gene HSP90AA1 are highly expressed in various malignant tumors, including breast cancer, liver cancer, lung cancer, colorectal cancer, ovarian cancer, cervical cancer, leukemia, endometrial cancer, and nasopharyngeal carcinoma [8–11]. This paper investigates the expression of HSP90 α in breast cancer patients through in vitro experiments and bioinformatics analysis, explores its diagnostic value in breast cancer, and discusses its clinical significance in monitoring the efficacy of breast cancer chemotherapy and prognosis.

2. MATERIALS AND METHODS

2.1 Study Population and Sample Collection

The study included 797 breast cancer patients and 764 patients with benign breast diseases (controls) treated at the Harbin Medical University Affiliated Tumor Hospital from March 2019 to November 2021. All patients were histologically confirmed to have breast cancer. The inclusion criteria required that patients had not received any antitumor treatments at the time of diagnosis, did not have other malignant tumors in different organs, and were free from cardiovascular diseases, infectious diseases, and autoimmune disorders. Additionally, all patients had complete clinical records. Follow-up for all patients continued until November 2022. For the analysis of therapeutic efficacy in advanced breast cancer, 85 patients receiving systemic chemotherapy were monitored. Peripheral venous blood samples were collected before treatment and after two cycles of therapy. Based on therapeutic outcomes evaluated by RECIST 1.1 criteria, these patients were divided into a clinical benefit group (CR + PR+SD) and a disease progression group (PD).

2.2 Measurement of Plasma Markers

Plasma levels of HSP90 α were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions. Plasma levels of CEA and CA15-3 were determined using electrochemiluminescence immunoassay methods. All measurements were performed in duplicate.

2.3 Bioinformatics Analysis of HSP90AA1

Expression and Survival Analysis: Gene expression data and clinical information were obtained from the TCGA-BRCA dataset. The "Limma" package in R was used to identify differentially expressed genes (DEGs) between high and low HSP90AA1 expression groups (cutoff: $|\log FC| > 1$, $FDR < 0.05$). Differential expression was visualized using box plots via the "ggplot2" package and validated using GEPIA2. Immunohistochemistry staining images validating protein expression in normal and tumor tissues were retrieved from the Human Protein Atlas (HPA). **Functional Enrichment and Interaction Networks:** To identify HSP90AA1-correlated genes, the LinkFinder module of the LinkedOmics database was used. Functional enrichment analysis for DEGs and co-expressed genes was performed using R packages and the clusterProfiler tool. A p -value < 0.05 was considered statistically significant. The Protein-Protein Interaction (PPI) network was constructed using the STRING database to identify top functional partners. **Methylation Analysis:** The correlation between HSP90AA1 expression and DNA methylation was analyzed using cBioPortal. Promoter methylation levels were compared between normal and tumor tissues using UALCAN. Visualization of specific CpG sites and their prognostic value was performed using the MethSurv tool. Additionally, the distribution of methylation sites was investigated using the SMART database. **Immune Infiltration Analysis:** Correlations between HSP90AA1 expression and tumor-infiltrating immune cells were analyzed using ssGSEA (Single Sample Gene Set Enrichment Analysis) and TIMER (Tumor Immune Estimation Resource). The association with immune subtypes and molecular subtypes was investigated using TISIDB. **GSEA (Gene Set Enrichment Analysis):** To explore biological pathways, GSEA software was used to analyze tumor characteristics between high and low HSP90AA1 expression groups. A p -value < 0.05 and $FDR < 0.25$ were considered statistically significant.

2.4 Cell Culture and Transfection

Human breast cancer T-47D cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO₂ humidified atmosphere. For functional assays, cells were transfected with small interfering RNAs (siRNAs) targeting HSP90 or overexpression plasmids using Lipofectamine 3000, with empty vectors or scrambled siRNA serving as negative controls (NC).

2.5 Western Blot and qRT-PCR

Western Blot: Total protein was extracted, separated by SDS-PAGE, and transferred to PVDF membranes. Membranes were incubated with primary antibodies against HSP90 and β -actin (loading control), followed by secondary antibodies. Bands were visualized using ECL reagents. **qRT-PCR:** Total RNA was isolated using Trizol reagent. Reverse transcription and quantitative PCR were performed to measure

HSP90 mRNA levels. Relative expression was calculated using the method with GAPDH as the internal control.

2.6 Cell Functional Assays

Cell Proliferation: Assessed using the CCK-8 assay. Absorbance at 450 nm was measured at 24, 48, 72, and 96 hours post-transfection. **Colony Formation:** Transfected cells were seeded in 6-well plates and cultured for 10–14 days. Colonies were fixed with methanol, stained with crystal violet, and counted. **Wound Healing Assay:** A scratch was created in the confluent cell monolayer. Migration distance was imaged and measured at 0 and 24 hours to calculate the wound closure rate.

2.7 Statistical Analysis

Statistical analyses were performed using SPSS 26.0, MedCalc, and GraphPad Prism. **Comparison of Groups:** Continuous variables were analyzed using the Student's t-test or Mann-Whitney U test for two groups, and one-way ANOVA or Kruskal-Wallis test for multiple groups. The paired-sample Wilcoxon signed-rank test was used to compare biomarker levels before and after chemotherapy. **Diagnostic Value:** Analyzed using ROC curves and Area Under the Curve (AUC). **Survival Analysis:** Survival curves were estimated using the Kaplan-Meier method and compared using the Log-rank test. **Prognostic Factors:** Univariate and multivariate Cox proportional hazards regression models were used to identify independent prognostic factors. **Nomogram:** A nomogram was constructed based on independent prognostic factors, and calibration plots were used to assess predictive accuracy. Significance levels are denoted as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. $****p < 0.0001$ was considered not statistically significant (N.S.).

3. RESULTS

3.1 Expression of HSP90 α , CEA and CA153 in breast cancer patients and their controls

Comparison of HSP90 α expression between breast cancer patients and benign control groups revealed that the levels of HSP90 α , CEA, and CA153 in the plasma of breast cancer patients were significantly higher than those in the benign control group, with statistically significant differences. Details are provided in Supplementary data Table. 1.

3.2 Relationship between HSP90 α and clinicopathological characteristics of BC patients

Analysis of the relationship between HSP90 α expression and various clinical pathological parameters revealed that plasma HSP90 α levels in breast cancer patients were significantly associated with lymph node metastasis, distant metastasis, and staging, with statistically significant differences. However, there was no significant correlation with age, molecular subtype, Ki-67, pathological type, or tumor size. Details are provided in Supplementary data Table. 2. In the subgroup analysis of the lymph node

metastasis group, it was found that HSP90 α expression levels in N2 and N3 were significantly higher than in N0 + N1 ($p < 0.05$), but there was no significant difference between N2 and N3 (Fig. 1A). In the distant metastasis subgroup, patients with distant metastases exhibited significantly elevated plasma HSP90 α levels compared to those without distant metastases. In the clinical staging subgroup analysis, stage IV patients showed significantly higher plasma HSP90 α levels compared to stages I + II and III (Fig. 1B) ($p < 0.05$). Regarding diagnostic utility (Fig. 1C, Supplementary data Table. 3), HSP90 α alone exhibited moderate performance, characterized by high specificity but comparatively low sensitivity. However, when combined with CEA and CA15-3, the area under the ROC curve increased, surpassing that of any individual marker, thereby indicating enhanced diagnostic efficacy. Additionally, as illustrated in Figs. 1D (Supplementary data Table. 4) and 1E (Supplementary data Table. 4), the survival analysis underscored the prognostic significance of circulating HSP90 α . Patients exhibiting elevated HSP90 α expression experienced significantly reduced progression-free survival (PFS, $p = 0.009$) and overall survival (OS, $p = 0.038$) compared to those with lower expression levels. The divergence in survival curves emerged early in the follow-up period, suggesting that increased HSP90 α levels are correlated with earlier disease progression and an elevated risk of mortality.

3.3 Therapeutic impact and prognostic significance of plasma HSP90 α in advanced breast cancer patients

Plasma levels of HSP90 α , CEA, and CA153 were measured in 85 patients with advanced breast cancer. Based on the therapeutic outcomes after chemotherapy, patients were divided into a clinical benefit group (CR + PR+SD) and a disease progression group. The results showed that the expression levels of HSP90 α , CEA, and CA153 were higher in the PD group, but the differences were not statistically significant. Details can be found in Supplementary data Table. 5. An analysis of the levels of plasma HSP90 α , CEA, and CA153 before and after chemotherapy was conducted in patients with advanced breast cancer to assess treatment efficacy. The results indicated that in the clinical benefit group (CR + PR+SD), levels of HSP90 α , CEA, and CA153 all decreased significantly after chemotherapy compared to before treatment. In the disease progression group, levels of plasma HSP90 α and CEA increased after chemotherapy compared to before, while CA153 levels decreased, though these changes were not statistically significant. Details are provided in Supplementary data Table. 6. Kaplan–Meier analysis demonstrated that patients exhibiting elevated plasma HSP90 α expression experienced significantly reduced progression-free survival (PFS) and overall survival (OS) compared to those with lower expression levels (both $P < 0.05$), as illustrated in Fig. 1C. To further assess its prognostic significance, a Cox proportional hazards regression model was employed, incorporating variables such as age, molecular subtype, Ki-67, pathology, tumor size, lymph node status, HSP90 α , CEA, and CA15-3. In the univariate analysis, HSP90 α , CEA, and CA15-3 were significantly correlated with decreased PFS ($P < 0.05$), whereas the other variables did not demonstrate significance. The multivariate analysis identified CEA as the sole independent predictor of PFS ($P = 0.015$), with HSP90 α and the remaining markers lacking statistical significance (Supplementary data Table. 7). Regarding OS, the univariate analysis similarly identified HSP90 α , CEA, and CA15-3 as significant adverse prognostic indicators ($P < 0.05$). In

the multivariate analysis, HSP90 α persisted as an independent predictor of poor OS ($P = 0.041$), while other variables, including CEA and CA15-3, did not achieve significance (Supplementary data Table. 8). Considering that plasma HSP90 α is indicative of the protein encoded by HSP90AA1, the expression of HSP90AA1 was subjected to further analysis using publicly available tumor datasets. Within the TCGA cancer cohort, HSP90AA1 was found to be upregulated across various tumor types, including breast cancer (Fig. 2A). Specifically in breast cancer, the expression of HSP90AA1 was markedly elevated in tumor tissues as opposed to normal tissues (Fig. 2B), with paired analyses of tumor and normal tissues corroborating the consistent overexpression observed in tumors (Fig. 2C). Additionally, immunohistochemistry data sourced from the HPA database revealed increased levels of HSP90AA1 protein in breast cancer tissues compared to corresponding normal samples (Fig. 2D–E).

3.4 The expression of HSP90AA1 is correlated with the clinical and pathological characteristics of BC

The relationship between the expression level of HSP90AA1 in the TCGA database and the prognosis of BC patients was analyzed. Combined with Kaplan-Meier survival curve analysis, it was shown that patients with higher expression levels of HSP90AA1 had shorter overall survival compared to those with lower expression ($P = 0.022$). We further explored the diagnostic value of HSP90AA1 in BC by establishing a ROC curve based on HSP90AA1 expression in the samples (AUC = 0.641) (Figs. 3A-B). The results indicate that using the expression of HSP90AA1 to predict the prognosis of BC patients is quite accurate.

Univariate Cox regression analysis showed that the expression of HSP90AA1 significantly affects the prognosis of BC patients. Multivariate Cox regression analysis indicated that HSP90AA1 is an independent prognostic factor irrespective of the clinical characteristics of BC patients. Subsequently, to predict the OS of BC patients, a nomogram was created based on the expression of HSP90AA1 and patient characteristics from the TCGA dataset (Fig. 3C). Calibration plots for 1-year, 3-year, and 5-year OS probabilities showed good consistency between observed OS and nomogram-predicted OS (Fig. 3D). To further investigate the biological relevance of HSP90AA1 overexpression, Gene Set Enrichment Analysis (GSEA) was performed to identify functional pathways associated with its high expression in breast cancer (Fig. 3E).

3.5 Functional Enrichment, Interaction Network, and Methylation Analysis of HSP90AA1 in Breast Cancer

To further investigate the biological role of HSP90AA1 in breast cancer, we employed the LinkFinder module available on the LinkedOmics portal to identify differentially expressed genes (DEGs) associated with HSP90AA1 within the TCGA-BC dataset. The co-expression analysis is depicted in Figs. 4A–C. Specifically, Fig. 4A presents a volcano plot illustrating the correlations between HSP90AA1 and other gene expressions, determined via the Spearman test; 8,212 genes (indicated by deep red dots) exhibit

positive correlation with HSP90AA1, whereas 11,942 genes (indicated by deep green dots) show negative correlation. The top 50 positively and top 50 negatively correlated genes are represented in the heatmaps in Figs. 4B and 4C, respectively. Functional enrichment analysis, as shown in Figs. 4D–G, involved Gene Ontology (GO) annotation and KEGG pathway analyses to elucidate the functions of DEGs related to HSP90AA1. In terms of Biological Processes (BP), the DEGs were predominantly enriched in RNA localization, organelle fission, and mitotic cell cycle phase transition (Fig. 4D). Regarding Cellular Components (CC), the genes were primarily located in the chromosomal region, transferase complexes, and microtubules (Fig. 4E). Molecular Functions (MF): The genes demonstrated significant enrichment in ATPase activity, catalytic activity related to RNA, and mRNA binding (Fig. 4F). KEGG Pathways: The analysis revealed that these differentially expressed genes (DEGs) are predominantly enriched in RNA transport, the cell cycle, and ubiquitin-mediated proteolysis pathways (Fig. 4G). Protein-Protein Interaction (PPI) Network (Figs. 4H–I): We performed a network analysis on HSP90AA1 utilizing the STRING database. Figure 4H illustrates the top 10 functionally associated partner genes with the highest connectivity: ERBB2, CDC37, AHSA1, STUB1, EGFR, DNAJB1, TP53, NR3C1, TOMM70, and FKBP4. Through combined Gene Ontology (GO) and KEGG enrichment analyses for these specific partners, we identified significant enrichment in pathways such as heat shock protein binding, chaperone binding, and protein folding, as depicted in Fig. 4I. Methylation and Expression Correlation (Figs. 4J–L): We further explored the relationship between gene expression and DNA methylation. Correlation: Analysis using the cBioPortal database indicated that the expression of HSP90AA1 is negatively correlated with its methylation level (Fig. 4J; Spearman $r = -0.44$, $P = 1.28e-38$). Expression Status: UALCAN analysis shows HSP90AA1 expression is significantly higher in primary tumors than in normal tissues (Fig. 4K). Methylation Sites: Using MethSurv, we visualized DNA methylation at specific CpG sites linked to HSP90AA1. Figure 4L's heatmap illustrates methylation differences across racial groups and expression levels, notably at sites cg06798479 and cg23268892.

3.6 The correlation between HSP90AA1 expression and immune infiltration

We further analyzed the correlation between HSP90AA1 expression and immune cell infiltration using ssGSEA and Spearman's r . In Fig. 5A, the numbers of Th2 cells, T gd, T helper cells, and Tcm showed a positive correlation with HSP90AA1 expression. Among them, Th2 cells and Tgd showed the strongest positive correlation with HSP90AA1 expression. Conversely, the expression of HSP90AA1 was negatively correlated with the numbers of pDC, CD8 T cells, NK cells, cytotoxic cells, iDC, T cells, DC, and NK CD56bright cells ($P < 0.05$), with the number of pDC showing the strongest negative correlation (Figs. 5B–D).

Breast cancer was classified into various immune subtypes, including C1 (wound healing), C2 (IFN- γ dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 (TGF- β dominant). The TCGA research network identified four prognostic molecular subgroups of BC: basal, HER2, LumA, LumB, and Normal. Using TISIDB, we studied the correlation of HSP90AA1 levels

with immune and molecular subtypes. In Fig. 5E, HSP90AA1 expression was significantly correlated with the C1, C2, C3, C4, and C6 subtypes ($P = 4.57\text{e-}20$). Furthermore, we also found that HSP90AA1 expression was significantly correlated with the basal, HER2, LumA, LumB, and Normal subgroups ($P = 6.87\text{e-}27$), as shown in Fig. 5F. These results suggest that HSP90AA1 expression is related to immune subtypes and molecular subgroups.

3.7 Functional impact of HSP90 on T-47D breast cancer cell proliferation and migration

Functional assays were performed to investigate the role of HSP90 in breast cancer cell behavior. Western blot and RT-qPCR confirmed effective HSP90 knockdown using two independent siRNAs and successful overexpression in T-47D cells (Fig. 6A–B). CCK-8 assays demonstrated that HSP90 silencing significantly reduced cell proliferation, whereas overexpression promoted cell growth (Fig. 5C). Consistently, colony formation assays showed fewer colonies in si-HSP90-treated cells and increased colony numbers following HSP90 overexpression (Fig. 6D–E). In addition, wound-healing assays revealed that HSP90 inhibition impaired cellular migration, while ectopic HSP90 expression enhanced wound closure rates (Fig. 6F).

4. DISCUSSION

HSP90 α is an important member of the heat shock protein family. Under physiological conditions, HSP90 α is not secreted or is secreted in small amounts, but its expression level can significantly increase under various adverse stress conditions or malignant tumor stimuli, and it can be secreted extracellularly. Various studies have shown that cancer cells can continuously secrete HSP90 α extracellularly when cultured, and extracellular HSP90 α can enhance the mobility and invasive capabilities of cancer cells, thereby promoting tumor metastasis to distant sites. Additionally, the production and high expression of HSP90 α play a suggestive role in the early occurrence and prognosis assessment of malignant tumors.

Multiple studies have found that HSP90 α is closely associated with the occurrence and development of cancer. In Zhang's study, it was discovered that R1OK3 interacts with heat shock protein 90 α , thereby promoting the binding of HSP90 α to isocitrate dehydrogenase 1 (IDH1), ultimately affecting the survival of colon cancer tumor cells in vivo [15]. Lee's research found that high expression levels of HSP90 α are significantly associated with lymphatic infiltration, lymph node metastasis, and advanced stages (TNM staging $\geq$ Stage III). Patients with larger and more aggressive tumors exhibit higher levels of HSP90 α expression compared to those with smaller and less aggressive tumors [16]. Li's research found that the combination of HAP90 α and AFP is the optimal strategy for the auxiliary diagnosis of metastatic liver cancer [17]. Wei's research found that plasma HSP90 α is positively correlated with CEA, CA125, and CA199 in colorectal cancer patients [18]. This study found that the expression levels of plasma HSP90 α , CEA, and CA153 are significantly elevated in breast cancer patients. The expression is not related to age, molecular subtype, Ki67, pathological type, or tumor size. However, as lymph node metastasis, distant

metastasis, and clinical staging progress, the expression levels of plasma HSP90 α gradually increase. Through ROC curve analysis and plotting the area under the curve, it was found that HSP90 α has a high specificity for breast cancer. HSP90 α can be combined with CEA and CA153 to improve the diagnostic efficacy for breast cancer. Correlation analysis shows that there is a positive correlation between HSP90 α and both CEA and CA153, and the results of this study are consistent with the aforementioned conclusions.

In the field of breast cancer research, the importance of immunotherapy is increasingly emphasized [19, 20]. HSP90AA1, a molecular chaperone protein, is typically associated with protein folding, protection, and functional regulation. Recent studies have begun to explore its role in the tumor microenvironment, particularly its potential impact on immune regulation.

In this study, we focused on the expression of HSP90AA1 in breast cancer and its relationship with immune cell infiltration. The results showed that the expression level of HSP90AA1 is significantly correlated with the infiltration of immune cells in breast cancer, involving various types of immune cells, including Th2 cells, T γ δ cells, T helper cells, and central memory T cells (Tcm). This broad correlation suggests that HSP90AA1 may regulate the tumor microenvironment by affecting the activity or infiltration of these immune cells.

Furthermore, data analyzed using the ESTIMA method showed that the expression of HSP90AA1 is positively correlated with the matrix score. The matrix score is an indicator that quantifies the components of the extracellular matrix in the tumor microenvironment, with high matrix scores typically associated with tumor aggressiveness and poor prognosis. The positive correlation between HSP90AA1 and high matrix scores further emphasizes its significant role in the tumor microenvironment, possibly by influencing the composition or function of the extracellular matrix to participate in tumor progression and immune escape. In summary, these findings suggest that HSP90AA1 not only plays a role in the survival and proliferation of breast cancer cells but also plays a key role in regulating the tumor immune microenvironment. This provides a new perspective for future research, which may help in developing immunotherapeutic strategies targeting HSP90AA1, thereby improving the treatment outcomes and prognosis for breast cancer patients.

In terms of efficacy analysis, significant differences were observed in the expression levels of HSP90 α before and after chemotherapy in patients with neoadjuvant and advanced breast cancer. The expression levels of HSP90 α , CEA, and CA153 were all reduced after chemotherapy in the clinical benefit group, whereas in the disease progression group, the expression levels increased after chemotherapy compared to before. Yao's study explored how indirubin exerts an antitumor effect on leukemia cells by inhibiting the expression of HSP90AA1 [21].

Therefore, by dynamically monitoring the levels of HSP90 α , CEA, and CA153 in plasma, these markers can be used jointly for monitoring the condition of breast cancer patients and assessing short-term treatment efficacy. Studies have found that the level of HSP90 α is a favorable predictive factor for PFS and OS in patients with advanced lung cancer [22, 23]. In our survival analysis of advanced breast

cancer, high expression of plasma HSP90 α was associated with poorer PFS and OS, making it an independent prognostic predictor of poor OS in patients with advanced breast cancer, and an important adverse factor for poor PFS. Thus, it can be used for the prognosis assessment of patients with advanced breast cancer. However, due to the short follow-up period in this study, no deaths were observed in the group with low expression of HSP90 α , indicating that longer follow-up is needed to confirm these findings.

In summary, we found that the expression of plasma HSP90 α and its encoding gene, HSP90AA1, are closely related to the occurrence and development of breast cancer. They can be used in conjunction with CEA and CA153 for the diagnosis of breast cancer and can also be utilized for monitoring chemotherapy efficacy and evaluating prognostic value.

Declarations

This study has been approved by Harbin Medical University Affiliated Cancer Hospital (KY2020-03). Patients have all provided informed consent. Sample collection was done after approval by the Institutional Ethics Committee of Harbin Medical University. This study was conducted in accordance with the Declaration of Helsinki.

6. Funding

This study was funded by the Beijing Medical Award Foundation (Grant Number YXJL-2023-0078-0053).

7. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All participants have consented for all images and clinical data and other data included in the manuscript to be published.

Authors' contributions

YZZ, YCC and YHT wrote the first draft of the manuscript. XWJ and SZYL contributed to conception. YL and TG collected data. HP contributed to review and editing. All authors contributed to manuscript revision, read, and approved the submitted version.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and ethical restrictions, but are available from the corresponding author on reasonable request. Publicly available datasets analyzed in this study were obtained from TCGA and other public databases as described in the Methods section. Supplementary data supporting the findings of this study are included in the article and its supplementary materials.

References

1. Liu J, Shu H, Xia Q, You Q, Wang L. Recent developments of HSP90 inhibitors: an updated patent review (2020-present). *Expert Opin Ther Pat* (2024) 34(1–2):1–15.
2. Chen Y, Chang Q, Fang Q, Zhang Z, Wu D, Bian L, et al. Genome-Wide Identification, Molecular Characterization, and Expression Analysis of the HSP70 and HSP90 Gene Families in *Thamnaconus septentrionalis*. *Int J Mol Sci* (2024) 25(11):5706.
3. Wang Q, Kong X, Guo W, Liu L, Tian Y, Tao X, et al. HSP90 Exacerbates Bone Destruction in Rheumatoid Arthritis by Activating TRAF6/NFATc1 Signaling. *Inflammation* (2024) 47(1):363–375.
4. Batko J, Antosz K, Miśków W, Pszczołowska M, Walczak K, Leszek J. Chaperones-A New Class of Potential Therapeutic Targets in Alzheimer's Disease. *Int J Mol Sci* (2024) 25(6):3401.
5. Hazra J, Vijayakumar A, Mahapatra NR. Emerging role of heat shock proteins in cardiovascular diseases. *Adv Protein Chem Struct Biol.* (2023) 134:271–306.
6. Hou L, Dong H, Zhang E, Lu H, Zhang Y, Zhao H, et al. A new insight into fluoride induces cardiotoxicity in chickens: Involving the regulation of PERK/IRE1/ATF6 pathway and heat shock proteins. *Toxicology* (2024) 501:153688.
7. Chen S, Yu Q, Zhou S. Plasmatic Levels of HSP90α at Diagnosis: A Novel Prognostic Indicator of Clinical Outcome in Advanced Lung Cancer Patients Treated With PD-1/PD-L1 Inhibitors Plus Chemotherapy. *Front Oncol* (2021) 11: 765115.
8. Wang J, Mijiti Y, Chen Y, Liu Z. Aryl hydrocarbon receptor is a prognostic biomarker and is correlated with immune responses in cervical cancer. *Bioengineered* (2021) 12(2):11922–11935.
9. Yang S, Bi J, Drnevich J, Li K, Nowak RA. Basigin is necessary for normal decidualization of human uterine stromal cells. *Hum Reprod* (2022) 37(12):2885–2898.
10. Irshad R, Batool F, Raj N, Karim S, Alkreathy HM, Manzoor N, et al. Multi-targeted effects of D-carvone against Non-Small Cell Lung Cancer (NSCLC): A network pharmacology-based study. *Toxicol Appl Pharmacol* (2024) 487:116978.
11. Iskandar K, Foo J, Liew AQX, Zhu H, Raman D, Hirpara JL, et al. A novel MTORC2-AKT-ROS axis triggers mitofission and mitophagy-associated execution of colorectal cancer cells upon drug-induced activation of mutant KRAS. *Autophagy* (2024) 20(6):1418–1441.
12. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* (2015) 12(5):453–7.
13. Zuehlke AD, Beebe K, Neckers L, Prince T. Regulation and function of the human HSP90AA1 gene. *Gene* (2015) 570(1):8–16.
14. Uhlen M, Fagerberg L, Hallstrom BM. Proteomics. Tissue-based map of the human proteome. *Science* (2015) 347(6220):1260419.
15. Zhang N, Dong L, Ning T, Du F, Zhao M, Xu J, et al. RIOK3 sustains colorectal cancer cell survival under glucose deprivation via an HSP90α-dependent pathway. *Oncogenesis* (2024) 13(1):12.

16. Lee HW, Kim KM. Clinical significance of heat shock protein 90 α expression as a biomarker of prognosis in patients with gastric cancer. *Niger j clin pract* (2019) 22(12): 1698–1705.
17. Li S, Wei W, Feng Z, Bian Y, Pan J, Mai J, et al. Role of Serum CYFRA 21 – 1 in Diagnosis and Prognostic in Colorectal Liver Metastases. *Cancer Manag Res* (2023) 15:601–614.
18. Wei W, Zhou J, Chen L. Plasma Levels of Heat Shock Protein 90 Alpha Associated With Colorectal Cancer Development. *Front Mol Biosci* (2021) 8(null): 684836.
19. Shiao SL, Gouin KH 3rd, Ing N, Ho A, Basho R, Shah A, et al. Single-cell and spatial profiling identify three response trajectories to pembrolizumab and radiation therapy in triple negative breast cancer. *Cancer Cell* (2024) 42(1):70–84.e8.
20. Jia D, Wang Q, Qi Y, Jiang Y, He J, Lin Y, et al. Microbial metabolite enhances immunotherapy efficacy by modulating T cell stemness in pan-cancer. *Cell* (2024) 187(7):1651–1665.e21.
21. Yao Y, Li X, Yang X, Mou H, Wei L. Indirubin, an Active Component of Indigo Naturalis, Exhibits Inhibitory Effects on Leukemia Cells via Targeting HSP90AA1 and PI3K/Akt Pathway. *Anticancer Agents Med Chem* (2024) 24(9):718–727.
22. Zhu Y, Dai Z. HSP90: A promising target for NSCLC treatments. *Eur J Pharmacol.* (2024) 967:176387.
23. Bian Y, Liu H, Huang J, Feng Z, Lin Y, Li J, et al. eHSP90 α in front-line therapy in EGFR exon 19 deletion and 21 Leu858Arg mutations in advanced lung adenocarcinoma. *BMC Cancer* (2024) 24(1):835.

Figures

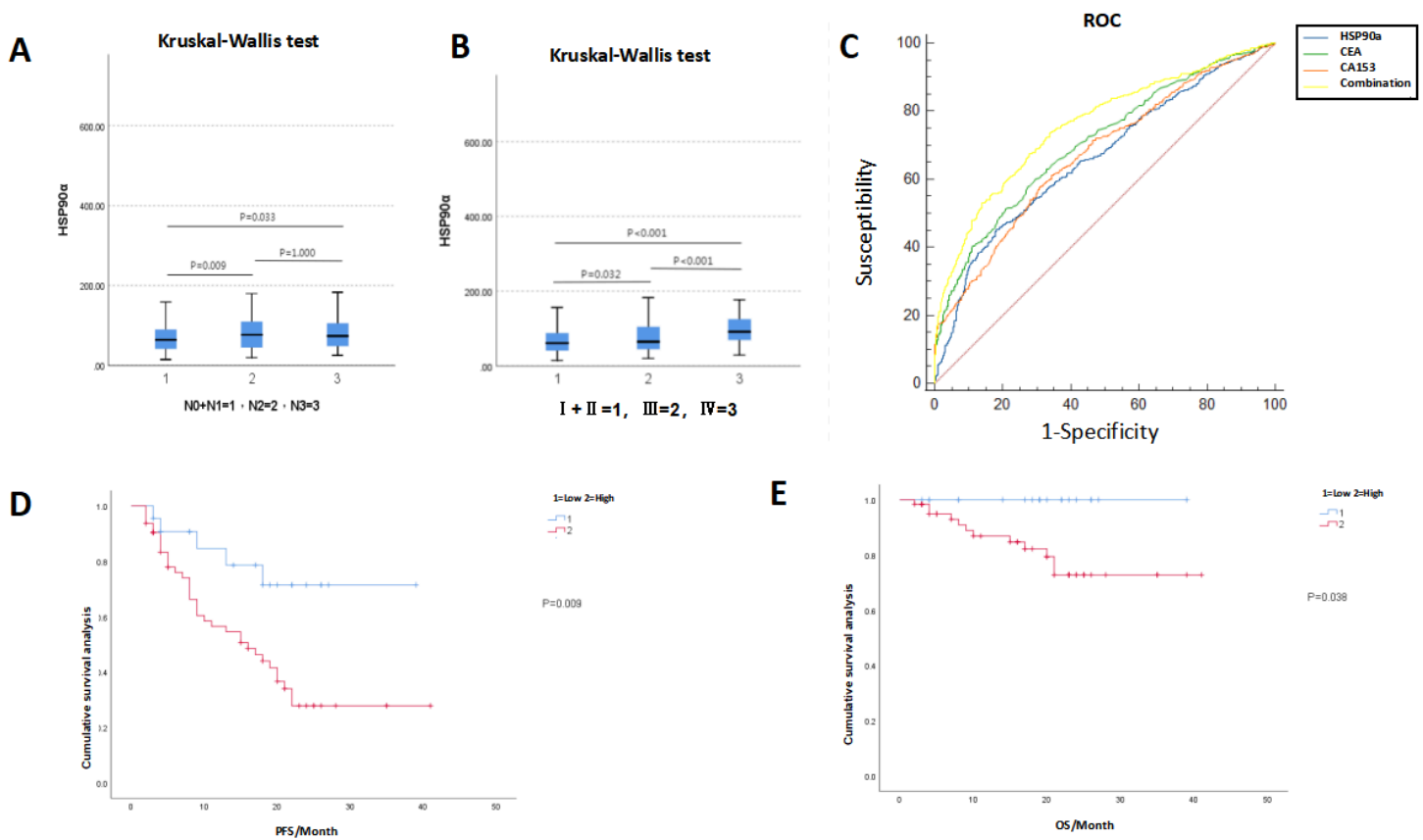


Figure 1

A: Expression of HSP90α in different lymph node metastasis groups. B: Expression of HSP90α in different staging groups. C: Receiver operating characteristic (ROC) curves for HSP90α, CEA, CA153, and their combined model in distinguishing breast cancer from benign breast disease. D: Kaplan–Meier analysis of progression-free survival (PFS) in patients grouped by HSP90α expression (high vs. low). E: Kaplan–Meier analysis of overall survival (OS) in patients stratified by HSP90α expression.

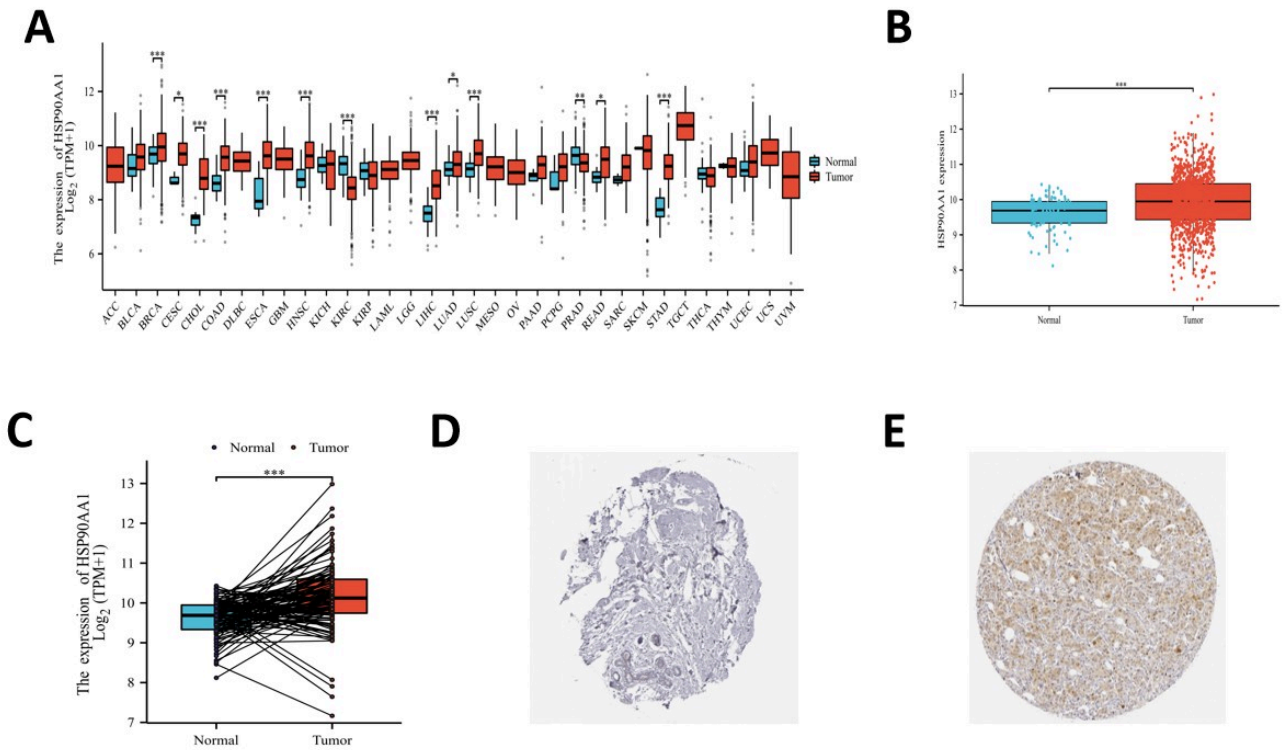


Figure 2

A: Comparison of HSP90AA1 in 33 different tumor tissues versus normal tissues. B: Box plot of differential expression of HSP90AA1 in the GEPIA database. C: Relationship of HSP90AA1 expression between normal tissues and paired breast cancer tissues. D-E: Immunohistochemical staining of HSP90AA1 in normal tissues and the same sample of breast cancer tissues downloaded from the HPA database. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

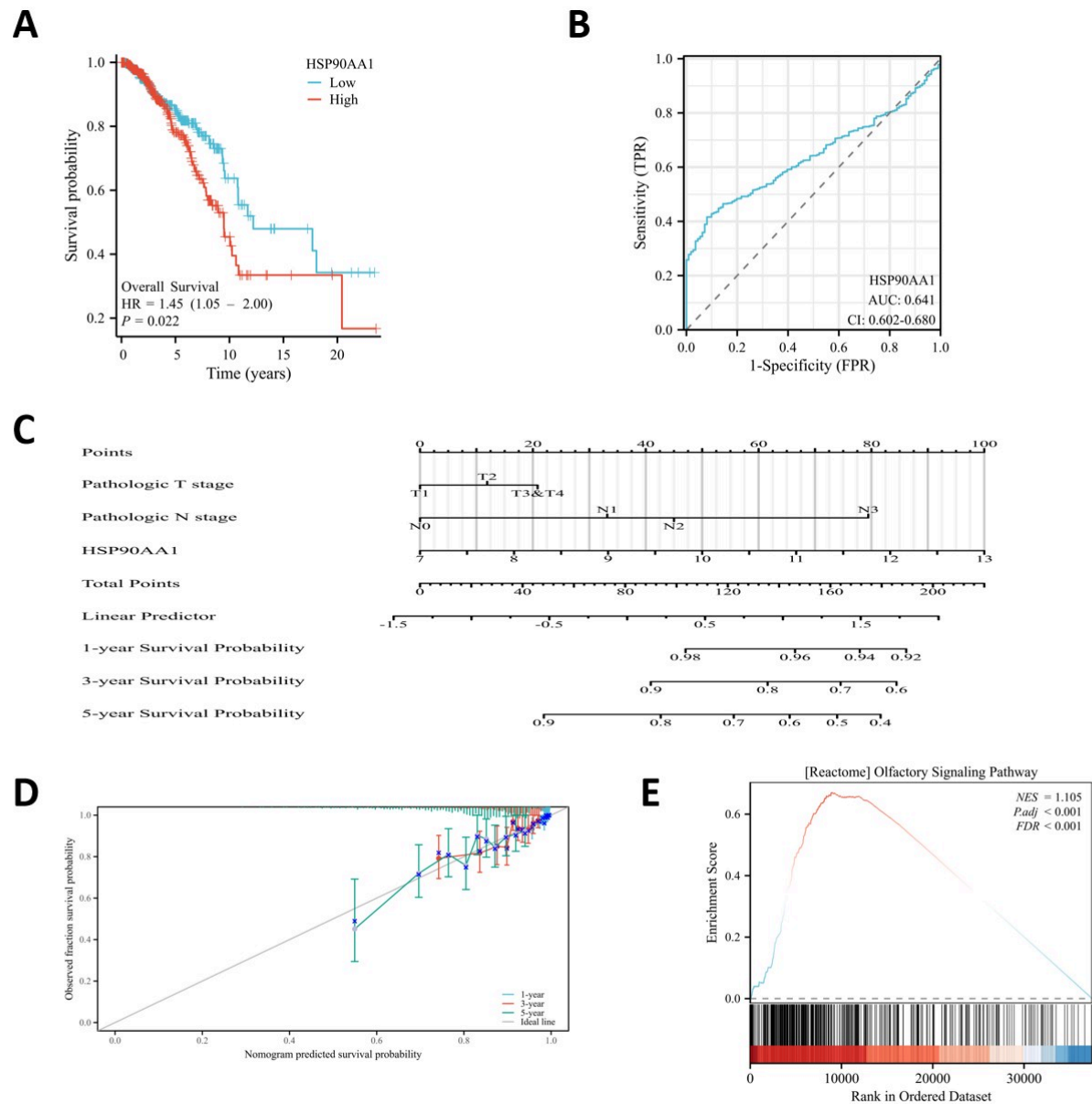


Figure 3

A: The correlation between HSP90AA1 expression and overall survival in BC. B: ROC curve for predicting patient survival based on HSP90AA1 expression. C: Nomogram for predicting BC prognosis based on the expression of HSP90AA1. D: Calibration plots for the nomogram predicting 1-year, 3-year, and 5-year OS probabilities. E: Gene set enrichment analysis (GSEA) indicates that tumor markers are enriched in the high expression group of HSP90AA1.

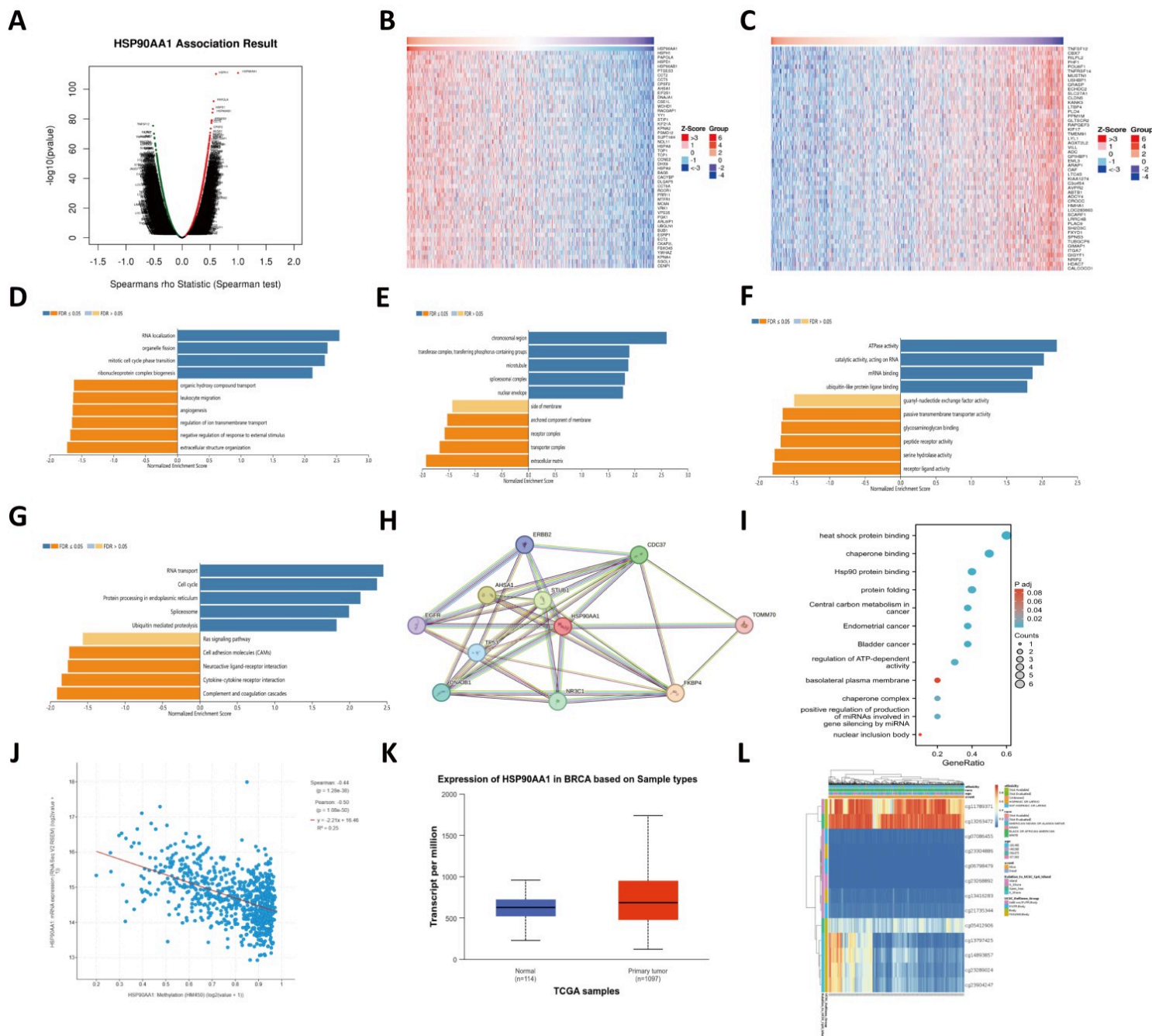


Figure 4

A: The heatmap shows that in BC, there is a negative correlation with HSP90AA1. Red indicates positively correlated genes, while blue indicates negatively correlated genes. B-C: The top 50 genes positively and negatively correlated with HSP90AA1 in BC. Red indicates positively correlated genes, while blue indicates negatively correlated genes. D: GO-Biological process, E:GO-Cellular Component, F:GO-Molecular Function G: KEGG patyway, H:Protein-protein interaction network obtained from STRING. I: GO term and KEGG enrichment analysis of 10 functional partner genes of HSP90AA1. J. Analyze the correlation between HSP90AA1 methylation and its expression levels using the cBioPortal database. K. Using UALCAN analysis, examine the methylation levels of the HSP90AA1 promoter in BC (breast

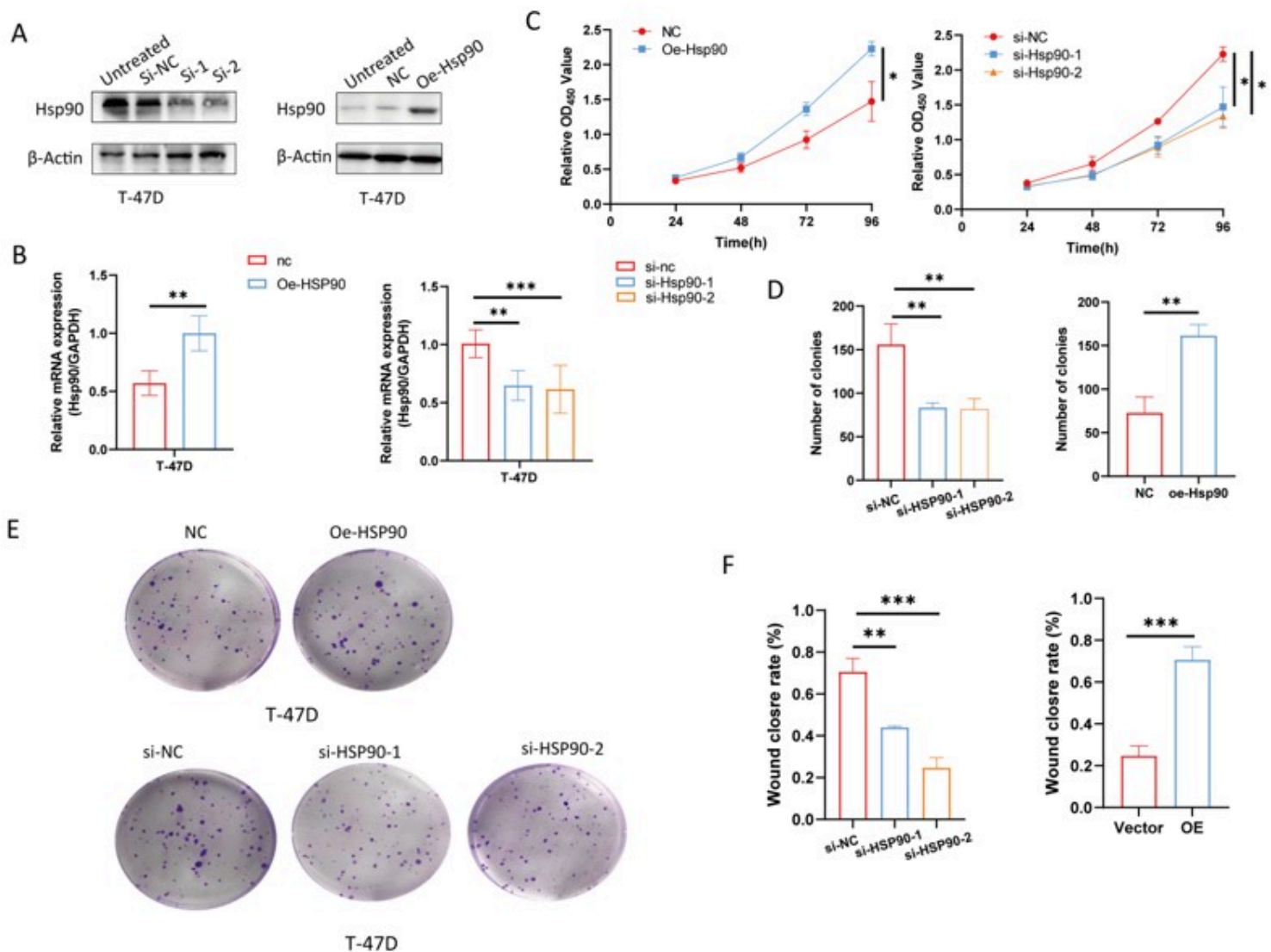


Figure 6

Impact of HSP90 on T-47D breast cancer cell growth and movement. (A) Western blot confirms HSP90 knockdown and overexpression in T-47D cells, using β -Actin as a control. (B) RT-qPCR shows changes in HSP90 mRNA levels post-knockdown and overexpression ($P < 0.01$, $P < 0.001$). (C) CCK-8 assays reveal decreased growth with HSP90 knockdown and increased growth with overexpression ($P < 0.05$, $P < 0.01$). (D) Colony assays show fewer colonies with HSP90 knockdown and more with overexpression ($P < 0.01$). (E) Crystal violet staining illustrates colony differences. (F) Wound-healing assays demonstrate reduced migration with HSP90 inhibition and increased migration with overexpression ($P < 0.01$, $P < 0.001$). NC/si-NC: negative control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [manuscript2.docx](#)
- [SupplementaryFileUncroppedWesternBlotsFigure6A..pdf](#)