Journal of proteome research

Article

Cite This: J. Proteome Res. 2017, 16, 3961-3968

pubs.acs.org/jpr

Mass Spectrometric Analysis of SOX11-Binding Proteins in Head and Neck Cancer Cells Demonstrates the Interaction of SOX11 and HSP90α

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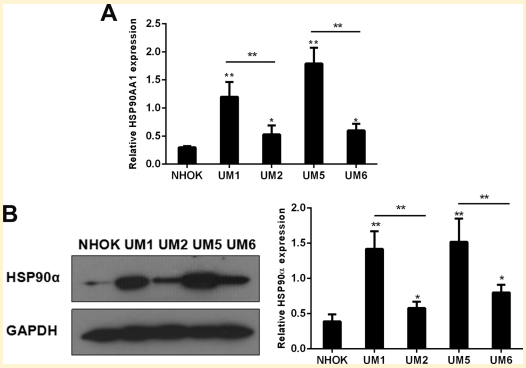
Supporting Information

ABSTRACT: Deregulated expression of SOX11 has been shown to be involved in the progression of various types of cancer. However, the role of SOX11 in head and neck cancer remains largely unknown. In this study, coimmunoprecipitation (Co-IP) and liquid chromatography−tandem mass spectrometry (LC−MS/MS) were performed to identify the proteins that bind to SOX11 at significantly higher levels in head and neck cancer cells than in normal human oral keratinocytes. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses indicated that many potential SOX11-binding partners were associated with protein synthesis, cell metabolism, and cell−cell adhesion. One of the identified proteins, heat shock protein 90 alpha (HSP90α), was selected for further investigation. The binding of HSP90α with SOX11 in head and neck cancer cells was validated by Co-IP with western blotting. In addition, HSP90α was found to be remarkably overexpressed in head and neck cancer cell lines when compared to its level in normal human oral keratinocytes, and knockdown of HSP90α inhibited the proliferation and invasion capacity of these cancer cells. On the basis of The Cancer Genome Atlas (TCGA) data analysis, HSP90AA1 gene was overexpressed in head and neck cancer tissues compared to normal controls and increased HSP90AA1 gene expression was positively associated with extracapsular spread and clinical stage. Head and neck cancer patients with higher HSP90AA1 expression had significantly poorer long-term overall and disease-free survival rates than those with lower HSP90AA1 expression. Collectively, our studies indicate that SOX11 binds to HSP90α, a highly overexpressed protein that may promote invasion and progression of head and neck cancer cells.



KEYWORDS: *HSP90α, head and neck cancer, SOX11*

INTRODUCTION

Head and neck cancer (HNC) is an aggressive malignancy that arises in the upper respiratory−digestive tract. It is the sixth most common type of cancer worldwide, and squamous cell carcinoma represents up to 90% of HNC.[1] Despite new advances for the treatment of HNC, the 5 year overall survival of patients with HNC remains at about 50%.[2,3] This highlights the importance of studying the molecular mechanisms underlying HNC initiation and progression and discovering molecular-targeted therapy to treat this disease.

The SRY (sex determining region Y)-related HMG (high-mobility group) box (SOX) family comprises 20 genes that have been shown to be involved in regulating many biological processes such as embryonic development, cell-fate decisions, and lineage commitment, determination, and differentiation.[4−6] This family of transcription factors is divided into nine subgroups based on the level of amino acid conservation within the HMG box and the presence of other motifs. SOX11 is a transcriptional activator that falls in subgroup C, which includes SOX4, SOX11, and SOX12.[4] Aberrant expression of SOX11 has been reported in various types of solid tumors such as glioma,[7] gastric cancer,[8] mantle cell lymphoma,[9] and epithelial ovarian tumor.[10] The role of SOX11 in the tumor microenvironment is cancer type-dependent. For instance, SOX11 functions as a tumor suppressor in prostate cancer,[11] whereas it plays an oncogenic role in aggressive mantle cell lymphoma.[12]

In this study, we first employed coimmunoprecipitation (Co-IP) and liquid chromatography−tandem mass spectrometry (LC−MS/MS) to identify the proteins that bind to SOX11 at significantly higher levels in HNC cells than in normal human oral keratinocytes (NHOKs). Then, one of the drastically changed targets, heat shock protein 90 alpha (HSP90α), was

Received: April 24, 2017
Published: September 15, 2017

DOI: 10.1021/acs.jproteome.7b00247
J. Proteome Res. 2017, 16, 3961−3968

chosen for further investigation. HSP90α is an abundant cellular protein, constituting about 1−2% of total protein in nonstressed tissues and about 4−6% in stressed tissues.[13,14] It functions to assist proper protein folding and prevent aggregation of non-native proteins.[15] We have demonstrated the direct binding between SOX11 and HSP90α in HNC cells and explored the functional role of HSP90α in HNC.

MATERIALS AND METHODS

Cell Culture

Four head and neck squamous cell carcinoma (HNSCC) cell lines, UM1, UM2, UMSCC5 (UM5), and UMSCC6 (UM6), were cultured in DMEM with 10% fetal bovine serum, 100 units/mL penicillin G, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA). NHOKs were cultured in keratinocyte basal media containing keratinocyte growth factors (Invitrogen). Cell cultures were maintained in a humidified atmosphere of 5% CO_2, 95% air at 37 °C, and the culture medium was changed every 3 days.

Coimmunoprecipitation

For Co-IP analysis, UM1, UM2, UM5, UM6, and NHOK cells were washed with PBS, harvested, and lysed with a buffer containing 50 mM Tris-base, 150 mM NaCl, 1 mM EDTA, 1% NP-40, and 1× protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Lysates were spun at 14 000*g* for 10 min at 4 °C, and the supernatant was harvested for Co-IP experiments. M-280 tosyl activated Dynabeads (Invitrogen) were placed on the magnet and washed (×3) with buffer A containing 0.1 M borate at pH 9.5. Anti-SOX11 and IgG (Santa Cruz Biotechnology, Dallas, TX) were then added, respectively. The tubes were incubated on a roller at 37 °C for 22 h after adding buffer A and buffer C (3 M ammonium sulfate in buffer A). The beads were placed back on the magnet followed by washing (×3) with buffer A. Buffer D containing 0.5% (w/v) BSA in PBS was then added to each tube, and the tubes were incubated at 37 °C for 1 h on a roller. Afterward, the beads were placed back on the magnet followed with washing (×3) with buffer E containing 0.1% (w/v) BSA in PBS. A cell lysate supernatant sample was then added to the beads and incubated at 4 °C for 48 h on a roller. Finally, the beads were placed back on the magnet followed by washing (×3) with buffer E, and the beads were eluted using citric acid (50 mM, pH, 2.9) followed by neutralization with 1 M NaOH. The eluates were then subjected to LC−MS/MS or western blot analysis.

Gel Electrophoresis

Samples eluted from Co-IP were separated on 10% SDS-PAGE gels, and the gels were fixed for 30 min with 50% methanol and 10% acetic acid. After fixation, the gels were stained with the Coomassie blue to visualize the protein bands. Gel lanes were excised for subsequent in-gel tryptic digestion.

LC−MS/MS and Database Searching

Proteins in SDS-PAGE gel pieces were reduced using 25 mM dithiothreitol (DTT) and alkylated with 50 mM iodoacetamide followed by in-gel trypsin digestion (protein/trypsin ∼ 50:1) at 37 °C for 16 h. The resulting peptides from each sample were desalted using a C18 cartridge prior to LC−MS/MS analysis. In brief, the peptides were separated on a C18 nano column (75 μm × 150 mm, 3 μm, 100 Å, C18(Dionex, Sunnyvale, CA) at a flow rate of 250 nL/min on an UltiMate 3000 LC system (Dionex) using a 3 h gradient of solvent A (2% acetonitrile in 0.1% FA) and solvent B (85% acetonitrile in 0.1% FA). The gradient settings were as follows: 1−5% B from 0 to 10 min, 30% B at 150 min, 50% B at 170 min, and 95% B at 180 min. The MS/MS spectra were acquired on a Q Exactive mass spectrometer (Thermo Scientific, Canoga Park, CA) using the data-dependent analysis mode, where the top 10 most abundant ions in each MS scan (m/z 400−2000) were selected for MS/MS analysis. The capillary temperature was set to 275 °C, and the spray voltage set to 2.15 kV. The resolution for the MS scan was 70 000 full width at half-maximum (fwhm), and it was 17 500 fwhm for the MS/MS scan. The automatic gain control (AGC) target for full MS was 1×10^6, and it was 5×10^4 for the MS/MS scan. The lock mass was used for accurate mass measurement. The MS/MS spectra were searched against the UniRef100 human database (120 982 entries) using the Mascot search engine (version 2.3) through the Proteome Discoverer platform. The mass tolerance was 10 ppm for MS and 0.1 Da for MS/MS. The variable modifications included methionine oxidation and protein N-terminus acetylation, whereas the fixed modification included cysteine carbamidomethylation. All proteins and peptides were identified with a false discovery rate of less than 1%. Relative quantitation was calculated based on a spectral counting method, where the spectral counts of identified proteins in cancer cells were divided by their corresponding spectral counts in NHOKs. To avoid a large ratio from the presence of zero in the denominator, we arbitrarily added 1 spectral count to both the nominator and denominator during the ratio calculation.

Western Blotting

Cells were lysed in rehydration buffer containing 7 M urea, 2 M thiourea, 50 mM DTT, 4% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 5% glycerol, and 10% isopropanol. The total concentration of protein was quantified using a micro BCA assay. Lysates were separated on a 4−12% Bis-Tris NuPAGE gel (Invitrogen), electrophoretically transferred to nitrocellulose membrane, and blocked with 5% nonfat milk (Santa Cruz Biotech) in TBS-Tween (1×). The membranes were then incubated with a mouse polyclonal antibody against human Hsp90α (Santa Cruz Biotech) at a dilution of 1:300 overnight, followed by incubation with HRP-linked goat anti-mouse IgG (1:2000; GE Healthcare, Marlborough, MA). The detection was performed with the ECL-Plus western blotting reagent kit (GE Healthcare).

Quantitative PCR

Total RNA was extracted from cells using the Quick-RNA Mini prep (Zymo Research, Irvine, CA) following the manufacturer's protocol. The Superscriptase III kit (Invitrogen) was used to synthesize first-strand cDNA. The cDNA was then amplified with Light Cycler 480 SYBR Green I MasterMix (Roche, Indianapolis, IN) on a CFX96 qPCR instrument (Bio-Rad, Hercules, CA). Human β-actin was used as the reference gene for normalization, and the $2^{-\Delta\Delta C_t}$ method was employed to calculate relative expression levels of mRNA. PCR reactions were conducted in triplicate, and data are representative of three separate experiments.

siRNA Knockdown of HSP90α in Cancer Cells

UM1/UM5 cells were transfected with siRNA in 96-well or 6-well plates using the Lipofectamine 3000 transfection reagent (Invitrogen) according to the company's instructions. Validated double-stranded siRNAs of HSP90α (sc-35608) or nontarget control siRNAs (Santa Cruz Biotech) were mixed with the transfection reagent and then added to the cell culture. After

DOI: 10.1021/acs.jproteome.7b00247
J. Proteome Res. 2017, 16, 3961−3968

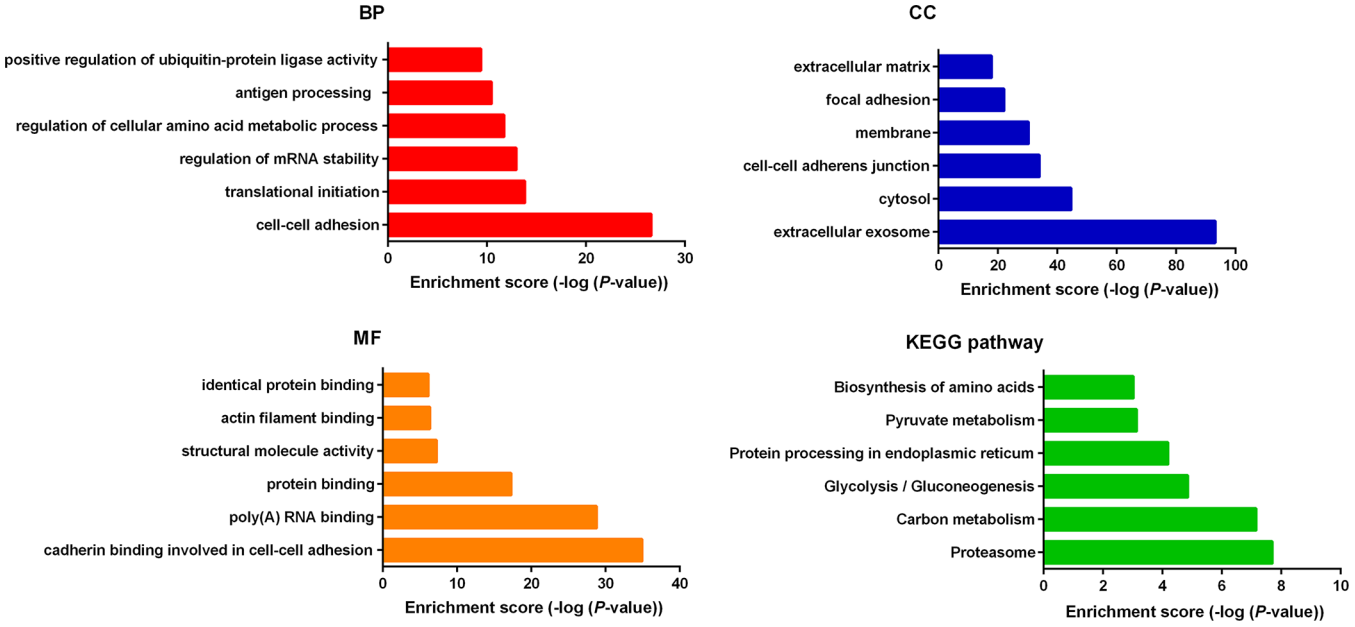


Figure 1. Gene Ontology and KEGG analyses of potential SOX11-binding proteins according to their biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways in UM1 cells. Similar analyses were also performed for UM2, UM5, and UM6 cells, and the results are shown in Figures S1, S2, and S3, respectively.

overnight incubation, the siRNAs were removed and the cells were further cultured in fresh media for 48 h.

Cell Counting Assay

Cells were harvested from 10 cm Petri dishes by 0.25% trypsin treatment to prepare single-cell suspensions, followed by counting the cells with a Vi-CELL cell counter (Beckman Coulter, Brea, CA). Then, the cells were seeded into 24-well plates at a density of 2.5 × 104 cells per well. At the indicated time points, cells were harvested and resuspended in 500 μL of complete media and subjected to cell counting analysis.

Invasion Assay

The invasion assays were performed with Matrigel-coated transwells (BD Biosciences, San Jose, CA). Following 24 h of serum starvation, cells were harvested and resuspended in DMEM containing 0.1% FBS, and 5 × 10^4 cells were then added to the upper chamber of the transwell inserts. DMEM containing 10% FBS was used as a chemoattractant in the lower chamber. After 24 h, cells that had migrated through the membrane were fixed and stained with the HEMA 3 staining kit (Thermo Fisher, Waltham, MA). The invaded cells in four random fields were counted under a light microscope and expressed as the average number of cells per field.

HSP90AA1 Expression in Tumor Samples and Its Clinical Significance

The clinical information and RNASeq V2 data sets of HNC cancer patients were obtained from The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/docs/publications/tcga/) to determine the clinical significance of HSP90AA1 gene expression in HNC. Briefly, the mRNA expression levels were $\log_2$-transformed, and X-tile software was used to find the best cutoff point to divide the cancer patients into high/low HSP90AA1 expression groups.[16] Kaplan−Meier overall survival curves were generated for patients whose clinical follow-up data were available. The log-rank test was used for survival analysis between the two groups.

Statistical Analysis

The data were expressed as the mean ± standard deviation and analyzed by an independent samples *t*-test using MedCalc (MedCalc Software, Ostend, Belgium). Gene Ontology and KEGG pathway enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics resource (https://david-d.ncifcrf.gov/). Again, the optimum cutoff point of mRNA expression in the TCGA patient cohort was calculated using the X-tile software. Briefly, the gene expression data of HSP90AA1 in HNC patients and the related patient survival information, including survival time and status (alive or dead), were loaded into the X-tile software. The cancer patients were then categorized into two groups (high and low HSP90AA1 expression) by running the Kaplan−Meier program. A chi-squared test was used to determine the association between the HSP90AA1 mRNA expression level and the clinicopathological parameters of the HNC patients. The Kaplan−Meier method in combination with the log-rank test was performed for survival analysis, and P values < 0.05 were considered statistically significant.

RESULTS

LC−MS/MS Identification of SOX11-Interacting Proteins

Using a LC−MS/MS and spectral counting method, we compared the proteins in the Co-IP samples (UM1, UM2, UM5, UM6, and NHOK cells) that potentially interacted with SOX11. In total, 919 proteins were identified with LC−MS/MS and database searching (Table S1). A number of SOX11-binding partners (n = 229) were found to be significantly (ratio ≥ 2) increased in all HNC cancer cell lines when compared to NHOKs. Gene Ontology and KEGG pathway enrichment analysis of the identified proteins were performed using the DAVID bioinformatics resource. For the "biological processes (BP)", cell−cell adhesion, translational initiation, regulation of mRNA stability, and regulation of cellular amino acid metabolic

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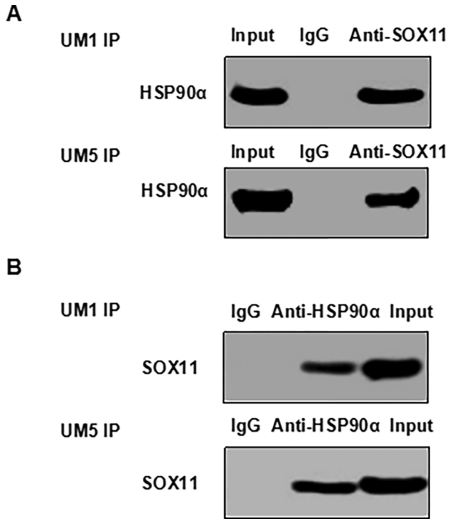

Figure 2. (A) Western blotting detection of HSP90α in whole cell lysates, anti-SOX11 pull-down samples, and negative control samples. The Co-IP assay suggested that SOX11 binds to HSP90α in UM1 and UM5 cells. (B) Western blotting detection of SOX11 in whole cell lysates, anti-HSP90α pull-down samples, and negative control samples. The Co-IP assay indicated that HSP90α interacts with SOX11 in both UM1 and UM5 cells.

process were the commonly enriched categories. For the "cellular component (CC)" ontology, commonly enriched categories among the cancer cell lines were associated with extracellular exosome, cytosol, cell−cell adherens junction, membrane, focal adhesion, and extracellular matrix. With regard to the "molecular function (MF)", the SOX11-binding partners mainly showed enrichment in the GO categories including cadherin-related cell−cell adhesion, poly(A) RNA binding, protein binding, and actin filament binding. Finally, the KEGG pathway analysis indicated that the SOX11-binding partners mapped to common pathways in HNC cell lines, such as proteasome, protein processing in endoplasmic reticulum, and carbon metabolism (Figures 1, S1, S2, and S3).

Binding of SOX11 to HSP90α

MS analysis showed that HSP90α is ranked as one of the most abundant proteins that bind to SOX11 in HNC cancer cells when compared to NHOK (UM1/NHOK = 9.8, UM2/NHOK = 8.4, UM5/NHOK = 8.6, and UM6/NHOK = 7.4). To determine if SOX11 interacts with HSP90α in HNC cells, we performed Co-IP/western blotting analysis with the protein lysates from UM1 and UM5 cancer cells. As shown in Figure 2, HSP90α was coimmunoprecipitated by the anti-SOX11 antibody in both UM1 and UM5 cells (Figure 2A). Meanwhile, SOX11 was also pulled down by the anti-HSP90α antibody in these two cell lines (Figure 2B).

Downregulation of HSP90α Inhibits the Proliferation and Invasion Capacity of HNC Cells

Quantitative PCR and western blot analyses both showed that HSP90α was significantly overexpressed in all four HNC cell lines when compared to NHOKs. Moreover, the expression level of HSP90α was higher in highly invasive cancer cells (UM1 and UM5) compared to the weakly invasive cancer cells (UM2 and UM6) at both the mRNA and protein levels ($^*P < 0.05$, $^{**}P < 0.01$) (Figure 3A,B).

The expression level of HSP90α was significantly suppressed in the UM1 and UM 5 cells transfected with HSP90α siRNA (siHSP90α) when compared to those transfected with scrambled control siRNA (siCTRL) (Figure 4A). Cell proliferation was determined by a cell counting assay in UM1 cells at 24, 48, 72, and 96 h after siRNA transfection. UM1 cells with siHSP90α transfection experienced a significant reduction in cell number when compared to the controls after 72 and 96 h ($^{**}P < 0.01$). Similarly, the proliferative capacity of UM5

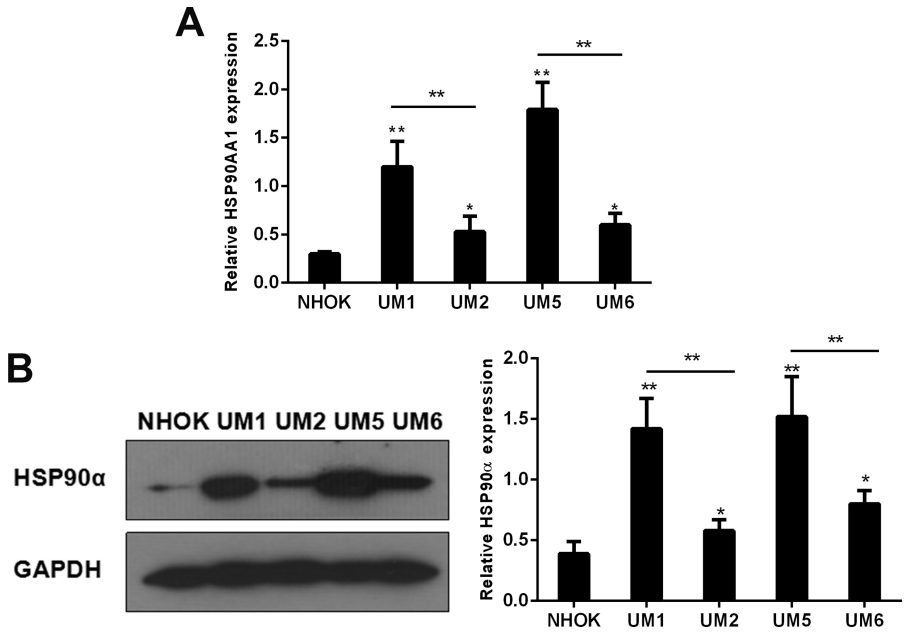

Figure 3. HSP90α upregulation in HNC cells. (A) Quantitative PCR analysis showed that HSP90α mRNA was significantly overexpressed in all four HNC cell lines compared to NHOKs. In addition, HSP90α mRNA levels were significantly higher in highly invasive cancer cell lines (UM1 and UM5) than weakly invasive cancer cell lines (UM2 and UM6). (B) Western blot analysis showed that HSP90α was significantly overexpressed in HNC cancer cells, particularly in UM1 and UM5 cells, which is in line with the quantitative PCR results, further confirming that HSP90α is overexpressed in these cancer cells.

DOI: 10.1021/acs.jproteome.7b00247
J. Proteome Res. 2017, 16, 3961−3968

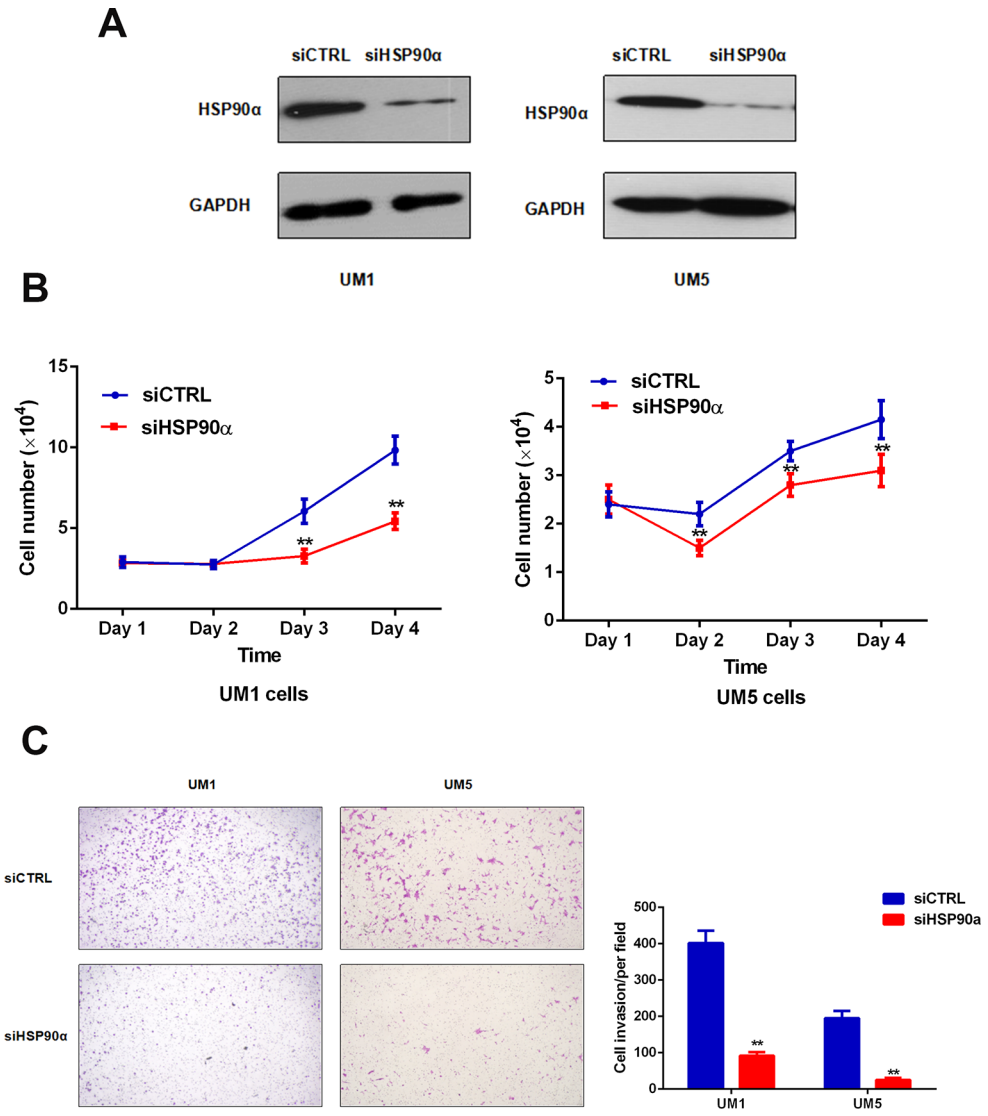

Figure 4. (A) Western blot analysis of the expression levels of HSP90α protein. Significant downregulation of HSP90α was seen in both UM1 and UM5 cells following siHSP90α transfection. (B) HSP90α downregulation inhibited the proliferation of HNC cells. The cell counting assay results showed that the number of UM1 cells transfected with siHSP90α was significantly lower than the cells transfected with siCTRL. Similar results were observed in UM5 cells. (C) HSP90α suppression inhibited the invasive capacity of HNC cells. The number of HSP90α knockdown UM1 cells that migrated through the membrane was significantly lower than the controls (**$P < 0.01$). Similarly, the number of HSP90α-suppressed UM5 cells that migrated through the membrane was significant lower than the cells in the controls (**$P < 0.01$). Each experiment was repeated in triplicate. All values are expressed as the mean ± standard deviation.

cells was significantly reduced following siHSP90α knockdown (**$P < 0.01$) (Figure 4B).

To investigate whether HSP90α is involved in regulating the invasive capability of HNC cells, a Matrigel invasion assay was used to evaluate the invasion capacity of UM1/UM5 cells after siHSP90α knockdown. Compared to control cells, knockdown of HSP90α expression in UM1/UM5 cells showed a significant reduction in the number of cells invading through the transwell membrane (**$P < 0.01$) (Figure 4C)

HSP90AA1 Gene Expression Is Upregulated in HNC and Associated with Poor Prognosis

The gene expression level of HSP90AA1 was found to be significantly increased in HNC tissues when compared to that in adjacent normal tissues ($P < 0.0001$) (Figure 5A). In addition, upregulated tissue HSP90AA1 expression was positively associated with extracapsular spread ($P = 0.0079$) and clinical stage ($P = 0.0377$) in HNC patients (Table 1). Moreover, the HNC patients in the high HSP90AA1 expression group had remarkably shorter long-term overall survival ($P = 0.0003$) and disease-free survival ($P = 0.0133$) when compared to those in the low HSP90AA1 expression group (Figure 5B).

DISCUSSION

HNC remains a significant public health problem in both developed and developing countries. The carcinogenesis of HNC is a multistep process characterized by genetic alterations that influence key cellular pathways of growth and development.[2] However, the underlying molecular mechanisms accounting for the initiation, promotion, progression, and metastasis of this malignancy remain largely unknown.

Our recent studies have demonstrated that SOX11 plays a tumor promotion role in the development of HNC.[17] However, determining what proteins bind to SOX11 in HNC cells remains an open question. In this study, we employed a LC–

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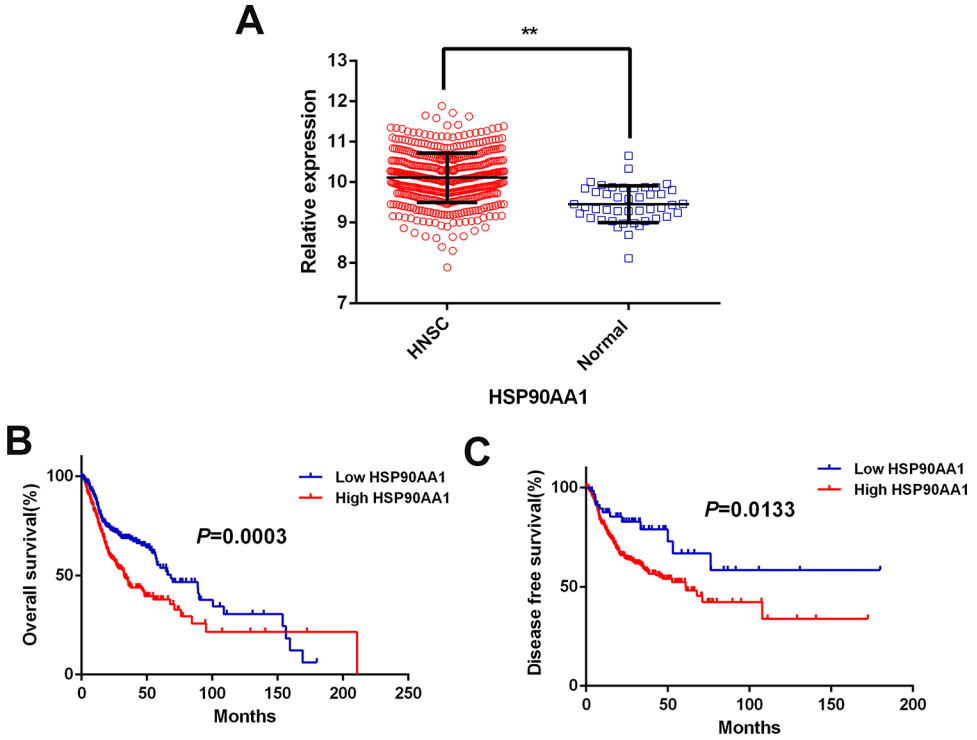

Figure 5. Gene expression level and clinical significance of HSP90AA1 in HNC. (A) HSP90AA1 was significantly upregulated in tissues of HNC patients compared to the normal controls ($P < 0.0001$). HNC patients in the high HSP90AA1 expression group suffered poorer (B) long-term overall ($P = 0.0003$) and (C) disease-free survival ($P = 0.0133$) than those in the low HSP90AA1 expression group.

Table 1. Association between HSP90α mRNA Expression Level and Clinicopathological Parameters of HNC Patients

	HSP90α mRNA expression		
clinicopathological feature	low	high	*P*
age	60.77 ± 12.10	60.99 ± 11.72	0.8377
gender			
female	77	61	0.1309
male	184	197	
T stage			0.1034
T1−T2	102	84	
T3−T4	150	167	
N stage			0.1771
N0	129	113	
N1−N3	120	134	
M stage			1.0000
M0	244	244	
M1	3	3	
stage			**0.0377**
I−II	69	49	
III−IV	184	203	
angiolymphatic invasion			0.2947
no	118	108	
yes	57	66	
extracapsular spread			**0.0079**
no	133	109	
yes	45	66	

MS based approach to identify novel targets that may interact with SOX11 in HNC cells. The proteins that strongly bind to SOX11 in HNC cells may be important for maintaining the activity, stability, and function of SOX11 or may be regulated by SOX11. On the basis of Gene Ontology and KEGG enrichment analyses of the identified proteins, "cell−cell adhesion" has the highest enrichment score in the "biological process" category. Cell adhesion related molecules have been demonstrated to play a significant role in the metastatic potential of HNC.[18,19] SOX11 is a transcriptional factor, and its upregulation in HNC cells may well affect a large number of proteins associated with "cell-to-cell adhesion","translational initiation", and "regulation of mRNA stability". For the "cellular component" category, "extracellular exosome" shows the highest enrichment score. Extracellular exosome contents may include proteases, growth factors, bioactive lipids, phosphoproteins, mutant oncoproteins, oncogenic transcripts, tumor suppressors, microRNAs, and DNA sequences.[20] Most cancer cells release a heterogeneous population of extracellular exosomes to promote carcinogenesis. In regard to the "molecular function (MF)" category, the most affected molecular function is cadherin binding involved in cell−cell adhesion. The perturbation of cadherin function causes temporal or permanent disaggregation of tumor cells and might promote the invasion and metastasis of cancer cells.[21] For the KEGG pathway analysis, most of the identified pathways are related to "biosynthesis of amino acids", "cancer metabolism protein processing", and "proteasome". Collectively, we speculate that upregulation of SOX11 might first activate the aggressive phenotype of HNC cells by modulating oncoprotein synthesis and altering cellular metabolism. Then, it might further promote invasion and metastasis by affecting the cell−cell adhesion system and the formation and release of extracellular exosomes.

As shown in Table S1, HSP90α represents one of the top candidates that may interact with SOX11, as revealed by Co-IP with LC−MS/MS. A few structural proteins such as type II cytokeratin, α-actinin, MSN protein, cytoskeleton-associated

DOI: 10.1021/acs.jproteome.7b00247
J. Proteome Res. 2017, 16, 3961−3968

protein, and myosin show a higher binding capacity to SOX11, which may result from nonspecific binding due to their highly abundant levels in cells. Other top candidates include annexin, GTPase activating protein 1, purine nucleoside phosphorylase, ubiquitin-like modifier activating enzyme 1, 14-3-3, plastin-2, expotin-2, DJ-1, elongation factor 2, and protein disulfide isomerase, which are all tumor-associated molecules. Besides HSP90α, other members of the heat shock protein family such as highly similar to HSP105, HSP70, and highly similar to HSP60 may also interact with SOX11 (Table S1).

HSP90α is a chaperone protein that assists protein folding, stabilizes proteins against heat stress, aids in protein degradation, and facilitates cell signaling. Previous studies have proven that HSP90α stabilizes proteins (e.g., EGFR, PI3K, and AKT) that are important drivers of oncogenic signaling.[22−24] In this study, we first demonstrated that SOX11 interacts with HSP90α in HNC cells, but this interaction barely occurs in NHOKs. Then, Co-IP with western blot analysis was used to further validate the binding between these two molecules in HNC cells. SOX11 is a transcriptional factor involving in regulating many important biological processes. Our finding suggests that SOX11 might serve as a new substrate of HSP90α, and studying the HSP90α/SOX11 axis in HNC cells may lead to a potential novel mechanism accounting for the oncogenic role of SOX11 in HNC. Thus, we further investigated the role of HSP90α in HNC.

Our results indicated that HSP90α was significantly overexpressed in HNC cells, especially in invasive cancer cell lines (e.g., UM1 and UM5). In addition, HSP90α suppression inhibited the proliferation and invasion of HNC cells. Moreover, the expression level of HSP90α was significantly increased in HNC tissues, and increased HSP90α gene expression was positively associated with adverse clinical parameters such as advanced clinical stage and extracapsular spread. HNC patients with higher HSP90α gene expression suffered worse long-term overall and disease-free survival rates than those with lower HSP90α expression. Therefore, our study provides strong evidence demonstrating that HSP90α might play an oncogenic role in the development of HNC. Consistent with our findings, the expression level of HSP90α was found to be significantly upregulated in HNC invasive cell lines.[25] Future studies are warranted to explore the tumor promotion function of HSP90α *in vivo* and validate its clinical significance in HNC.

The molecular chaperone HSP90α is frequently deregulated in many types of cancers. For instance, the expression of HSP90α mRNA was significantly higher in breast cancer tissues than in noncancer tissues. In addition, a positive correlation was observed between HSP90α and cyclin D1 in cancer tissues, indicating that HSP90α might be important for the proliferation of breast cancer cells.[26] HSP90α was demonstrated to be a downstream target of the IL-6R/STAT3 and MAPK signaling pathways, which are important for maintaining the survival of multiple myeloma cells, and inhibition of HSP90α was sufficient to induce the apoptosis of the cancer cells.[27] HSP90AA1 expression was upregulated in primary gastric cancer (GC) when compared to normal tissues, and it was also significantly increased in metastatic GC compared to primary GC, indicating that HSP90α might be a valid disease biomarker and therapeutic target for GC.[28]

In conclusion, our studies have identified a number of proteins that may interact with SOX11 in HNC cells, including HSP90α. A biochemical interaction was validated between SOX11 and HSP90α through Co-IP with western blot analysis. In addition, we found that downregulation of HSP90α inhibits the malignant phenotype of HNC cells and that HSP90α upregulation is significantly associated with worse clinical outcome of HNC, suggesting that HSP90α might serve as a potential prognostic biomarker and therapeutic target for HNC.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.7b00247.

Comparison of identified proteins and their relative quantitation in Co-IP samples (Table S1) (PDF)
Gene Ontology and KEGG analysis of the potential SOX11-binding proteins according to the biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways in UM2 cells (Figure S1) (PDF), UM5 cells (Figure S2) (PDF), and UM6 cells (Figure S3) (PDF)

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Notes

The authors declare no competing financial interest.

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DOI: 10.1021/acs.jproteome.7b00247
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