

Silencing Heat Shock Protein 27 Decreases Metastatic Behavior of Human Head and Neck Squamous Cell Cancer Cells in Vitro

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Abstract: The small heat shock protein 27 (Hsp27) is a molecular chaperone that is involved in a variety of cellular functions in cancer cells. The purpose of this research is to study Hsp27 in vitro metastatic behaviors of head and neck squamous cell carcinoma cells (HNSCC). The expression of Hsp27 in primary and metastatic cell lines derived from the primary HNSCC and a synchronous lymph node metastasis in the same patient was determined using real-time PCR and Western blotting. Proliferation of the primary and metastatic HNSCC cell lines was evaluated using the MTS proliferation assay. Metastatic behavior was assessed using migration and invasion assays. siRNA knockdown of Hsp27 was performed in the highly migratory metastatic HNSCC cell line. MTS assays showed that the primary (UM-SCC-22A) and metastatic (UM-SCC-22B) HNSCC have similar proliferation rates. However, UM-SCC-22B derived from the metastasis showed 2.3- to 3.6-fold higher migration ability and 2-fold higher invasion ability than UM-SCC-22A. Real-time PCR demonstrated that Hsp27 mRNA is 22.4-fold higher in metastatic UM-SCC-22B than primary UM-SCC-22A. Similarly, Western blotting showed that Hsp27 is rarely detectable in UM-SCC-22A whereas UM-SCC-22B expresses a 25-fold higher level of Hsp27 protein. siRNA-mediated knockdown of Hsp27 in UM-SCC-22B reduced Hsp27 mRNA expression by nearly 6-fold and protein expression by 23-fold. Furthermore, siRNA knockdown of Hsp27 decreased metastatic behaviors of UM-SCC-22B by 3- to 4-fold in migration and 2-fold in cell invasion reducing cell invasion and migration to levels similar to the primary HNSCC UM-SCC-22A. These data indicate that Hsp27 may regulate metastatic potential of HNSCC cancer cells. Targeting Hsp27 may decrease metastasis in head and neck squamous cell cancer cells.

Keywords: Heat shock protein 27; human head and neck squamous cell cancer; metastasis; siRNA

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent cancer type worldwide with an incidence

of more than 500,000 cases annually and a high mortality rate, making it the fifth leading cause of cancer related death.¹ In the United States, it accounts for 6% of all cancer diagnoses and results in an estimated 14,000 deaths annually.² Despite the advanced therapeutic regimens used in treating HNSCC, oral cavity cancer survival during 1996–2003

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was less than 50% on average according to the data from American Cancer Society. Furthermore, patients with recurrent or metastatic HNSCC have median survival of approximately 6 months.^{1,2} The major contributing factors for low survival include local-regional relapse, lymph node or distant metastatic spread of the primary tumor.^{3,4}

Tumor progression to the invasive and metastatic stage represents the most ominous challenge in the management of HNSCC. Metastasis of HNSCC is a complex process associated with multiple biochemical and genetic changes. Increased cell motility and invasive growth are considered to be important in the metastatic cascade. Degradation of extracellular matrix and tumor angiogenesis may also contribute to HNSCC metastasis. Several proteins have been studied in HNSCC metastatic disease such as Hsp90, HER2,⁵ MMP2,^{6,7} MMP9,⁷ TGF- β 1.⁸ However, the actual mechanisms driving metastasis of HNSCC remain unclear.

Recent studies suggest that high levels of heat shock proteins (HSPs) and heat shock factor 1 (HSF1) that regulates HSP expression promote prostate adenocarcinoma tumor cells to invade and to spread to distant organs.^{9,10} Clinical studies showed a high correlation between HSF1 overexpression and HSPs including Hsp27 and Hsp70 which have

also been shown to be correlated with invasion and/or metastasis of prostate cancer.^{10–12}

Hsp27 acts as a molecular chaperone in cellular responses for a variety of stresses such as heat shock, toxicants, and oxidative stress.¹³ Hsp27 has also been shown to have various functions in signal transduction, regulation of growth, differentiation, and tumorigenesis. High levels of Hsp27 have been found in prostate cancer,¹⁴ human hepatocellular carcinoma,^{15,16} renal cell carcinoma,¹⁷ oral tongue squamous cell carcinoma,¹⁸ and breast cancer.¹⁹ Garrido et al. overexpressed Hsp27 in REG colon cancer cells and increased REG cell tumorigenicity in the syngeneic host.^{20,21} Furthermore, overexpression of Hsp27 has also been linked to the metastasis of several tumor types including prostate cancer,¹⁴ hepatocellular cancer,¹⁵ tongue squamous cell cancer.¹⁸ For example, a high level of Hsp27 expression was associated

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with tongue squamous cell cancer invasion and metastasis.²² Curiously, Nakajima et al. showed that patients with Hsp27-negative esophageal squamous cell carcinoma tended to have a poorer prognosis compared with patients with Hsp27-positive tumors.²³ The function of Hsp27 in head and neck squamous cell cancer has not been fully understood.

We observed that Hsp27 was overexpressed in a head and neck squamous cell carcinoma from a metastatic lymph node, but rarely expressed in the primary cancer cells from the same patient. We hypothesize that Hsp27 potentially may be involved in the metastasis of head and neck squamous cell carcinoma cells (HNSCC). These head and neck squamous cancer cell lines served as paired controls to study Hsp27 function. We first studied the expression level of Hsp27 in both metastatic and primary head and neck squamous cell cancer cells, and then we correlated the expression levels of Hsp27 to cell motility and invasion. siRNA was used to knock down Hsp27 in metastatic head and neck squamous cell carcinoma cells to study its function in metastasis.

Material and Methods

Materials and Cell Culture. UM-SCC-22A and UM-SCC-22B cells were isolated from a head and neck cancer patient who presented with synchronous primary and metastatic cancer to the head and neck cancer program at the University of Michigan. The primary head and neck squamous cancer cells (UM-SCC-22A) were obtained from the surgical resection specimen of the primary cancer of the hypopharynx, while the metastatic head and neck squamous cancer cell line (UM-SCC-22B) was established from a metastatic lymph node of the same patient. This was an aggressive tumor that progressed and caused the death of the patient within 8 months of diagnosis. All cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Grand Island, NY) supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin (100 units/mL) and streptomycin (100 µg/mL), and 100 nM nonessential amino acids. Both cell lines were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

MTS Cell Proliferation Assay. Various numbers (10–5000) of UM-SCC22A and UM-SCC22B cells in 200 µL of DMEM/well supplemented with 10% fetal bovine serum were seeded to the wells of a 96-well plate, cultured for 24 h/48 h. The medium was allowed to equilibrate for 1 h, and then 25 µL/well of combined MTS (3-(4,5-dimethylthiazol-

2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt)/PMS (phenazine methosulfate) solution was added. After culturing at 37 °C in a humidified 5% CO₂ atmosphere for 2.5 h, the absorbance at 490 nm was measured using Bio-Tek Elisa plate reader.

Real-Time PCR Assay. RT-PCR was carried out as described previously.²⁴ Briefly, TRIzol reagents (Invitrogen, Carlsbad, CA) were used to extract total cellular RNAs as described in the protocol provided by the manufacturer. Superscript III first strand synthesis kit from Invitrogen was used to reverse transcribe the cDNA. Then the real-time PCR is carried out in ABI PRISM 7900T real-time PCR system (Perkin-Elmer, Branchburg, NJ) using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). The primers used in RT-PCR are as follows: Hsp27, forward, 5'-GTC CCT GGA TGT CAA CCA CT-3'; reverse, 5'-CTT TAC TTG GCG GCA GTC TAC-3'; internal standard β-actin, forward, 5'-GCT CGT CGT CGA CAACGG CTC-3'; reverse, 5'-CAA ACA TGC TCT GGG TCA TCT TCT-3'.

mRNA levels were calculated as fold change compared to control. After completion of the RT-PCR, Ct values (cycle numbers in which signal intensity equal to the threshold value) will be obtained from the software. For each sample, ΔCt is calculated as $\Delta C_t = C_{t_{Hsp27}} - C_{t_{\beta-actin}}$. Then ΔΔCt is calculated as $\Delta\Delta C_t = \Delta C_{t_{treatment}} - \Delta C_{t_{control}}$. The fold change of the Hsp27 mRNA levels relative to control is calculated as $2^{-\Delta\Delta C_t}$.

Western Blot. The procedure for the Western blot analysis was briefly described as follows. Cells were washed twice with ice-cold PBS, collected in RIPA lysis buffer (Thermo Scientific, USA) supplemented with a protease inhibitor mixture (Sigma; added at a 1:100 dilution), and incubated on ice for 30 min. The lysate was then centrifuged at 14000g for 15 min. Protein concentration was detected by BCA protein assay kit (Thermo Scientific, USA). Equal amounts of total protein were subjected to 20% SDS-PAGE electrophoresis, and proteins were transferred for 1 h using a Bio-Rad Semi-Dry apparatus in a transfer buffer. The PVDF membranes were incubated in blocking buffer (0.01 M PBS, 0.05% Tween-20 with 5% nonfat dry milk) for 1 h, probed with various antibodies against HSP27 (Cell Signaling Technology, USA, 1:1000), Hsp90, Hsp70, Akt, cdk4, β-actin (Santa Cruz Biotechnology, 1:1000) overnight at 4 °C, and incubated with HRP-conjugated secondary antibody for 1 h at room temperature. Peroxidase activity on the PVDF membrane was visualized on X-ray film using ECL Western blotting detection system.

Small Interfering RNA (siRNA) Knockdown of Hsp27. Gene silencing by small interfering RNA (siRNA) uses a small double-strand RNA that degrades target mRNA. HSP27 siRNA duplexes that target the sequences as de-

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scribed previously²⁵ (sense, 5'-UGAGAGACUGCCGC-CAAGUAA-3'; antisense, 5'-UUACUUGGCGGCAGUCU-CAUU-3') were synthesized by Dharmacon (Lafayette, CO). Transfection of siRNA was carried using Lipofectamine 2000 (Invitrogen). Briefly, one day before transfection, cells were plated in 5 mL of growth medium without antibiotics. On the day of transfection, the media in 60 mm plates was replaced with 5 mL of media without antibiotics and 1 mL of Lipofectamine 2000–siRNA complex. In 1 mL of Lipofectamine 2000–siRNA complex, 10 μ L of Lipofectamine 2000 in 500 μ L of Opti-Mem (Gibco) and 200 pmol of siRNA in 500 μ L of Opti-Mem were mixed together for a final volume of 1 mL. The mixture was incubated at room temperature for 20 min. In each 60 mm plate, the final siRNA concentration was 33 nM. The cells were incubated in a 37 °C 5%CO₂ for 5 h. The media was replaced with 5 mL of media without antibiotics. Two controls (Lipofectamine and scrambled siRNA) were included in the experiments.²⁶ The scrambled siRNA (5'-AATTCTCCGAACGTGTCACGT-3') were purchased from Dharmacon (Lafayette, CO); this scrambled sequence does not match any human genome sequence. After 24–48 h, the cells were collected to measure the mRNA and protein, or the cells were used in invasion and wound healing assays.

Cell Migration Assay. Cell migration ability was monitored using a wound-healing assay. Cells were seeded at a high density on 6-well cell culture plate. After serum-free incubation for 18 h, wounds were made by scraping through the cell monolayer with a sterile micropipet tip. Cells were further incubated in cell culture medium for up to 72 h with or without siRNA transfection. Images were taken ($\times 40$) under the microscope to measure cell migration using the widths of wound in cell monolayer.

Cell Invasion Assay. The Matrigel-coated filter system (Becton Dickinson Labware, Bedford, MA) was used to assess cell invasion. The Matrigel matrix is a reconstituted and solubilized basement membrane. The Matrigel invasion chambers have pore size of 8.0 μ m (1×10^5 pores/cm²). Because the Matrigel matrix occludes the pores of the polyethylene terephthalate (PET) membrane, noninvasive cells are blocked from migrating through the membrane. The chambers were rehydrated with 500 μ L of serum-free media for 2 h at 37 °C. After rehydration, the chambers were placed in the lower compartment, which was previously loaded with 680 μ L of cell culture medium with 10% FBS. The cells were placed in the upper chamber of the Transwell with a density of 2×10^4 cells in cell culture medium with 1%

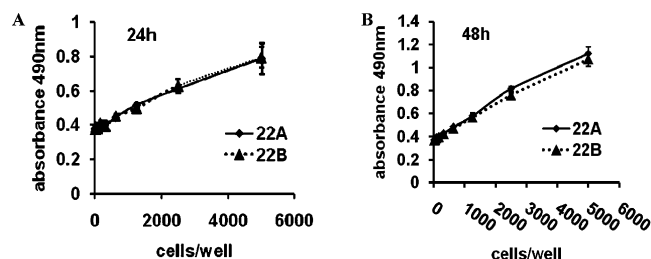


Figure 1. Growth rate of UM-SCC-22A and UM-SCC-22B. Various numbers of UM-SCC22A and UM-SCC22B cells were seeded in a 96-well plate, cultured for 24/48 h. MTS was used to measure the cell proliferation. Each point represents the mean \pm SD of 3 replicates. The correlation indicates a linear response between cell number and absorbance at 490 nm. The background absorbance shown at zero cells/well was subtracted from these data.

FBS per 500 μ L per well. The cells were allowed to invade the Matrigel at 37 °C in 5% CO₂ for 40 h. Then the cells were fixed with cold methanol, and stained with 0.5% Crystal Violet for 10 min. Cells in the upper surface of filter were removed using a cotton swab, and the cells that invaded through the Matrigel, which were located on the lower side of the filter, were counted (8 fields/filter). Experiments were repeated three times. The invasive cells were counted under the microscope, and cells in 8 different view fields were counted along a line across the center point.

Results

Metastatic Head and Neck Squamous Cell Carcinoma Cells (HNSCC) Showed Higher Invasion and Migration, but Similar Proliferation Ability Compared to Primary HNSCC. To determine the proliferation ability of metastatic head and neck squamous cell carcinoma cancer cells (UM-SCC-22B) and primary cells (UM-SCC-22A), MTS assay was used to determine the number of viable cells in proliferation after 24 and 48 h. When various cell numbers (10–5000 cells/well) were plated in a cell culture plate, UM-SCC-22A and UM-SCC-22B showed similar growth rate after 24–48 h culture ($P > 0.05$) (Figure 1). These experiments indicate that the primary (UM-SCC-22A) and metastatic (UM-SCC-22B) head and neck cancer cells have similar proliferation rates.

Next, we tested the metastatic potential of the primary and metastatic head and neck cancer cell lines using in vitro invasion and in vitro migration assays. In the invasion assay, 2×10^4 cells were added to the upper chamber in culture medium containing 1% FBS with medium containing 10% FBS in lower chamber. At 40 h, cells were fixed and stained, and then the cells on the lower surface of filter were counted under a microscope (Figure 2A). Eight fields were counted in each plate, and six experiments were conducted. The results showed that invading cell numbers of metastatic head and neck cancer cells UM-SCC-22B through Matrigel were 53.56 ± 26.71 ($n = 6$), whereas the invading cells of primary head and neck cancer cells UM-SCC-22A through Matrigel were 25.45 ± 14.57 ($n = 8$) (Figure 2B).

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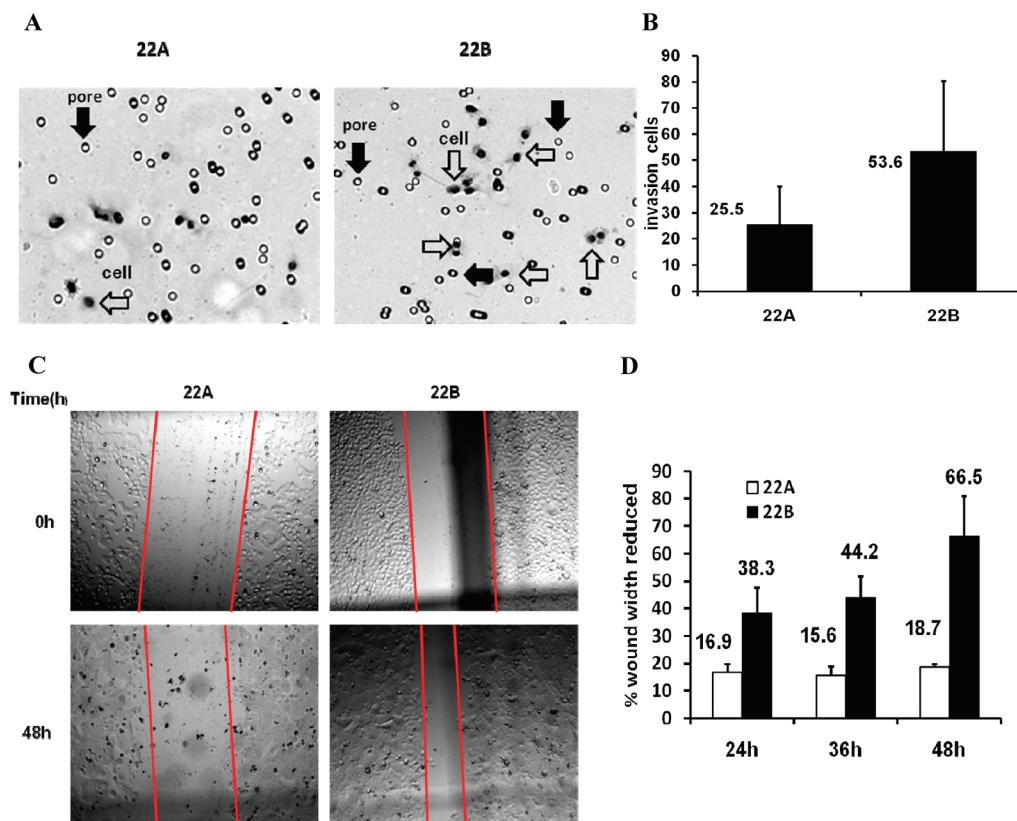


Figure 2. Cell invasion and migration of UM-SCC-22A and UM-SCC-22B. (A) Microscopy image ($\times 100$) of invading cells in Matrigel filter invasion assay from one representative experiment. Open arrows, pore; solid arrows, cell. In the fields shown, 9 cells are apparent in the UM-SCC-22A culture and 16 are apparent in the UM-SCC-22B culture. (B) Bar graph represents number of invading cells (mean \pm SD) of UM-SCC22A and UM-SCC22B from six cell invasion experiments ($*p < 0.01$). (C) Microscopy image ($\times 40$) of cell migration of UM-SCC-22A and UM-SCC-22B from one representative experiment. (D) Bar graph represents the percentage of reduced wound width (%) compared to time 0 of UM-SCC-22A and UM-SCC-22B from six cell migration experiments. Blank bar, UM-SCC-22A; filled bar, UM-SCC-22B.

In the migration assay, a gap was generated in the cell layer using a micropipet tip, and then the cells were allowed to grow and migrate for 24, 36, and 48 h (Figure 2C). After 24, 36, and 48 h, the gap width of metastatic head and neck cancer cells UM-SCC-22B was reduced by $38.30 \pm 9.30\%$, $44.24 \pm 7.31\%$ and $66.50 \pm 14.36\%$, respectively. In contrast, after 24, 36, and 48 h, the gap width of primary head and neck cancer cells UM-SCC-22A remained unchanged and was reduced only by $16.85 \pm 2.81\%$, $15.61 \pm 3.17\%$ and $18.69 \pm 1.02\%$, respectively. These data showed that UM-SCC-22B migrate 2.3- to 3.6-fold faster than UM-SCC-22A (Figure 2D). These data suggest that metastatic head and neck squamous cell carcinoma cells (UM-SCC-22B) indeed showed higher metastatic potential although it has similar proliferation ability compared to primary head and neck squamous cell carcinoma cells UM-SCC-22A.

Hsp27 mRNA and Protein Levels Are Higher in Metastatic Head and Neck Squamous Cell Carcinoma Cells (HNSCC). To investigate the expression of several heat shock proteins and oncogenic proteins in primary and metastatic head and neck squamous cell carcinoma cells (UM-SCC-22A and UM-SCC-22B), we used Western blotting to detect the level of Hsp90, Hsp70, and Hsp27, AKT,

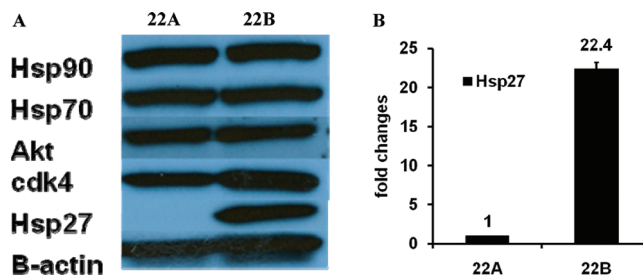


Figure 3. (A) Western blotting to detect protein levels of molecular chaperones and oncogenic proteins in UM-SCC-22A and UM-SCC-22B. The cells were lysed, and the soluble protein ($35 \mu\text{g}$ of protein per lane) was separated by SDS-PAGE and detected by Western blotting using the specific antibodies. β -Actin was used as control for protein loading. (B) Real-time PCR to detect the Hsp27 mRNA level in UM-SCC-22A and UM-SCC-22B. Fold change was calculated by $2^{-\Delta\Delta Ct}$.

and Cdk4. The results revealed that Hsp27 protein level was rarely detectable in primary UM-SCC-22A cells, while metastatic UM-SCC-22B showed 25.3-fold higher Hsp27 protein expression than UM-SCC-22A (Figure 3A). In comparison, we also monitored the expression levels of other

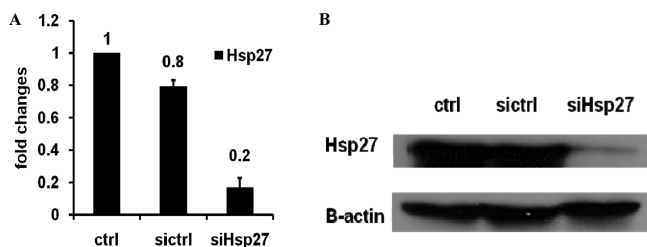


Figure 4. (A) Real-time PCR to detect Hsp27 mRNA level in UM-SCC-22B cells after siRNA transfection: ctrl, cell treated with transfection reagent Lipofectamine 2000; sictrl, cells transfected with scrambled siRNA; siHsp27, transfected with siRNA of Hsp27. (B) Western blotting to detect protein levels of Hsp27 in UM-SCC-22B cells after siRNA transfection. The cells were transfected with scrambled siRNA (sictrl) or Hsp27 siRNA (siHsp27) for 48 h. The resulting cells were lysed, and the soluble protein (35 μ g of protein per lane) was separated by SDS-PAGE and detected by Western blotting using the specific antibodies. β -Actin was used as control for protein loading.

chaperones (Hsp90 and Hsp70) and a few other oncogenic proteins (AKT, cdk4). The Western blotting result showed similar levels of these proteins in these two cell lines. To confirm the different expression levels of Hsp27 in two head and neck squamous cell carcinoma cell lines, real-time PCR was used to measure the mRNA levels of Hsp27. Real-time PCR result showed that Hsp27 mRNA expression level was (22.4 ± 0.84)-fold higher in metastatic UM-SCC-22B compared to primary UM-SCC-22A (Figure 3B). This pair of head and neck squamous cell carcinoma cells with different Hsp27 expression levels and metastatic potential provides an excellent cell model to elucidate the possible function of Hsp27.

siRNA Transfection Knocks down Hsp27 mRNA and Protein Expression in Head and Neck Squamous Cell Carcinoma Cells (HNSCC). To obtain insight into whether Hsp27 is directly linked to HNSCC cell invasion and migration ability, Hsp27 knockdown with small interference RNA (siRNA) was performed in the metastatic UM-SCC-22B cell line with high Hsp27 expression level. UM-SCC-22B cells were transfected for 24 h with 33 nM siRNA, and total RNA was extracted from cells by TRIzol reagents. Real-time PCR was used to measure Hsp27 mRNA level, and Western blotting was used to quantify the protein level of Hsp27. Real-time PCR showed that siRNA knocked down Hsp27 mRNA expression level by 83% in metastatic UM-SCC-22B compared with control (Figure 4A). Western blotting revealed that siRNA transfection decreased Hsp27 protein level 23-fold in metastatic UM-SCC-22B compared to control transfected cells (Figure 4B). Thus silencing Hsp27 with small interfering RNA successfully reduced the expression of Hsp27 in mRNA and protein level.

Silencing Hsp27 Inhibits Migration and Invasion of Head and Neck Squamous Cell Carcinoma Cells (HNSCC). To study whether silencing of Hsp27 in HNSCC could reduce invasion and migration, in vitro Matrigel

invasion assay and wound-healing migration assays were conducted with UM-SCC-22B after Hsp27 siRNA knock-down. Cells were transfected with Hsp27 siRNA for 5 h. For the invasion assay, 2×10^4 cells were added to the upper chamber in culture medium containing 1% FBS with medium containing 10% FBS in the lower chamber. At 40 h, the cells on the lower surface of the filter were counted under a microscope (Figure 5A). Eight fields were counted in each plate. Six experiments were conducted. The results showed that the cells number of UM-SCC-22B cells invading through Matrigel was 53.6 ± 26.7 ($n = 6$), whereas the invading cells number of UM-SCC-22B with Hsp27 siRNA transfection was 26.9 ± 11.2 ($n = 6$) (Figure 5B). This is comparable to the primary HNSCC, UM-SCC-22A (with rarely Hsp27 expression), which had 25.5 ± 14.6 (Figure 2B) invading cells on the lower surface of the filter.

In the migration assay, after the wound gap was generated, the cells were cultured for 24–72 h (Figure 5C). After 24, 48, and 72 h, the metastatic UM-SCC-22B migrated significantly faster and the gap width was reduced by $34.2 \pm 8.2\%$, $64.4 \pm 7.3\%$, $71.7 \pm 6.8\%$, respectively. In comparison, when metastatic UM-SCC-22B was transfected with Hsp27 siRNA, the gap width was reduced by only $11.6 \pm 9.0\%$, $13.1 \pm 3.0\%$, $18.3 \pm 7.9\%$ at 24, 48, and 72 h (Figure 5D). Similarly, the migration ability of UM-SCC-22B with siRNA transfection is comparable to the primary HNSCC, UM-SCC-22A (with rarely Hsp27 expression) (Figure 2D). These data suggest that silencing Hsp27 in the metastatic cell line indeed diminishes the motility and invasion of UM-SCC-22B.

Discussion

The small heat shock protein 27 (Hsp27) belongs to the heat shock protein family, and acts as a molecular chaperone. Hsp27 is localized in the cytosol of most human cells at a low level.²⁷ Expression of Hsp27 can be induced by environmental stress, such as heat shock, heavy metals, oxidants, infection, inflammation, ischemia.^{28,29} Once Hsp27 is induced, it may be phosphorylated and translocated from cytoplasm into or around the nucleus.^{30–32} Hsp27 has been

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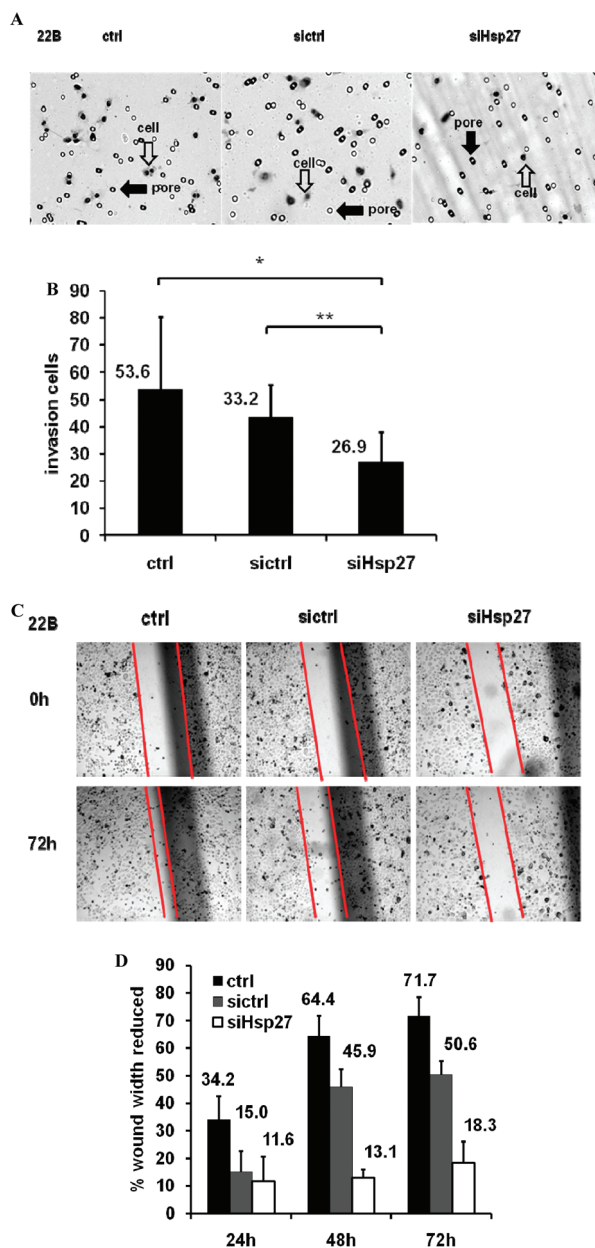


Figure 5. (A) Microscopy image ($\times 100$) of cell invasion in UM-SCC-22B after siRNA transfection in one representative experiment. Open arrows, pore; solid arrows, cell. (B) Bar graph represents a summary of six experiments of invading numbers of UM-SCC-22B cells after transfection with siHsp27, or control siRNA. Mean \pm SD ($n = 6$). UM-SCC-22B cells treated with 33 nM Hsp27 siRNA have a 49.76% ($*p < 0.01$) reduction in cell invasion compared to control cells and 18.99% ($**p < 0.01$) relative to control siRNA treated UM-SCC-22B cells. (C) Microscopy image ($\times 40$) of cell migration of UM-SCC-22B after siRNA transfection in one representative experiment. (D) Bar graph represents the percentage of reduced wound width (%) after 24–72 h in UM-SCC-22B cells after siRNA transfection. A–D: ctrl, cells treated with transfection reagent Lipofectamine 2000; siCtrl, cells transfected with scrambled siRNA; siHsp27, transfected with Hsp27 siRNA.

shown to have various cellular functions to promote differentiation, proliferation,²⁹ cell growth, and motility.¹⁷ In addition, it has been reported that Hsp27 is associated with various carcinomas. However, studies showed that Hsp27 is overexpressed in some cancer types, but downregulated in others.^{33,34} Increased levels of Hsp27 was detected in various cancers, such as breast,^{35,36} endometrial,³⁷ prostate,^{38,14} ovarian,³⁹ oral squamous cell,²⁹ gastric,^{40,41} and liver cancer,⁴² in these cancer types, Hsp27 is considered to be a negative prognostic factor. A recent study identified 16 proteins associated with primary hepatocellular carcinoma (HCC) with or without metastases.¹⁵ Among these proteins, Hsp27 was found to be correlated with HCC metastasis. Therefore, overexpression of Hsp27 serves as a unique feature of metastatic HCC. In addition, higher levels of Hsp27 expression are also considered to be associated with invasion and metastasis in tongue squamous cell cancer.²²

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On the other hand, Hsp27 has also been reported to be a good prognosis indicator. In patients with malignant fibrous histiocytoma,¹⁹ neuroblastoma,⁴³ endometrial adenocarcinomas^{37,44} and esophageal cancer,^{23,45} high levels of Hsp27 have been correlated with good prognosis. Nakajima et al.²³ showed that patients with Hsp27-negative esophageal squamous cell carcinoma tended to have a poor prognosis compared with patients with Hsp27-positive tumors. Hsp27 did not correlate with cancer and metastasis in renal cell carcinoma.¹⁷

To date, Hsp27 has not been conclusively studied in head and neck squamous cell cancers. Our data indicates that a higher level of Hsp27 is a negative prognostic factor. We found that Hsp27 is overexpressed in human head and neck squamous cell cancer cells with high metastatic ability.

Since cancer and its metastasis are complex processes, various cancer types and their metastasis processes have different oncogenic pathways involved.¹⁸ Different cancers may have distinct biomarker signatures that contribute their metastatic behavior. The literature reports that the process of metastasis is regulated by a variety of internal and external signals via complex signal transduction cascades. A variety of molecules including FAK, Src, MMPs, Her-2, and actin cytoskeleton⁴⁶ are involved in cell migration. Our data indeed showed that primary cancer cells (22A) still express these proteins (such as Her-2, data not shown). Although two different cell lines with different metastasis ability show dramatic difference in Hsp27 expression, we cannot rule out the contribution of the other molecule for metastasis. Therefore, the expression level of Hsp27 is only one of the reasons to manifest migratory behavior for 22A. In addition, it is also not surprising that Hsp27 may play different roles in different cancer types. And thus it is rather challenging to compare Hsp27 function in different cancer types.

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To simplify the model, we selected a pair of head and neck cell lines from the same patient, but with different metastatic potential to dissect the functions of Hsp27. This pair of cell lines will limit individual difference and minimize the genetic variation. The cancer cell lines were isolated from surgical specimens of a previously untreated patient with a primary tumor in the throat and a metastatic lymph node in the neck. The present study using these two cell lines from the same patient provides an excellent model to study function of Hsp27 in metastasis without other interference. In these two cell lines from the same patient, we monitored the cell migration and invasion ability, Hsp27 expression levels, siRNA knockdown of Hsp27. Indeed, the metastatic head and neck squamous cell cancer cell line UM-SCC-22B has higher invasion and migration ability compared to the primary squamous cell cancer cell line UM-SCC-22A, although these two cell lines have similar proliferation rates. Furthermore, Hsp27 is overexpressed in the metastatic UM-SCC-22B, but is rarely detectable in the primary UM-SCC-22A. Silencing Hsp27 by siRNA in metastatic UM-SCC-22B cells reduced its invasion and migration ability to levels similar to that of the primary cancer cells UM-SCC-22A. In this regard, detection of Hsp27 in head and neck cancers may help us understand one of the molecular mechanisms of metastasis, and provide a biomarker for the prognosis of head and neck cancer after treatment.⁴⁷ It is worth noting that further clinical evaluation is warranted to validate these findings.

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