# Upregulation of Nuclear Heat Shock Factor 1 Contributes to Tumor Angiogenesis and Poor Survival in Patients With Non–Small Cell Lung Cancer

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*Background.* The aim of this study was to detect the expression of heat shock factor 1 (HSF1) and microvessel density (MVD) in patients with non–small cell lung cancer (NSCLC) and to examine the relevance between HSF1 and angiogenesis, clinicopathologic factors, and prognosis.

*Methods.* Immunohistochemical staining was used to examine the level of expression of HSF1 and CD34. MVD was used to detect the number of microvessels by counting $CD34^+$ endothelial cells. Their relationship with clinicopathologic factors and prognosis in patients with NSCLC was determined using IBM SPSS Statistics, version 19 (SPSS Inc, Chicago, IL).

*Results.* The level of expression of HSF1 was increased significantly in NSCLC. HSF1 overexpression was observed in 45 patients and was significantly associated with MVD ($p = 0.005$). The overexpression of HSF1 was not associated with patient sex, age, tumor size, histologic type, or differentiation, but it was significantly associated with node metastasis ($p = 0.005$) and clinical stage ($p = 0.006$). HSF1 overexpression and high MVD were significantly associated with poor 5-year disease-free survival ($p = 0.001$ and $p = 0.006$, respectively). Patients with HSF1 overexpression and high MVD had a significantly poor overall survival ($p = 0.006$ and $p = 0.019$, respectively) and disease-specific survival ($p = 0.005$ and $p = 0.016$, respectively). Multivariate analysis showed that HSF1 overexpression was an independent prognosticator for poor overall, disease-specific, and disease-free survival ($p = 0.040$, $p = 0.046$, and $p = 0.004$, respectively).

*Conclusions.* Overexpression of HSF1 is common and significantly correlated with tumor angiogenesis in NSCLC. Patients with high HSF1 expression had poorer overall, disease-free, and disease-specific survival. These current findings suggest that HSF1 may serve as a novel prognostic marker and potential therapeutic target molecule for anti-angiogenic therapy in patients with NSCLC.

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The incidence of lung cancer has increased and is attributed to heavier air pollution and smoking [1–3]. In 2012, of the 14.1 million new cancer cases, lung cancer was the most common (1.8 million [13%]) and the leading cause of cancer deaths (1.6 million [19.4%]) worldwide [4]. The 2014 American Cancer Society's recent investigation found the estimated incidence of new cases to be 14%: 13% in male individuals and 1% in female individuals; the estimated mortality was 26% [5]. Non–small cell lung cancer (NSCLC) accounts for approximately 75% to 80% of cases [6]. Despite recent therapeutic advances, traditional surgical resection, chemotherapy, and radiotherapy have not improved the long-term survival of patients with NSCLC satisfactorily. Approximately 40% of patients with NSCLC die of recurrence or metastasis within 5 years after potentially curative resection. Owing to the discovery of tumor biomarkers, advanced molecular targeted therapy brings hope [7, 8]. However, further studies are required to discover and identify novel and specific biomarkers of NSCLC for benefit in patient survival and quality of life.

Heat shock factor 1 (HSF1) is known as a main transcriptional regulator of heat shock response (HSR) that is involved in protection of cells and organisms from heat, ischemia, hypoxia, inflammation, and some other noxious stressors [9]. When HSR is activated by some stressors, HSF1 trimerizes, translocates to the nucleus, and binds to heat shock element sites of promoters of heat shock protein (HSP) genes in a hyperphosphorylated form that is able to activate transcription [10]. In addition, lots of studies have revealed that to mediate this process, HSF1 and its associated HSPs play critical roles in cell transformation, tumorigenesis, tumor development, and drug resistance in a wide range of human tumors [11–13]. One study reported that HSF1 was upregulated in patients with hepatocellular carcinoma, which promotes invasion and metastasis of hepatoma cells by enhancing cell motility through HSP27 and correlates significantly with a

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**Abbreviations and Acronyms**

HSF1 = heat shock factor 1
HSP = heat shock protein
HSR = heat shock response
MVD = microvessel density
NSCLC = non–small cell lung cancer

poor prognosis [14]. Another in vitro study also indicated that knockdown of HSF1 with small hairpin RNA induced the expression of tumor suppressor retinoblastoma protein, resulting in attenuated hepatoma cell growth and colony formation [15]. Immortalized mouse embryo fibroblast cells derived from $HSF1^{-/-}$ animals were deficient in both basal and epidermal growth factor–induced migration and concluded that HSF1 was necessary for mitogen-activated protein kinase signaling, which in turn affected epidermal growth factor–induced cell migration [16]. HSF1 deficiency impeded NF1-associated carcinogenesis by attenuating oncogenic RAS/MAPK signaling [17]. These data suggested that HSF1 may act as a novel candidate for the development of new cancer prognostic biomarkers.

Tumor angiogenesis is a crucial and proven pathologic process that is an essential requirement for tumorigenesis, progression, and metastasis of human malignant tumors [18]. The therapeutic potential of targeting tumor endothelium and vascular supply is now widely recognized; solid tumors cannot grow beyond the size of a few millimeters without inducing the proliferation of endothelium and formation of new blood vessels [19]. HSF1 promotes cardiac angiogenesis through suppressing p53 and downstream upregulation of hypoxia-inducible factor-1a to maintain cardiac adaptation [20]. In another pathway, HSF1 contributed to ischemia-induced angiogenesis by regulating the mobilization of bone marrow stem/progenitor cells in cardiovascular disease [21]. Few studies have been carried out to elucidate the correlation of HSF1 with angiogenesis. Elevated HSF1 significantly increased tumor angiogenesis in mammary cancers by promoting expression of human antigen R and decreasing degradation of hypoxia-inducible factor-1a indirectly [22], indicating that HSF1 may be used as a potential antiangiogenesis molecule candidate. However, it is unclear whether and how HSF1 contributes to angiogenesis in NSCLC.

To explore the functions of HSF1 in tumor angiogenesis and biological behaviors, we determined the level of HSF1 and its correlation with different clinicopathologic variables, tumor angiogenesis, and prognosis to elucidate whether HSF1 was an independent significant factor influencing long-term survival for patients with NSCLC.

## Patients and Methods

### *Patients*

One hundred five NSCLC specimens were obtained from patients with NSCLC who underwent complete surgical lobectomy or pneumonectomy and regional lymph node dissection at the department of thoracic surgery at Qilu Hospital from January 2003 to August 2007. The systematic lymph node dissection routinely contained stations 5, 6, 7, and 10 on the left and stations 2, 4, 7, and 10 on the right. Nineteen patients with N2 disease were diagnosed as having preoperative clinical stage N2. All chosen patients were not treated with neoadjuvant chemotherapy or chemoradiotherapy to avoid interference before the operation. These patients included 62 men and 43 women, and the median age was 49 years (range, 25–74 years). This study was approved by the Ethics Committee of Qilu Hospital. Informed consent was obtained from all patients for the collection of lung cancer specimens and postoperative adjuvant chemotherapy in accordance with the guidelines of Qilu Hospital.

### *Immunohistochemical Staining for HSF1 and CD34*

The formalin-fixed paraffin-embedded slides (4 μm) were baked at 60°C, dewaxed, and rehydrated with xylene and graded alcohol and then immersed in 3% hydrogen peroxide to quench endogenous peroxidase. High-temperature antigen retrieval was carried out with citrate buffer (pH 6.0) in a microwave oven to enhance immunoreactivity. The slides were incubated in blocking serum to reduce nonspecific binding. The serum was removed and anti-human HSF1 monoclonal antibody (Protein Tech Group, Inc, Chicago, IL; 1:200) and CD34 (Santa Cruz Biotechnology, Inc, Dallas TX; 1:100) were applied overnight at 4°C. Biotinylated second antibodies and streptavidin-peroxidase conjugate were applied, and antibody-specific binding was visualized with 3,3′-diaminobenzidine solution. Finally, the slides were counterstained with hematoxylin and mounted with neutral balsam. Sections of a prostatic cancer tissue known to overexpress HSF1 were used as a positive control [23]. Slides were incubated with phosphate-buffered saline instead of antibodies and were used as a negative control.

Immunostained sections were reviewed by light microscopy and scored visually, with a value assigned to each individual. Scoring was based on a semiquantitative scoring system according to both staining intensity: (0, no nuclear staining; 1, light yellow staining; 2, pale brown staining; 3, medium brown staining) and percentage of positive cancer cells (0, ≤10%; 1; 11%–25%; 2, 26%–50%; 3, 51%–75%; 4, ≥76%). The final staining score was the sum of staining intensity and percentage of positive cells, so it was further graded as follows: 0–1, (–); 2–3, (+); 4–5, (++); 6–7, (+++). Therefore the expression of HSF1 was divided into a non-overexpressing group (– or +) and an overexpressing group (++ or +++). Microvessels were recorded by counting the $CD34^{+}$ stained endothelial cells according to the international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid tumors [24, 25]. Simply, immunostained sections were initially scanned at a low power (×100) to identify "hot spots," which are areas with the highest vascularity. Subsequently, the stained microvessels were examined with 3 consecutive high-power fields (×200) within the hot spot. Any immunostained endothelial cell or endothelial cell cluster that was clearly separate from adjacent microvessels could be considered a single