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Heat shock protein 70 as a predictive marker for platinum-based adjuvant chemotherapy in patients with resected non-small cell lung cancer☆

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ABSTRACT

Objectives: Although adjuvant platinum-based chemotherapy improves survival in completely resected non-small cell lung cancer (NSCLC), its effect is limited. We evaluated whether the expression of heat shock protein 70 (Hsp70) is associated with clinical outcomes in patients with completely resected NSCLC who were treated with or without adjuvant platinum-based chemotherapy.
Patients and methods: Patients who underwent curative resection for NSCLC and diagnosed as stage IIA through IIIA were included. Immunohistochemical staining for Hsp70 was performed on surgical specimens and survival rates were compared by Hsp70 expression and adjuvant platinum-based chemotherapy.
Results: Of 327 enrolled patients, Hsp70 expression was positive in 220 (67.3%). For patients who did not receive adjuvant chemotherapy, Hsp70 expression did not significantly affect survival. However, for patients who received adjuvant chemotherapy, those with Hsp70-positive tumors had a longer disease-free survival outcome than cases with Hsp70-negative tumors (not reached vs. 27.3 months; $P=0.002$), although there was no significant difference in overall survival (97.0 vs. 58.9 months, $P=0.080$). In the adjuvant chemotherapy group, multivariate modeling showed that patients with Hsp70-postitive tumors had a lower risk of recurrence and death after adjusting for age, sex, performance status, pathologic stage, and histological type (disease-free survival: adjusted hazard ratio, 0.537; 95% CI, 0.362–0.796; $P=0.002$; overall survival: adjusted hazard ratio, 0.663; 95% CI, 0.419–1.051; $P=0.080$).
Conclusion: Hsp70 is a positive predictive factor in completely resected NSCLC with received platinum-based adjuvant chemotherapy.

1. Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide [1]. Although surgical resection is the curative treatment for early stage non-small cell lung cancer (NSCLC), adjuvant treatment is still required since 5-year survival rates for complete surgical resection are disappointing [2]. Recently, several large randomized trials and a meta-analysis showed a survival benefit with platinum-based adjuvant chemotherapy in resected NSCLC compared with surgery alone [3–6]. However, the effect of

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platinum-based adjuvant chemotherapy on survival is limited, with an absolute improvement in the 5-year survival ranging from 4 to 5.4% [7,8]. Furthermore, adjuvant chemotherapy is not effective in all patients and potentially has significant toxicity.

In recent years, various prognostic or predictive markers, such as epidermal growth factor receptor (EGFR) and the oncogene *k-ras*, have been proposed for the customization of chemotherapy in NSCLC patients [9,10]. In particular, expression of excision repair cross-complementation group 1 (ERCC1) protein, a DNA repair enzyme, has been suggested as a prognostic factor for resected NSCLC without adjuvant chemotherapy [11]. However, no significant difference in survival was found between patients with ERCC1-negative tumors and those with ERCC1-positive tumors in a cisplatin-based adjuvant chemotherapy group [11]. Additionally, there are four different isoforms of ERCC1, and the unique functional ERCC1 isoform (ERCC1-202) cannot be discriminated with the use of currently available ERCC1 antibodies [12].

The heat shock protein (Hsp) family maintains protein homeostasis as a molecular chaperone by modifying the structures and interactions of other proteins. Hsps are particularly in demand when cells are exposed to extracellular stimuli, such as heat, oxidative stress, or other protein-damaging events [13]. Interestingly, Hsp70 is either not expressed or expressed at very low levels in normal cells and is highly induced by intracellular stress, such as that of cancerous conditions [14]. Because Hsp70 is highly expressed in cells or tissues from a wide range of tumors, overexpression of Hsp70 may be an important indicator of cancer and treatment prediction. However, it is unclear whether an elevated Hsp70 level is related to prognosis or drug response in lung cancer, although several studies on other cancers have been conducted on this issue [14]. Therefore, we hypothesize that expression of Hsp70 in the tumor could predict a survival benefit from platinum-based adjuvant chemotherapy in resected NSCLC.

2. Patients and methods

2.1. Patients and study design

For this retrospective study, a total of 327 patients were recruited from four Korean centers (Korea Cancer Center Hospital, Hallym University Sacred Heart Hospital, Seoul National University Bundang Hospital, and Asan Medical Center). All patients underwent curative resection for NSCLC between January 1996 and December 2010 and were diagnosed with stage IIA through IIIA (the American Joint Committee on Cancer sixth edition). All participants had adequate tumor specimens for immunohistochemial staining and detailed prognosis records. Patients with other primary cancers that could affect survival were excluded. Patients who died of perioperative complications within 3 months after surgery were also excluded. Clinical, pathological, and radiological data were retrospectively reviewed, as well as follow-up information obtained until March 2012. The study protocol was approved by the institutional review board of each center.

2.2. Adjuvant treatment

Each participating center determined the adjuvant treatment approach in accordance with the pathological stage of the disease, the performance status of the patient, and the will of the patient to receive the adjuvant treatment. Each center could determine the subjects of adjuvant platinum-based chemotherapy, the drug that was combined with platinum, the dose of drugs given per cycle.

2.3. Follow-up and outcome evaluation

Patient follow-up was performed at each center. Tumor recurrences were assessed on computed tomography (CT), magnetic resonance imaging (MRI), or bone scans. The primary end point was to assess whether expression of Hsp70 affected survival in terms of disease-free survival (DFS) and overall survival (OS). DFS was defined as the time from resection to locoregional or distant recurrences or death from any cause (whichever was earlier), and OS was defined as time from resection to death.

2.4. Immunohistochemical staining of Hsp70 and analysis of Hsp70 expression

Paraffin-embedded tumor samples were collected from patients and depraffinized. After rehydrating with alcohol, immunohistochemical staining for Hsp70 was performed with anti-Hsp70 antibody (Thermo Fisher Scientific, Fremont, CA). Immunohistochemical analyses were evaluated centrally at the Korea Cancer Center Hospital. Expression levels of Hsp70 were scored semiquantitatively according to standard protocols [15]. The percentage of positively stained tumor cells was scored as follows: 0 (<5%), 1 (5–25%), 2 (25–50%), or 3 (>50%). Staining intensity was scored as follow: 0 (no staining), 1 (weakly stained), 2 (moderately stained), or 3 (strongly stained). Based on the immunohistochemical staining score, which was obtained by adding the positive proportion score to the intensity score, tumors were classified into Hsp70-negative tumors (score, 0–3; Fig. 1A) and Hsp70-positive tumors (score, 4–6; Fig. 1B).

2.5. Statistical analyses

Comparisons of demographic and tumor characteristics between patients with and without Hsp70 expression or with and without chemotherapy were performed using Pearson's Chi-square test or Fisher's exact test for categorical variables and Student's *t*-test for continuous variables.

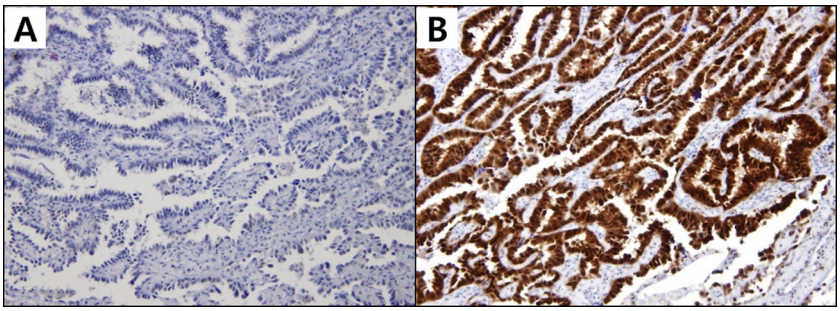

Fig. 1. Assessment of Hsp70 expression levels by immunohistochemical staining scores. (A) Hsp70-negative tumors (score 0–3); (B) Hsp70-positive tumors (score 4–6). The score was calculated as the sum of the score for the percentage of positively stained tumor cells (0, <5%; 1, 5–25%; 2, 25–50%; 3, >50%) and the score for the intensity of staining (0, no staining; 1, weakly stained; 2, moderately stained; 3, strongly stained).