Lung Cancer (2005) **49**, 55—62

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Expression of endoplasmic reticulum molecular chaperone Grp78 in human lung cancer and its clinical significance

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Received 29 July 2004; received in revised form 7 December 2004; accepted 9 December 2004

KEYWORDS
Grp78;
Lung cancer;
Immunohistochemical analysis;
Prognostic factor

Summary All eukaryotic cells respond to the accumulation of unfolded proteins in the endoplasmic reticulum (ER) by signaling an adaptive pathway termed the unfolded protein response (UPR). Glucose-regulated protein (Grp) 78 is a molecular chaperone involved in the UPR. The aim of this study was to detect Grp78 expression in lung cancer using immunohistochemical (IHC) staining, and also to evaluate the relationship between the Grp78 expression level and the prognosis of patients with lung cancer. We used immunohistochemistry to analyze the protein expression of Grp78 in paraffin-embedded tumor samples from 132 well-characterized lung cancer patients and compared the expression level of Grp78, clinical variables and survival outcome. A positive expression of Grp78 was detected in the cytoplasm of tumor cells in 88 of the 132 patients (66.7%) with lung cancer. No significant difference was observed between the Grp78 expression and the gender, age at operation, histological type, pathologic stage, pathologic T status, and pathologic N status. Lung cancer patients with a positive Grp78 expression tended to show a better prognosis than those with a negative Grp78 expression. In addition, a multivariate analysis of the clinicopathologic characteristics of lung cancer indicated a positive expression of Grp78 to be a significant factor for predicting a favorable prognosis ($p < 0.001$, risk ratio = 2.35). A positive expression of Grp78 may thus be a useful marker for predicting a favorable prognosis in patients undergoing a resection of lung cancer. The ER stress pathway mediated by Grp78 may therefore be responsible for controlling the growth of lung cancer cells.

Abbreviations: ER, endoplasmic reticulum; UPR, unfolded protein response; Grp, glucose-regulated protein; IHC, immunohistochemical; CEA, carcinoembryonic antigen; ATF4, activating transcription factor 4; NSCLC, non-small cell lung cancer

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doi:10.1016/j.lungcan.2004.12.011

1. Introduction

Lung cancer is the leading cause of cancer death worldwide [1]. The current stage classification of lung cancer is based on the clinicopathologic features [2]. However, such clinical informations can be sometimes incomplete or misleading to predict prognosis of patients [3,4].

Molecular diagnostics may offer precise, objective, and systematic human cancer classification. However, standard of molecular markers for most solid tumors to predict prognosis have not yet been identified. The potential prognostic implications of several biological and molecular parameters including K-ras [5—7] mutation, and c-erbB2 [8] overexpression, and p53 [9,10] mutation, have been reported in patients with lung cancer from our laboratory.

When unfolded proteins accumulate in the ER, which is a highly evolved folding factory where proteins attain their final folded conformation without any excessive misfolding and/or aggregation, the homeostatic response termed the UPR, which is conserved from yeast to man is activated. The primary targets of the UPR are molecular chaperones and folding enzymes localized in the ER; the induction of these proteins augments the capacity of the protein folding system, thus leading to keep homeostasis of the ER. In addition, some of the proteins involved in the ER-associated degradation system, which are used to eliminate misfolded proteins from the ER, are up-regulated by the UPR. Accumulating evidences indicate that the UPR is of fundamental importance in the quality control of proteins in the ER, under not only environmental stress condition, but also normal growth condition [11—14]. Grp78, a member of the Hsp70 family, is known to be induced in the ER compartment by a variety of stresses. These stress signals include glucose starvation, ER Ca^{2+} depletion, accumulation of misfolded proteins in the ER, and reductive stresses [11]. Expression of Grp78 has been reported to be regulated by transcription factors ATF6 [15,16] and XBP1 [17]. We investigated the Grp78 expression in lung cancer and its significance of predicted value for prognosis of patients with lung cancer.

2. Materials and methods

2.1. Patients and follow-up

We examined 132 consecutive patients with stage I—III lung cancer who underwent surgical resection between April 1993 and July 1996 at the University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan. Clinicopathologic data were obtained by a retrospective chart review. The tumor stage was classified according to Revisions in the International System for Staging Lung Cancer [2]. The subjects included 93 men and 39 women in this series, with a mean age of 66.2 years (range, 40—84). The pathologic types includes 76 adenocarcinoma, 44 squamouse cell carcinoma, 2 adenosquamous cell carcinoma, 3 carcinoid, 5 large cell carcinoma, and 2 small cell carcinoma. According to the pathologic staging, 26 patients were at stage IA, 41 at stage IB, 2 at stage IIA, 16 at stage IIB, 31 at stage IIIA, and 16 at stage IIIB. Twenty (15.2%), 17 patients (12.9%), and 3 (2.3%), of 132 patients had previously received chemotherapy, radiotherapy or both, respectively. Seven patients had received induction cisplatin-based chemotherapy, which means that they had a tumor preoperatively. Institutional review board-approved informed consent was obtained either from all patients or from the patient's legal guardians (someone who is legally responsible for looking after the patients) for use of tumor tissue specimens collected at the time of surgical resection.

For the postoperative follow-up, the patients were examined every month within the first year and approximately at 2- to 4-month intervals thereafter. The evaluations included a physical examination, chest roentgenography, an analysis of blood chemistry, and measurements of tumor markers such as carcinoembryonic antigen (CEA), SCC, and SLX. Chest, abdominal, and brain computed tomographic scans and a bone scintiscan were performed every 6 months for 3 years after surgery. If any symptoms or signs of recurrence appeared based on these follow up studies, additional examinations to evaluate the precise site of the recurrence were performed. Survival data were updated in November 2003. A follow-up was available for all patients. The median observation periods were 50.4 months.

2.2. Immunohistochemical staining

A formalin-fixed, paraffin-embedded, 3-μm section was obtained from each of the 132 samples of primary lesions. All specimens were stained with hematoxylin and eosin for histopathologic diagnosis. IHC staining was performed by a streptavidin—biotin—peroxidase complex method [18]. The sections were briefly immersed in citrate buffer (0.01 mol/l citric acid pH 6.0) and incubated for two 5-min intervals at 100 °C in a microwave oven for antigen retrieval. They were then incubated with polyclonal Grp78 antibody (Santa Cruz Biotechnology Inc., CA) diluted at 1:500 in