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Expression of the enhancer of zeste homolog 2 in biopsy specimen predicts chemoresistance and survival in advanced non-small cell lung cancer receiving first-line platinum-based chemotherapy

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ABSTRACT

Objectives: Enhancer of zeste homolog 2 (EZH2) plays a key role in tumorigenesis and cancer progression through epigenetic gene silencing and chromatin remodeling. The objective of this study was to investigate the correlation between EZH2 expression and platinum-based chemotherapy response as well as survival of patients with advanced non-small cell lung cancer (NSCLC).
Materials and methods: We identified 360 consecutive stage IIIB and IV NSCLC patients who underwent first-line platinum-based chemotherapy. Immunohistochemical analysis of EZH2 on the paraffin-embedded pre-treatment tumor samples was performed and correlated with chemotherapy response and survival.
Results: EZH2 was positive in 204 of 360 patients (56.7%). Of the 204 positive EZH2 patients, 72 (35.3%) responded to chemotherapy with either complete response, or partial remission. Of 156 negative EZH2 patients, 90 (57.7%) exhibited a response to chemotherapy. The difference in response to therapy between positive and negative EZH2 patients was statistically significant ($p < 0.01$). Univariate survival analysis indicated that patients with positive EZH2 had a significantly lower disease-free survival (DFS) and overall survival (OS) than those patients with negative EZH2 expression. Multivariate Cox regression analysis demonstrated that positive EZH2 expression was an independent prognostic factor for both DFS and OS. Kaplan–Meier survival curves further confirmed that positive EZH2 expression correlates with poor survival in NSCLC patients.
Conclusions: Our results indicate that advanced NSCLC patients with positive expression of EZH2 exhibited resistance to cisplatin-based chemotherapy. EZH2 may be a predictive and prognostic factor for cisplatin-based therapy response and disease survival in advanced NSCLC.

1. Introduction

Lung cancer is a leading cause of cancer deaths in the world. Approximately 80–85% of all lung cancers are non-small-cell lung cancer (NSCLC) [1,2]. Most of the patients have advanced stage IIIB or IV disease when first diagnosed, and patients with advanced NSCLC are candidates for systemic chemotherapy. A combination of cisplatin or carboplatin with third generation agents, such as gemcitabine, paclitaxel, docetaxel, or vinorelbine, represents the standard first-line treatment of patients with advanced disease [3–5]. However, appreciable clinical response to chemotherapy is achieved in only 30–40% of patients, probably because of relatively higher chemoresistance to lung cancer. Therefore, identifying molecular markers related to cisplatin resistance would be helpful to understand the mechanisms of chemoresistance and make individual chemotherapy strategies for NSCLC patients.

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The mechanisms of chemoresistance in NSCLC patients are likely to involve multiple gene products. For example, excision repair cross-complementing 1 (ERCC1) mRNA expression is resistant to cisplatin in NSCLC patients [6], breast cancer susceptibility gene l (BRCA1) haplotype is associated with survival of NSCLC patients received with platinum-based chemotherapy [7]. Recently, it was suggested that transglutaminase 2 is a novel cisplatin resistance marker in NSCLC [8]. With these resistance molecules, much effort is still needed to find biomarkers with potential utility in predicting the chemoresistance of NSCLC patients.

The gene of enhancer of zeste homolog 2 (EZH2), encoding a polycomb group protein, plays an important role in tumorigenesis and cancer progression through epigenetic gene silencing and chromatin remodeling. Overexpression of EZH2 has been reported in various malignant tumors, and considered to have prognostic value [9–15]. Recent studies indicated that EZH2 is involved in drug resistance in ovarian cancers [16], and EZH2 overexpression is detected in cisplatin-resistant lung cancer cells [17]. However, the relationship between the EZH2 expression and the development of chemotherapy resistance to NSCLC is still unknown. In this study, we investigated the expressions of EZH2 in patients with advanced NSCLC, and assessed the predictive and prognostic values of this marker to first-line platinum-based chemotherapy.

2. Materials and methods

2.1. Patients

A total 360 of stage IIIB to IV NSCLC patients who received platinum-based chemotherapy at the Nanjing Chest Hospital between January 2007 and September 2011 because they had a performance status (PS) of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) scale. All patients had tumor samples from bronchoscopic biopsies. Adequate tumor biopsy specimens were collected before chemotherapy. The histological classification was based on the Union for International Cancer Control (UICC-7) staging system for lung cancer [18]. Clinical staging was based on an initial evaluation that consisted of a clinical assessment, computed tomography of the chest and abdomen, magnetic resonance imaging of the brain, and bone scintigraphy. Patients were eligible for the study according to the following criteria: they were not indicated for definitive chemoradiotherapy with either borderline surgically respectable tumors or malignant pleural effusion, they had previously untreated stage IIIB or IV diseases, they received at least 2 courses of platinum-based doublet, they had measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) [19], and their paraffin-embedded pre-treatment tumor blocks were available for this study. Exclusion criteria were patients who had previously known history of cancer, or patients who had no subsequent clinical follow-up data.

All patient data, including staging information, treatment, and outcome variables, were collected retrospectively. Follow-up information was obtained by phone investigations. The median follow-up of surviving patients at the time of analysis was 14 months (range, 3–81 months). The date of the last follow-up was February 20, 2014. The protocol for chart and radiography review and archived tumor specimen use was approved by the institutional review board at the Nanjing Chest Hospital.

2.2. Chemotherapy regimens and evaluations

Three hundred patients received gemcitabine, given as two 1250 mg/m^2 doses on days 1 and 8, and cisplatin, given as a 75 mg/m^2 dose on day 8. Sixty patients received docetaxel given as two 60 mg/m^2 doses on days 1 and 8, and cisplatin, given as a 75 mg/m^2 dose on day 1. Both gemcitabine/cisplatin (GP) and docetaxel/cisplatin (DP) treatment paradigms were repeated on a 28 day cycle. Patients received a total of four to six chemotherapy courses. Fifty-four patients received palliative radiotherapy; thirty received radiotherapy for bone metastases and twenty-four received radiotherapy for cranial metastases before the first chemotherapy course.

Computerized tomography (CT) scanning of the chest including liver was performed before and every month during treatment until completion of chemotherapy, and every 3–6 months thereafter until disease progression. At the clinicians' discretion, radiological tumor assessment could be repeated early on the basis of clinical need or suspicion of disease progression. The sum of maximal diameters of all measurable tumor lesions was recorded at baseline and after treatment. These measurements were used to calculate the largest percentage of shrinkage or smallest percentage of growth using the baseline assessment as reference. The best response to therapy was defined according to the RECIST as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) [19]. For data analysis, CR and PR were combined as response that was sensitive to chemotherapy, while SD and PD were grouped as non-response.

2.3. Immunohistochemical analysis

Immunohistochemical (IHC) analyses were performed with rabbit polyclonal antibody to EZH2 (1:100; Cell Signaling Inc., Danvers, MA, USA). Tissue sections of 4 μm in thickness were cut from each paraffin block. Deparaffinized tissue sections were immersed in 10 mM citrate buffer (pH 6.0) and autoclaved for 15 min at 121 °C for antigen retrieval. Endogenous peroxidase activity was blocked by 0.03% hydrogen peroxide. Following incubation with 0.01 M phosphate-buffered saline (PBS) (pH 7.2) containing 5% bovine serum albumin (BSA) blocking buffer, the sections were incubated with primary antibody over night at 4 °C. After rinsing with 0.05 M Tris–HCl (pH 7.6), the sections were sequentially incubated with biotinylated secondary antibody (Histofine kit; proteintech, Wuhan, China) for 30 min, streptavidin peroxidase for 15 min and 3,3-diaminobenzidine for 15 min with an LSAB2 Kit (DAKO). The sections were then counterstained with Mayer's hematoxylin.

Cancer cell staining was regarded as positive or negative and all slides were independently evaluated by two pathologists who were unaware of the clinical and pathological information of the subjects. The EZH2 immunoreactivity level was classified by the proportion of positive cells: 0, <5% positive cells; 1+, 5–30% positive cells; 2+, 30–50% positive cells; 3+, >50% positive cells. The intensity of EZH2 expression was also scored: 0, negative to weak; 1, moderate; 2, strong. The score was the sum of the intensity and the percentage of positive cells. A score of $\leq$3 was applied as a cut-off point for negative of EZH2 expression.

2.4. Statistical analysis

SPSS version 18 was used to analyze the results. The correlations between immunohistochemical expression and the clinical variables and response to chemotherapy were evaluated by the Chi-square test or Fisher's exact test. Overall survival (OS) was measured from the start of chemotherapy to the date of death or the date the patient was last known to be alive. Disease-free survival (DFS) was measured from the start of chemotherapy to the date of recurrence. Survival curves were plotted by the Kaplan–Meier method and compared using the log-rank test. Survival data were evaluated using univariate and multivariate Cox regression analysis. Values of $p < 0.05$ were considered to indicate statistical significance.