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Evaluation of Circulating Tumor Cells in Predicting Therapeutic Response in Small Cell Lung Cancer Patients

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Background and Aims. Circulating tumor cells (CTCs) have prognostic significance in patients with metastatic cancer, but their utility in predicting the response to tumor therapy is unknown. This study examined the correlation of CTCs with the therapeutic response in small cell lung cancer (SCLC).

Methods. Clinical and pathological data from 96 SCLC patients were evaluated in this study. CellSearch kits were used to detect CTCs in peripheral blood samples. Statistical analysis was performed using Fisher exact test and Mann-Whitney *U* test.

Results. At baseline, 47 (50.0%) SCLC patients had detectable CTC counts. Serum neuron-specific enolase (NSE) was found to be associated with CTC thresholds. However, no significant differences were observed for an association of any threshold CTC count with the treatment response, with gender, age ($\leq$60 or $>$60 years), smoking status, syndrome of inappropriate antidiuretic hormone (SIADH), or Ki67 expression.

Conclusion. Detection of CTCs in SCLC patients was associated with serum NSE but not with response to cancer therapy.

Key Words: Circulating tumor cells, SCLC, Neuron-specific enolase, Therapeutic response.

Introduction

Small cell lung cancer (SCLC) is a rapidly progressive disease that accounts for ~15% of all lung cancers (1). Classically, SCLC is divided into limited disease stage (LD, localized disease) and extensive disease stage (ED, metastatic disease). Approximately 60–70% of SCLC patients present with extensive-stage disease (2). Mortality is high even in patients with LD, with a 5-year survival of only 10–26% (2,3). A number of clinical biomarkers have been used as prognostic markers for SCLC patients. These include neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), and pro-gastrin-releasing peptide (ProGRP). Elevated levels of these markers have been associated with SCLC diagnosis or poor prognosis (4–7). Among them, NSE is the most sensitive tumor marker in SCLC (6,7).

A biomarker that could predict the response to tumor therapy would be a useful clinical tool. Certain molecular characteristics of tumors such as oncogenic mutations (8,9), loss of tumor suppressor genes (10–12), or loss of mismatch repair genes (13–15) have been evaluated as prognostic factors, but none has been successfully applied to clinical practice.

Circulating tumor cells (CTCs) are thought to represent cells that are shed by primary or metastatic tumors. Significant interest has focused on examining CTCs as prognostic or predictive biomarkers in several malignancies including SCLC (16–18). It has been suggested that detection of CTCs may also provide useful information in predicting treatment efficacy (19). Several methods have been developed to detect CTCs in the blood of cancer patients, but they show variable sensitivity and specificity. CellSearch, a semi-automated immunomagnetic diagnostic system, has been shown to be reliable and has gained the approval from the U.S. FDA.

In this communication we investigated the prevalence of CTCs in patients with SCLC and determined whether the CTC count in SCLC patients was associated with the

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response to tumor therapy. To our knowledge, this work represents the first large scale study to study whether CELLSEARCH® technology was valuable in evaluating CTCs in Chinese patients with SCLC.

Materials and Methods

Study Population

This study was approved by the institutional ethics committee of the First Hospital of Jilin University. Patients were treated at the Cancer Center of the First Hospital of Jilin University between July 2012 and January 2014. Eligibility criteria included: a) age >18 years; b) primary SCLC confirmed by pathology or cytology; c) Eastern Cooperative Oncology Group (ECOG) performance status equal to 0 or 1; and d) no previous anti-tumor treatment. Exclusion criteria included: a) secondary (metastatic) lung cancer and b) double or multiple primary cancers. Confirmation of clinical stage was based on the results of examination by computed tomography (CT) of the chest and abdomen. In some cases, other procedures were also used including brain magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning or radionuclide bone scanning. The clinical staging was divided into LD and ED using the criteria as previously described (20).

Treatment and Efficacy Evaluation

Treatment regimens included a) chemotherapy, in which all the patients received 4−6 cycles of etopside plus platinum-based doublet first-line chemotherapy, and b) radiotherapy for limited stage patients, in which 54 GR of thorax radiotherapy was used after the third cycle of chemotherapy. Extensive stage patients were also given palliative radiotherapy. In addition, all patients received symptomatic and supportive treatment such as antiemetic and anti-inflammatory therapies. After every two cycles of chemotherapy, the tumor response was evaluated. Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors v. 1.0. The endpoints of the study included objective response rate (ORR = CR + PR) and clinical benefit rate (CBR = CR + PR + SD) after first-line therapy.

CTC Analysis

Blood samples were collected in 10 mL CellSave Preservation tubes, stored at room temperature, and processed within 96 h of collection according to the manufacturer's instructions (Veridex). CTC analysis was performed using the CELLSEARCH® System (Veridex). To be considered a CTC, a cell was CD45 negative, contained a nucleus, and exhibited positive cytoplasmic staining for cytokeratins (CK-8, CK-18, and CK-19).

Ki67 Analysis

Ki67 was assessed in 56 patients. Cell proliferation was determined using a monoclonal antibody against Ki67. Formalin fixed, paraffin-embedded tumor specimens were cut 4-mm thick and affixed to glass slides. They were then stained with immunohistochemicals to detect the expression of Ki67 using the rabbit monoclonal antibody (MAIXIN-BIO Inc., China).

Neuron-specific Enolase (NSE) Analysis

NSE was assessed in 66 patients. For measurement of NSE, serum was separated from 2 mL blood that was allowed to coagulate for 10 min, and NSE was determined by Luminex xMAP (luminex200, Luminex, USA). The upper limit of the normal value was 20 ng/mL.

Diagnosis of Syndrome of Inappropriate Antidiuretic Hormone (SIADH)

Criteria to define SIADH were serum sodium <135 mmol/L associated with serum hypo-osmolality (<275 mosm/kg), continued urinary excretion of sodium (urine sodium >25 mEq/L), less than maximally dilute urine (urine osmolality >serum), serum glucose <250 mg/dL, serum albumin >3.0 g/dL, and normal renal (serum creatinine <1.5 mg/dL), adrenal, and thyroid function in a non-edematous patient. Tests of endocrine function included either a normal morning serum cortisol or appropriate response to cortrosyn stimulation and a normal serum level of thyroxine or thyroid-stimulating hormone. Patients receiving diuretics or other medications known to cause hyponatremia were excluded, according to the report from List et al. (21).

Statistical Analysis

Statistical analysis was performed using statistical software SPSS v.19.0 (SPSS, Inc., Chicago, IL). Significance statements refer to a p value of <0.05. Statistical tests applied were Fisher exact test and Mann-Whitney U test.

Results

Patient Characteristics

Ninety-six patients with SCLC were recruited between July 2012 and January 2014. Compared with normal NSE, elevated serum NSE was associated with CTC count when CTC thresholds ≥2 or 3/7.5 mL used (Table 1). However, no significant differences were observed for the association between any threshold CTC counts and gender, age (≤60 or >60 years), smoking status, clinical staging, and Ki67.