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Surfactant protein gene expressions for detection of lung carcinoma cells in peripheral blood

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Summary
Background: Inflow of tumor cells to circulation is an essential step for metastasis of primary tumors. To know its state may contribute to therapeutic strategy. However, methodology to detect lung carcinoma cells floating in peripheral blood has not been established. Pulmonary surfactant protein (SP)-A, SP-C and Clara cells-10 kd protein (CC10) are specific to the lung and often expressed in primary lung carcinomas. We evaluated the worth of these gene expressions for the detection of carcinoma cells in peripheral blood.
Methods: The expressions in 5 ml of venous blood were tested by RT-PCR. Ninety-nine patients with non-small cell lung carcinoma (NSCLC) and 17 with small cell lung carcinoma (SCLC) were compared to 13 with secondary lung tumor, 48 with non-malignant respiratory diseases and 19 healthy volunteers.
Results: The mRNA expressions of SP-A and SP-C were completely specific to NSCLC when compared to SCLC and secondary lung tumors. All of the healthy volunteers and patients with non-malignant respiratory diseases showed negative for these mRNA expressions, except for one sample. The positive rate of SP-A, SP-C and CC10 mRNA in patients with NSCLC was 33.3%, 14.1%, 3.3%, respectively. The rates of SP-A and SP-C mRNA were higher than that (11.1%) in CEA mRNA. The increased positive rate of mRNA of SP-A and SP-C was significantly dependent on the clinical stage and the existence of distant metastasis.

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Conclusion: These results demonstrate that the detection of mRNA of SP-A and SP-C would give clinicians valuable information suggesting the presence of blood-floating carcinoma cells as a step toward metastasis.

Introduction

Primary lung carcinoma is a disease with a very poor prognosis. Even if absolute resection is successfully performed, only 60–70% of patients with stage I primary lung carcinoma according to the TNM classification have survived for 5 years.[1,2] This poor prognosis may be caused by late diagnosis which was obtained after the occurrence of micrometastasis. Although the entry of tumor cells from the primary site into the peripheral blood plays an important step in the multi-step process of metastasis, the detection of tumor cells in peripheral blood using the cytological method does not appear to be practical because of its low sensitivity. Using several markers, including cytokeratin (CK) and carcinoembryonic antigen (CEA), reverse transcriptase-polymerase chain reaction (RT-PCR) has recently been used to detect circulating lung carcinoma cells in peripheral blood.[3] However, employing these markers can sometimes cause serious problems; mixing of the skin epithelial cells at the time of sample extraction,[4,5] and the existence of pseudogenes.[6,7] Furthermore, since these markers are not specific for the lung, they do not appear to be suitable for distinguishing primary lung carcinomas from metastatic neoplasms of the lung. In the search for a candidate marker which could overcome these problems, we focused our study on surfactant protein (SP)-A, SP-C and Clara cell 10 kd protein (CC10). SP-A is a marker for both alveolar type II cells and Clara cells in peripheral lung tissues, while SP-C and CC10 provides a particularly high specificity to alveolar type II cells and Clara cells, respectively.

By using anti-SP-A monoclonal antibodies, we have previously demonstrated that SP-A was positively detected in 50% of primary lung adenocarcinoma tissues but was not present at all in tissues of metastatic lung tumor.[8] Nomori and colleagues[9] also reported that the expression rates of SP-A and CC10 in tissues of primary lung adenocarcinoma were 65.3% and 10.2%, respectively. Several cell lines of lung carcinoma expressed mRNA for SP-A and SP-C.[10,11] In pleural effusions from patients with primary lung adenocarcinoma, RT-PCR for SP-A mRNA showed a sensitivity of 83% and a specificity of 100%, suggesting that SP-A could be a worthy indicator for differential diagnosis.[12]

The purpose of this study was to examine whether mRNAs of the lung specific proteins are detected in peripheral blood of patients with lung carcinomas and whether the detection rates are associated with clinical stage and distant metastasis.

Materials and methods

Patients

We enrolled 116 patients with lung carcinomas who visited Sapporo Medical University Hospital; 78 with adenocarcinomas, 18 with squamous cell carcinomas, 2 with adenosquamous carcinomas, 1 with large cell carcinoma and 17 with small| cell lung carcinomas (SCLC). The 116 patients with lung carcinoma were classified according to the TNM classification.[13] As controls, the 48 patients with non-malignant respiratory diseases and from the 19 healthy volunteers were examined. The 13 metastatic tumor patients were also examined. Interstitial pulmonary fibrosis (IPF) and lung carcinoma are known to be frequently associated. The chest-X-ray findings of IPF often show a homogenous increase in density, which makes an early diagnosis of lung carcinoma difficult. We therefore excluded IPF cases from the control samples in our study.

Mononuclear cell Isolation and RNA extraction

This study was approved by the Sapporo Medical University Ethics Committee, and informed consent was obtained from each subject. The samples from patients with lung carcinoma were extracted before medical treatment. We collected peripheral venous blood samples (5 ml) in EDTA tubes. Mononuclear cells from peripheral blood samples were isolated using Ficoll gradient centrifugation. Briefly, blood samples were centrifuged to separate plasma. The plasma was stored at −30 °C until SP-A measurement. The remainder was diluted with PBS and then layered on 5 ml of Ficoll gradient solution. The gradient was centrifuged at 350*g* for 30 min. Total RNA extraction was performed by using Trizol reagent (Life Technologies, Inc. Rockville, MD) according to the manufacturer's instructions.