Prognostic Significance of OCT4 Expression in Adenocarcinoma of the Lung

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Objective: The purpose of this study was to detect the presence of cancer stem-like cells with bronchioalveolar stem cells (BASCs) properties and investigate the clinicopathological role of expression of OCT4 as well as the correlation with clinical outcomes in adenocarcinoma of the lung.

Methods: Specimens of 112 cases of Stage IB–IIIA lung adenocarcinoma after radical surgery were collected from June 1999 to June 2002. The putative cancer stem cells in tumor sections were visualized immunofluorescently by using the antibodies against three bronchioalveolar stem cells markers: surfactant protein C (SPC), Clara cell secretory protein (CCSP) and Octamer-4 (OCT4). Cancer stem-like cells with bronchioalveolar stem cell properties in human lung adenocarcinoma were subdivided into two phenotypes: OCT4⁺BASC (SPC⁺CCSP⁺OCT4⁺) and OCT4⁻BASC (SPC⁺CCSP⁺OCT4⁻).

Results: Cancer cells with CCSP⁺SPC⁺BASC phenotype were detected in 107 cases, 80 cases with OCT4⁺BASC phenotype (SPC⁺CCSP⁺OCT4⁺) and 27 cases with SPC⁺CCSP⁺OCT4⁻. There was a correlation between differentiation and OCT4 expression (P = 0.047). The pattern of survival curves shows the expected trend of decreasing survival with increasing stage at diagnosis (P = 0.015) and with OCT4⁺BASC expression (P = 0.019). Multivariate Cox’s analysis reveals that pathological stages of TNM (P = 0.008) and bronchioalveolar stem cells phenotypes (P = 0.015) are the independent prognostic factors.

Conclusions: The cancer cells with bronchioalveolar stem cells phenotype are detectable in adenocarcinoma of the lung and the expression of self-renewal regulatory gene OCT4 in these cells indicated the worse clinical outcomes.

Key words: lung adenocarcinoma – cancer stem cells – OCT4

INTRODUCTION

The incidence of lung cancer in 2007 is estimated to be 213,380 with 160,390 deaths in the USA. It will contribute to 31% of male and 26% of female cancer-related deaths and is the largest cause of cancer-related mortality in both men and women (1). Lung cancers are generally categorized as small cell carcinomas (SCLC) and non-small cell carcinomas (NSCLC), and the later can be further divided into three subtypes (e.g. adenocarcinoma, squamous cell carcinoma and large cell carcinoma). In recent years, the incidence of lung adenocarcinoma has been increased significantly and has become the most prevalent subtype of NSCLC. Further, the long-term survival rate of patients with lung adenocarcinoma remains unsatisfactory. So, there is an urgent need for further understanding the pathophysiology and progression of NSCLC, especially lung adenocarcinoma (2).

Currently, a particularly attractive hypothesis suggests that cancers originate from the adult stem cells in various human...
organs via a process of malignant transformation. These transformed stem cells are called cancer stem cells (CSCs). CSCs are believed to be the category of primitive and non-differentiated cells that possess the self-renewal and differentiation into various types of cancer cells (3). CSCs have also been reported to have an increased risk to metastasize and are markedly resistant to cytotoxic agents. It is also believed that CSCs are responsible for the metastasis and re-growth of tumors after unsuccessful treatment (4). Therefore, the presence of CSCs in tumors might be associated with the prognosis of patients. Recently, Kim et al. (5) provided the first evidence that the bronchioalveolar stem cells (BASCs) at the bronchiolar–alveolar duct junction (BADJ) of adult airway represent an initiating cell source for lung adenocarcinoma. The studies with mouse model have suggested that BASC has a phenotype characteristic of co-expressing markers related to the bronchiolar Clara cells (Clara cell secretary protein, CCSP) and the alveolar type 2 (AT2) cells (surfactant protein C, SPC), and the BASC possesses the potential to differentiate into Clara or AT2 cells. These primitive cancer cells also exhibit the capacity to self-renewal and the expression of Octamer-4 (OCT4), a member of the POU (Pit-oct-unc) family transcriptional factors, which is essential for maintenance of the stem character in the embryonic stem cells (ESCs).

On the basis of these findings, it is reasonable to expect that BASCs represent an initiating cell source of human lung adenocarcinoma. This hypothesis is yet to be validated. It remains unclear whether human lung adenocarcinoma originates from the same type of stem cells as their mouse counterparts. In order to validate the presence of cancer stem-like cells with BASC properties in human lung adenocarcinoma and to evaluate their probable implication to the prognosis of patients with this disease, the specimens of 112 cases of lung adenocarcinoma after radical surgery were collected and detected. In this study, we detected the expression of SPC, CCSP and OCT4 in the cancer tissues of lung adenocarcinoma and we found that cancer stem-like cells with BASC properties in human lung adenocarcinoma could be subdivided into two phenotypes: OCT4⁺BASC (SPC⁺CCSP⁺OCT4⁺) and OCT4⁻BASC (SPC⁺CCSP⁻OCT4⁻).

PATIENTS AND METHODS

TISSUE SAMPLES AND PATIENT CHARACTERISTICS

Between June 1999 and June 2002, 252 patients were diagnosed as lung adenocarcinoma in Shanghai Chest Hospital in this period. A total of 112 consecutive and non-selected patients who underwent definitive surgery for lung adenocarcinoma at the Shanghai Chest Hospital with a confirmed pathologic stage of Stage IB, II or IIIA were reviewed retrospectively. Among these 112 cases, 57 were male and 55 were female, with a near 1:1 ratio of male to female. Patients’ age at the time of surgery ranged from 22 to 81 (median age: 57 years). Surgical-pathologic staging was carried out according to (sixth edition) TNM Classification of Malignant Tumours by the International Union Against Cancer (UICC) (6), and patients were categorized into Stages IB (n = 28), II (n = 32) and IIIA (n = 52). Depending on the glandular architecture, cellular pleomorphism and mucosecretion of the predominant pattern, lung adenocarcinoma was divided into three degrees of differentiation: well, moderately and poorly differentiated (Fig. 1). In well-differentiated tumors, the glandular structures are easily demonstrated on routine microscopy, the malignant glands are composed of tall columnar or mucinous epithelium, with large, round nuclei and prominent nucleoli, and mitotic figures are commonly present. In moderately differentiated tumors, the glands are not as well developed as in well-differentiated neoplasms. Poorly differentiated adenocarcinomas are composed of a neoplastic cell population showing very poor glandular development, with small abortive or distorted glands scattered among sheets or solid islands of poorly differentiated epithelial cells.

No patient had received radio- or chemotherapy before tumor excision. Postoperatively, all patients received four cycles of adjuvant chemotherapy of navelbine 25 mg/m² d1, 8, and cisplatin 75 mg/m² d1, q3w. Among these cases, there were 25 cases of well differentiation, 65 cases of moderate differentiation and 22 cases of poor differentiation carcinoma. This study was conducted under the regulations of the Institutional Review Board. All these patients were successfully followed up at least for 5 years after operation.

Figure 1. Depending on the glandular architecture, cellular pleomorphism and mucosecretion of the predominant pattern, lung adenocarcinoma presents three degrees of differentiation: well (A), moderately (B) and poorly (C) differentiated.
STATISTICAL ANALYSIS

Correlation between CCSP, SPC and OCT4 expression and clinicopathologic factors was analyzed using Fisher’s exact probability test or χ² test. Univariate analysis was performed by modeling Kaplan–Meier survival curves. The log-rank test was used to evaluate the statistical significance of differences in survival distributions among prognostic groups.

Multivariate analysis was carried out by use of the Cox proportional hazard model. A P value of <0.05 was considered to be significant. The statistical data were obtained using an SPSS software package (SPSS 11.5 Inc., Chicago, IL, USA).

RESULTS

IMMUNOFLUORESCENT STAINING FOR CCSP, SPC AND OCT4

We analyzed the expression of CCSP, SPC and OCT4 in 112 lung adenocarcinoma samples by immunofluorescent Staining. Immunofluorescent staining showed that SPC expression was found in all the cancer cells of lung adenocarcinoma in 112 cases (Fig. 1), 107 cases showed cells positive for CCSP, whereas only 5 cases showed cells for CCSP™. According to the phenotype characteristics of BASC, SPC™CCSP™ tissues of such five cases could be classified into the category in which BASC could not be tested and identified, so the five cases could be excluded from statistical analysis. In the rest of 107 cases, CCSP+ cells of 80 cases made simultaneous expressions of OCT4 (OCT4™BASC), the rest 27 cases did not express OCT4 (OCT4™BASC) (Fig. 2).

CORRELATIONS BETWEEN CCSP, SPC AND OCT4 EXPRESSION AND CLINICOPATHOLOGIC FEATURES

The association between clinicopathologic features of the 107 patients with lung adenocarcinoma and CCSP, SPC and OCT4 expression is shown in Table 1. There was a correlation between differentiation and OCT4 expression (P = 0.047). OCT4+ BASC phenotype was mostly found in poorly differentiated tumors, whereas OCT1+ BASC phenotype was mainly found in well-differentiated tumors. There was no obvious correlation between cancer stem-like cell phenotype and the age, gender, smoking experience and pathological stages of patients.

DETERMINATION OF INDEPENDENT FACTORS AFFECTING PROGNOSIS

For the 107 patients with lung adenocarcinoma, the 5-year overall survival rate was 31.8%. And the 5-year survival rate of patients with OCT4™BASC was only 22.5%, whereas that of those with OCT4™BASC was 59.3%. In univariate analysis by log-rank test, the pattern of survival curves shows the expected trend of decreasing survival with increasing stage at diagnosis, which is statistically significant (P < 0.015), and notably, patients with OCT4+ BASC expression show a more highly significant increase in mortality than those with OCT4™BASC (P = 0.019) (Table 2 and Fig. 3). The result of multivariate Cox’s analysis reveals that pathological stages of TNM (P = 0.008) and BASC phenotypes (P = 0.015) are independent prognostic factors (Table 3). There is no statistically significant correlation between survivals and factors like age, gender, smoking experience and differentiation.
DISCUSSION

Currently, CSCs are increasingly accepted as the initiating cells for malignant tumors (9). These tumor-initiating cells have been proposed to originate from the transformation of somatic stem/progenitor cells within the organ where the tumor develops (10). The research on CSC is a newly emerging discipline since 1990s. In 1994, researchers led by Lapidot separated and verified CSC from human acute leukemia for the first time in history (11). Singh et al. (12) and Al-Hajj et al. (13) verified that CSC exists in human glioblastoma and breast cancer, respectively. In the past 5 years, great achievements have been made in the research on CSC in solid tumors. Currently, there are only a few studies dealing with CSC in human lung cancer. Ho et al. (14) first reported that the CSC from NSCLC including adenocarcinoma was enriched in the SP fraction, whereas Eramo et al.
(15) identified the CD133 antigen as a marker of CSC in NSCLC. More recently, Chen et al. (16) reported that OCT4, a key transcriptional factor which is essential for the self-renewal and multipotency in ESCs, is present and functional in the CD133⁺ stem cell-like population isolated from NSCLC tissues and cell lines. Nevertheless, such research outcome does not fit in the theory of tumor stem cells, which insists that CSC originates from the malignant transformation of adult stem cells and that CSC should share the same phenotype with adult stem cells.

Kim et al. (5,17) provided the first evidence that in the mouse lung, the resident stem cells have been identified along the adult airway epithelium. For example, the Basal cells site in the submucosal gland duct and the inter-cartilaginous zone of the bronchi, and the variant Clara cells located in pulmonary neuroepithelial bodies of the bronchioles and at the BADJ (18). The variant Clara cells at BADJ (also named as the BASCs) have experimentally proven to be the initiating cells of lung adenocarcinoma (5). The mouse lung BASCs co-express markers related to the bronchiolar Clara cells (CCSP) and the AT2 cells (SPC), and possess the potential to differentiate into Clara or AT2 cells. Kim et al. (5) reported that in the mouse lung, enforced expression of mutant K-ras oncogene under the control of CCSP promoter initiates the formation of adenomatous hyperplasia and adenocarcinoma as well. Ji et al. (19) further reported that the lung adenocarcinoma initiated by K-ras oncogene is not metastatic, and the acquirement of metastasis potency needs an additional inactivation in tumor suppressor genes, such as LKB1 and P16INK4A. This 'two-hit' transformation of stem cells is in concordance to the classic multistep model of carcinogenesis.

On the basis of these findings, it is reasonable to expect that BASCs represent an initiating cell source of human lung adenocarcinoma. In this study, we provided the evidence that the CSC with BASC properties is present in human lung adenocarcinoma tissue samples, which could be divided into two phenotypes as OCT4⁺BASC and OCT4⁻BASC. The data from this report reveal that CSC with BASC properties can be tested and verified in 95.5% (107/112) of lung adenocarcinoma, which have BASC-like lung adenocarcinoma stem cell expression and BASC phenotype, and 74.8% (80/107) of which have simultaneous expression of OCT4, a member of the POU family transcriptional factors, which is essential for maintenance of the stemness status in the ESCs (20). However, it seems that little work has been done on the research over the correlation between CSC and prognosis of patients. The 5- to 7-year follow-up on the above-mentioned 107 cases of lung adenocarcinoma indicates a close correlation between the patient survival and the phenotype of OCT4⁺BASC, which shows an unfavorable prognosis of those with OCT4⁺BASC expression.

The self-renewal and multipotency is the corner stone of CSC, but the underlying mechanisms remain largely unknown (21). In ESCs, the stemness status is maintained by a group of regulatory genes. Studies with human ESCs indicate that OCT4 and the homeobox protein Nanog are the key transcription factors that cooperatively maintain the pluripotency (22,23). Other studies further document that OCT4 can mediate the re-programming of functional genomics in terminally differentiated somatic cells and can stimulate them to convert into the pluripotent cells (24,25). Interestingly,
OCT4 has also been detected in CSC from several solid tumors (10). Chen et al. described that knock-down of OCT4 expression in the CD133\(^+\) stem-like lung cancer cells significantly inhibit the abilities of tumor invasion and colony formation, and increase apoptotic activities. The existence of BASC features in these CSCs, however, has not been examined by the authors (15).

In the current study, we provided the first evidence that the human lung adenocarcinoma stem cells with BASC properties express OCT4 protein. In 107 cases of tumor sections tested, cancer cells with OCT4\(^+\)BASC phenotype were detected in 80 cases. A significant association between survival and the OCT4\(^+\)BASC phenotype was noticed (\(P = 0.019\)). Even after adjusting for BASC phenotype and tumor stage, the OCT4\(^+\)BASC phenotype was still significantly associated with a poor prognosis. This data pointed out that the expression of self-renewal regulatory gene OCT4 in these cells indicated the worse clinical outcomes. A further research on the regulatory mechanism of OCT4 and a randomized controlled trial are warranted in this setting.

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**Conflict of interest statement**

None declared.

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