Original Article

A severe combined immunodeficiency disease mouse model of human adenocarcinoma with lepidic-predominant growth

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1. Introduction

Lung cancer, particularly pulmonary adenocarcinoma, has become the leading cause of cancer death in the world, including the United States and Japan. It is still the general consensus that the poor prognosis of pulmonary adenocarcinoma reflects its aggressive biological nature and the paucity of basic studies on its molecular carcinogenesis. Useful in vitro and in vivo models that imitate the histological and clinical characteristics of this tumor are necessary to determine the molecular mechanisms of pulmonary adenocarcinogenesis.

The conceptual validity of orthotopic models was first suggested by Paget in 1889 [1]. The organ site-specific implantation of tumor cells is essential for achieving optimal tumor growth and progression in vivo. This idea is widely supported by numerous studies using various tumor models including lung cancer. In a previous study, the biological characteristics of representative human pulmonary adenocarcinoma cell lines were investigated using the intrabronchial orthotopic propagation method [2]. However, existing lung cancer cell lines were established by in vitro culture or from subcutaneous xenografts. These develop into tumors in nude mice or mice with severe combined immunodeficiency disease (SCID); however, the tumors are all moderately or poorly differentiated adenocarcinoma and do not mimic the basic characteristics of pulmonary adenocarcinoma, namely lepidic (previously called bronchioleolar) spread.

The purpose of this study was to establish a xenograft model using SCID mice that recapitulates the histopathological characteristics of the original human lung tumor.

2. Materials and methods

2.1. Patient samples

Surgically resected tissue from 16 patients with pulmonary adenocarcinoma who attended the University Hospital of Tsukuba (Ibaraki, Japan) or Ibaraki Prefectural Central Hospital (Ibaraki, Japan) was used in this study. Histologically, the tumor specimens included 14 adenocarcinomas with subtypes of lepidic (n = 2), acinar (n = 4), papillary (n = 3), micropapillary (n = 1) and solid (n = 4), and 1 case each of invasive mucinous adenocarcinoma and fetal adenocarcinoma [3]. The pathological stages were T1aN0M0 (n = 1), T1cN0M0 (n = 1), T2aN0M0 (n = 3), T2bN0M0 (n = 1), T2N1M0 (n = 1), T3N0M0 (n = 2), T1cN2M0 (n = 2), T2aN2M0 (n = 1), T2bN2M0 (n = 1), T4N0M0 (n = 1) and T2bN0M1b (n = 2) [4]. This study was approved by the Regional Committee for Medical Research Ethics (University of Tsukuba and Ibaraki Prefectural Central Hospital). All patients gave their informed consent before sample collection, in accordance with the institutional guidelines.

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2.2. Experimental animals

Five-week-old female SCID mice, with a CB-17 genetic background, were obtained from CLEA Japan, Inc. (Tokyo, Japan). The mice were free of known pathogens at the time of the study, and were housed in sterilized filter-topped cages with ad libitum access to autoclaved food and water. The animal experiments were carried out humanely after receiving approval from the Institutional Animal Committee of the University of Tsukuba and were in accordance with the Regulations for Animal Experiments of our university and the Fundamental Guidelines for Proper Conduct of Animal Experiments.

2.3. Fresh tumor cell implantation and histopathological studies

After removing a specimen in the operating room, the specimen was transferred to the Department of Pathology, where a pathologist immediately cut the specimen at the tumor site using a cutter. A fresh tumor fragment (approximately 8 mm³) was then removed from the necrotic area of the resected tumor. The fragment was minced as finely as possible under sterile conditions using serum-free RPMI 1640 containing antibiotics. This suspension, which contained 10,000 protease units of dispase (GODO SHUSEI, Tokyo, Japan), was heated in a shaking water bath for 60 min at 37 °C to make a single-cell suspension. After cooling the suspension to 37 °C, a volume of 0.1 ml of medium was injected into the bronchus in each of two SCID mice. Tumor cells were implanted in 27 SCID mice by the tracheal puncture and instillation method. Less than 5 min were required to implant the tumor cells for each animal. The rate of surgery-related mortality was < 8% (2/27). SCID mouse tumors mimicking the primary histology of adenocarcinoma were established for four of the 16 primary tumors (25%; Table 1). Four tumors developed at the injection site in 27 animals (14.8%). One of the four SCID mice (H8-13) was sacrificed on day 180 after implantation; the remaining three (H11-18, H12-20, H13-23) were sacrificed on day 365 after implantation. The former animal showed dyspnea and weight-loss when it was sacrificed; the others showed no abnormalities during the observation period. All of the tumors in these animals developed in the peripheral regions of the lung. The microscopic appearance of the tumor cells in these animals showed a very similar morphology to the original surgically resected tumors from which they were derived. No extra-pulmonary metastasis was observed in the heart, liver, adrenal gland, spleen, kidneys, brain, or mediastinal lymph nodes. Immunohistochemically, the protein expression of CEA, TTF-1, SPA and p53 in the tumor cells was also similar to that of the original human tumor specimens (Table 2). The tumor cells of the H8-13 SCID mouse showed acinar growth in the peripheral regions of the lung accompanied by multiple pleural dissemination. The tumor cells of the H11-18 SCID mouse showed solid-predominant growth with partial acinar growth. Surprisingly, the tumor cells of the H12-20 and H13-23 SCID mice showed the replacement (lepidic) growth patterns of alveolar structures accompanied by multiple intrapulmonary lesions in several lobes (Figs. 2 and 3). In the H13-23 SCID mouse, the tumor cells of the lepidic growth occupied the whole area of one lobe, mimicking a pneumonic type adenocarcinoma with a lepidic growth pattern (previously called bronchioloalveolar) pattern. As for the tumor cell types of H12-20 and H13-23, the tumor cells of H12-20 were classified as goblet cell type and those of H13-23 were Clara cell type or type II pneumocyte type based on the morphological criteria [5–7]. Moreover, we hypothesized that the tumor cells in H13-23 were Clara cell type or type II pneumocyte type, since they are immunohistochemically-positive for anti-

Fig. 1. The intrabronchial propagation technique (see Materials and methods).
For many years, implantation of subcutaneous xenografts has been a standard method for establishing animal tumors for human cancer research. Although such tumors have helped us to understand the biological nature of human cancer, they do not truly represent the histological characteristics of human cancer [9]. Many previous reports have suggested that the organ site-specific microenvironment is an important factor for the growth of implanted human cancer cells in immunodeficient animals [10,11].

In this study, the orthotopic implantation method was modified for human pulmonary adenocarcinoma of the peripheral regions using the lungs of SCID mice. Tumor cells from two of the four human cases were successfully implanted in SCID mice and developed into lepidic adenocarcinoma in the SCID mouse lung. It is very interesting that the two SCID mouse tumors showed lepidic growth, while the original tumors included an acinar component beside a lepidic component. We hypothesized that the method of orthotopic implantation influenced the tumorigenicity of the lepidic and acinar components. When adenocarcinomas developing in the peripheral lung tissue are subdivided into two groups showing lepidic or non-lepidic growth, approximately 75% of tumors are considered to show total or partial lepidic growth of pure bronchioloalveolar carcinoma (previously called BAC [12]) or "adenocarcinoma of mixed subtype with a BAC component" [13]. It is therefore extremely important to understand the growth and/or progression patterns and the molecular carcinogenesis of lepidic growth adenocarcinoma. However, to date there have been no reports on human lung cancer cell lines that show lepidic growth. This method of orthotopic implantation using SCID mice allows us to develop cell lines showing lepidic growth, which is the main characteristic of peripheral type lung carcinoma. Some limitations associated with the present study warrant mention. First, lepidic pulmonary adenocarcinoma shows a gradient of atypia, with milder atypia in the peripheral region than in the central region. However, the tumor cells in the xenograft monotonously spread over the alveolar walls, in the same manner as that...
observed in H13-23 SCID mouse tumor. Tumor cells of the SCID mouse models might be characterized as a metastatic tumor with alveolar spreading similar to a micropapillary subtype with a malignant potential against lepidic adenocarcinoma. Second, there were only two lepidic adenocarcinomas in the resected specimens, and none of the tumor cells were established in the SCID mouse investigated in this study. In a future study, to establish tumor cells with mild atypia of lepidic adenocarcinoma, many lepidic adenocarcinoma cells obtained from original human specimens may be required to establish cells which mimic human lepidic adenocarcinoma in the SCID mouse.

5. Conclusions

SCID mouse models with human pulmonary adenocarcinoma tumors showing lepidic spread were established using an orthotopic intrabronchial implantation method. The method provides a useful model of lepidic carcinoma, the major histological type of human lung cancer.

Conflict of interest

The authors declare no conflicts of interest in association with the present study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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