Establishment of an orthotopic lung cancer model in nude mice and its evaluation by spiral CT

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ABSTRACT

Objective: To establish a simple and highly efficient orthotopic animal model of lung cancer cell line A549 and evaluate the growth pattern of intrathoracic tumors by spiral CT.

Methods: A549 cells (5×10^6 mL^{-1}) were suspended and inoculated into the right lung of BALB/c nude mice via intrathoracic injection. Nude mice were scanned three times each week by spiral CT after inoculation of lung cancer cell line A549. The survival time and body weight of nude mice as well as tumor invasion and metastasis were examined. Tissue was collected for subsequent histological assay after autopsia of mice.

Results: The tumor-forming rate of the orthotopic lung cancer model was 90%. The median survival time was 30.7 (range, 20-41) days. The incidence of tumor metastasis was 100%. The mean tumor diameter and the average CT value gradually increased in a time-dependent manner.

Conclusions: The method of establishing the orthotopic lung cancer model through transplanting A549 cells into the lung of nude mice is simple and highly successful. Spiral CT can be used to evaluate intrathoracic tumor growth in nude mice vividly and dynamically.

KEY WORDS Lung cancer; orthotopic lung cancer model; spiral CT; A549

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Introduction

Lung cancer is by far one of the malignant tumors with the highest incidence and mortality all over the world. Lung cancer can be assigned into small cell and non-small cell lung cancer, among which the latter accounts for 85% with a 5-year survival rate of only about 15% (1). Over the past few decades, research on lung cancer has made great progress, but its response rate and survival improvement have been very slow. Selecting a simple and feasible tumor-bearing animal model is one of the important ways to study the pathogenesis and curative effect of lung cancer. The ectopic subcutaneous tumor-bearing animal model of lung cancer is convenient to be established and conducive to observation of tumor growth. It has been extensively applied for the drug sensitivity assay of lung cancer (2). Nonetheless, the ectopic subcutaneous tumor-bearing animal model does not represent the real tumor in real environment, since the growth and dissemination of tumor cells *in vivo* are organ-specific (3). Compared with the tumor model constructed by ectopic subcutaneous inoculation, orthotopic lung cancer can mimic natural environment of tumorigenesis (3). Thus, it is of necessity to establish an appropriate orthotopic animal model of non-small cell lung cancer, to observe the characteristics of tumor growth and evaluate its efficacy.

Many researchers have constructed orthotopic tumor models of urologic neoplasms and digestive tract tumors in immunodeficient SCID mice and nude mice making use of corresponding malignant tumor cells (4,5). McLemore *et al.* was the first establishing an orthotopic model of human lung cancer in nude mice through endobronchial injection (6). Later researchers adopted injection of tumor cells via the tail vein (7,8) and intrapulmonary inoculation of the tumor mass (9). However,

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the abovementioned methods have certain limitations because of great technical difficulties, which require the experiment operator to have considerable proficiency. In addition, their success rates are not high. This study aims at building up an orthotopic animal model of lung cancer in BALB/c nude mice by intrapulmonary inoculation of cancer cell suspension, so as to learn intrapulmonary tumor growth and changes of nude mice coupled with dynamic spiral CT through observation of the survival time and status of treated mice.

Methods and materials

Cells and cell cultivation

Lung adenocarcinoma cell line A549 was obtained from the Shanghai Institute of Cell Biology of the Chinese Academy of Sciences, which was incubated using DMEM medium containing 10% calf serum (US Hyclone Cooperation, Logan, US) (serum derived from Brazil and manufactured in Logan, Utah, US, article no. sv30087.01, batch no. NvM0347) in a 5% CO_2 incubator at 37 ℃ and routinely sub-cultured.

Animal and animal feeding

A total of 20 male nude BALB/*c nu/nu* mice aged 6-8 weeks and weighing 20-22 g were purchased from the Guangdong Provincial Medical Experimental Animal Center, the certificate no. of qualified animals SCXK (Yue) 2008-0002, raised in SPF environment at room temperature (25+2) ℃ and given aseptic full-price nutritional pellet feed and sterile water.

Methods

A total of 20 nude BALB/*c nu/nu* mice were intraperitoneally injected with pentobarbital sodium (10 mg/kg) to induce anesthesia and fixed in the right lateral decubitus position after anesthesia. Then 100 μL A549 single cell suspension (5×10^6 mL^{-1}) prepared with the 1ml injector was percutaneously inoculated into the upper margin of the sixth intercostal rib on the right anterior axillary line to a depth of about 5 mm rapidly and after that, the needle was promptly pulled out. Nude mice were maintained in the right lateral decubitus position after injection and observed until complete recovery.

Spiral CT scanning

Mice were fixed on a plane plate in a supine position following intraperitoneal injection of pentobarbital sodium (10 mg/kg) to induce anesthesia. The Toshiba Aquilion16-slice CT scanner was adopted to perform routine thin-slice plain CT scan from the mouse neck to abdomen, slice thickness 1mm, reconstruction interval 0.5-0.8 mm, tube voltage 100 kV and tube current 90-110 mA.

Survival index detection

Body weight

Body weight of each mouse was measured using the electronic balance every 4 days (precision: 0.1 g) and data were stored in EXCEL for inspection.

Survival time

Survival time was the time from modeling to natural death of mice.

Gross observation

Lung cancer cell invasion and metastasis in thoracic, mediastinal and pericardial parts as well as pleural effusion of mice were observed in the operation group.

Tissue embedding and pathological detection

After anesthesia, mice were sacrificed by cutting off the neck under anesthesia to remove the intrathoracic heart, bilateral lungs, pleura, lymph nodes and mass etc, which were fixed in 10% formalin solution and embedded in paraffin to make into tissue sections, subjected to HE staining and observed under a microscope.

Data analysis

Data were processed using SPSS 13.0 software package and expressed as $\bar{x}\pm s$. The Kaplan-Meier method was used to calculate the survival rate and median survival time, deriving the survival curves using median values.

Results

Survival index results of nude mice

Intrathoracic implantation via puncture succeeded in all the 20 nude mice. Gross anatomy and pathological confirmation following termination of experimental observation showed that the success rate of tumor implantation was 90% (18/20). Figure 1 shows the trend in mean body weight changes at different time points of observation after inoculation of tumor cells. It can be seen that the mean body weight of nude mice continually declined at around 10 days following intrathoracic inoculation of tumor cells. At around two weeks following inoculation of tumor cells, tumor-bearing nude mice developed similar cachectic symptoms as observed in clinical patients with tumors and symptoms of labored mouth breathing could be observed in dying nude mice. Figure 2 shows the survival curves of nude mice and the median survival time is 30.7 (range, 20-41) days. Figure 3 shows the image of the gross specimen of tumor-bearing nude mice, within which the right lung mass is clearly visible and there are visible

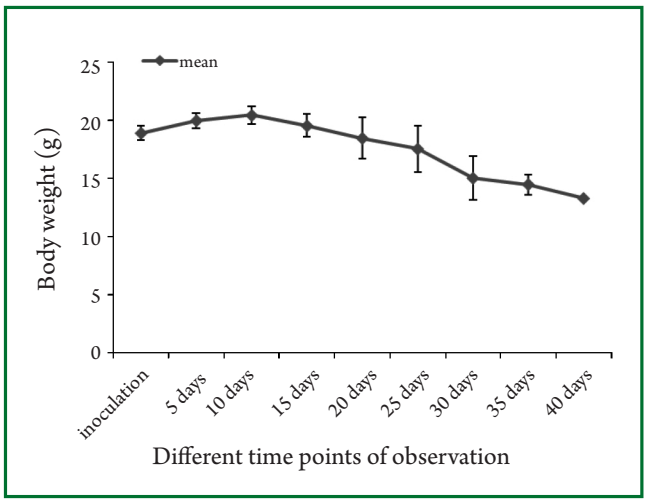

Figure 1. Weight trends of nude mice inoculated with A549.

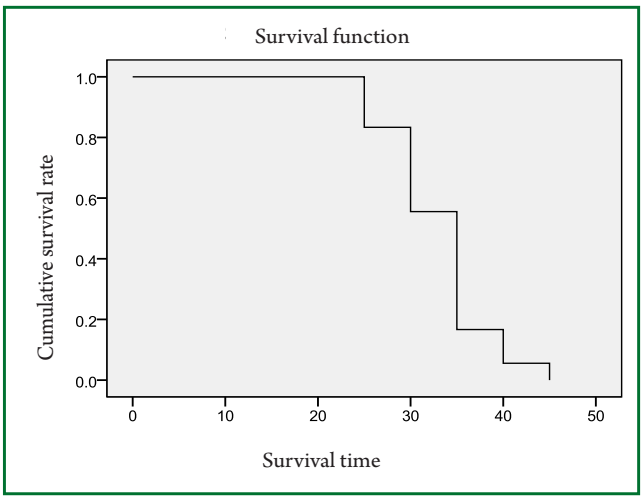

Figure 2. Survival curves of nude mice inoculated with A549.

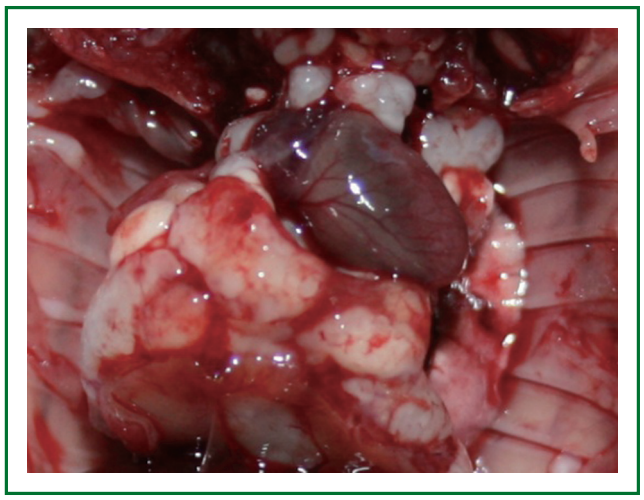

Figure 3. Macroscopic features of the thoracic cavity of tumor-bearing nude mice.

metastatic foci in contralateral lung tissue, mediastinal tissue and chest wall. The intrathoracic tumor metastasis rate of nude mice was 100% (18/18). Figure 4 is the diagram for HE staining of histopathological sections of lung tissue in nude mice. Figure 4A indicates that tumor tissue is located in the central region of the visual field, which developed accompanying alveolar tissue and blood vessels. Figure 4B indicates irregular tumor cell alignment, nuclear hyperchromatism and obvious heteromorphism after magnifying.

Observation of orthotopically implanted tumor growth in nude mice using spiral CT

Spiral CT scan was performed at day 7, 14 and 21 on tumor-bearing nude mice after intrathoracic injection of lung tumor cell suspension into the right chest for three consecutive weeks, to observe intrathoracic tumor growth in nude mice dynamically. Intrathoracic tumor diameters for the three times of spiral CT scanning were respectively: (1.1±0.4) , (2.7±0.7) and (4.8±1.4) mm. Intrathoracic tumor CT values for the three times of spiral CT scanning were respectively (32±12) , (68±15) and (89±13) HU. Figure 5 shows the results of spiral CT scanning of the orthotopically implanted tumor of lung cancer for the same nude mouse. Figure 5A shows small right lung nodules one week after intrathoracic injection of tumor cell suspension and gradual growth of intrathoracic tumor mass over time. Figure 5C shows right lung mass enlarged for several times and widened mediastinal shadow at week 3, which was confirmed as mediastinal metastasis by gross anatomy.

Discussion

Our studies have led to the development of a rapid and reproducible model of orthotopic lung cancer in which human lung tumor-derived cell lines can be engrafted throughout the pulmonary parenchyma. Among the several orthotopic lung cancer modeling methods commonly seen by far, the tumor-forming rate of injection via the tail vein is as high as 90-100%, but this method may lead to pulmonary vascular embolism by tumor cells thus causing death (7,8); intratracheal injection is intractable and its tumor-forming rate is only around 80% (10,11). In this study, direct intrathoracic injection was applied to establish the orthotopic lung cancer animal model using common human lung adenocarcinoma cell line A549 and the tumor-forming rate of modeling was 90%, similar to those obtained using other lung cancer cell lines (2,12,13). An advantage of this model includes the simple and easy implantation procedure by direct puncturing through the intercostal space to lung parenchyma, without thoracotomy or intubation.

In this study, spiral CT was also adopted to perform real-time evaluation of tumor formation following intrathoracic

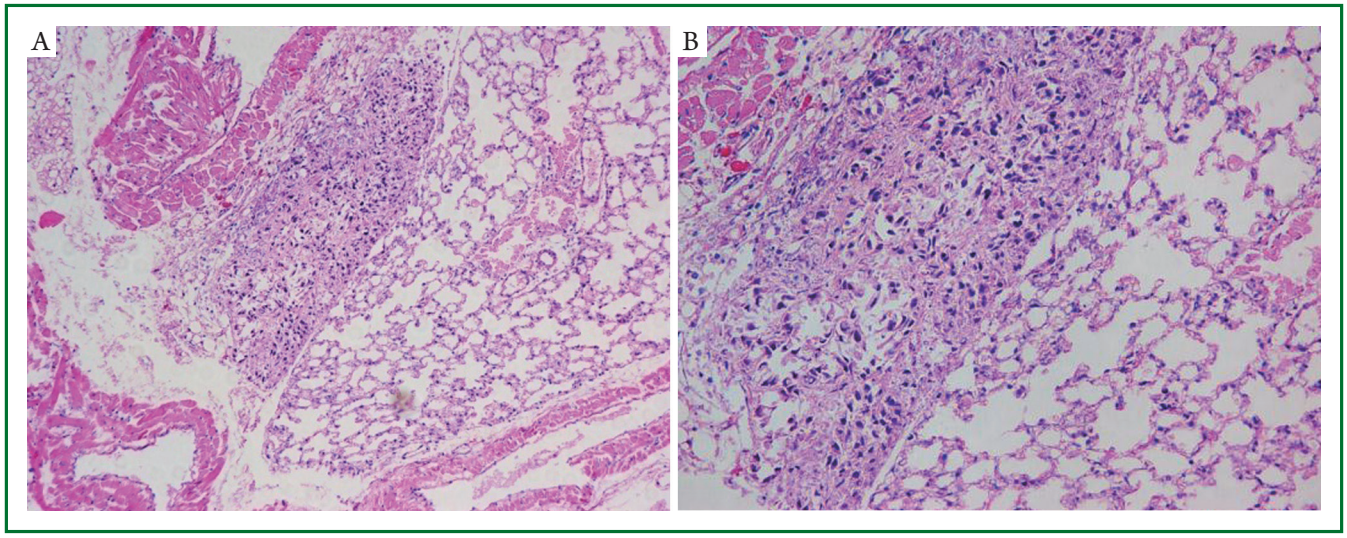

Figure 4. Microscopic features of the mouse lung. H/E stain of the paraffin-embedded sections of tumor developed within the mouse lung. (A ×100; B ×200).

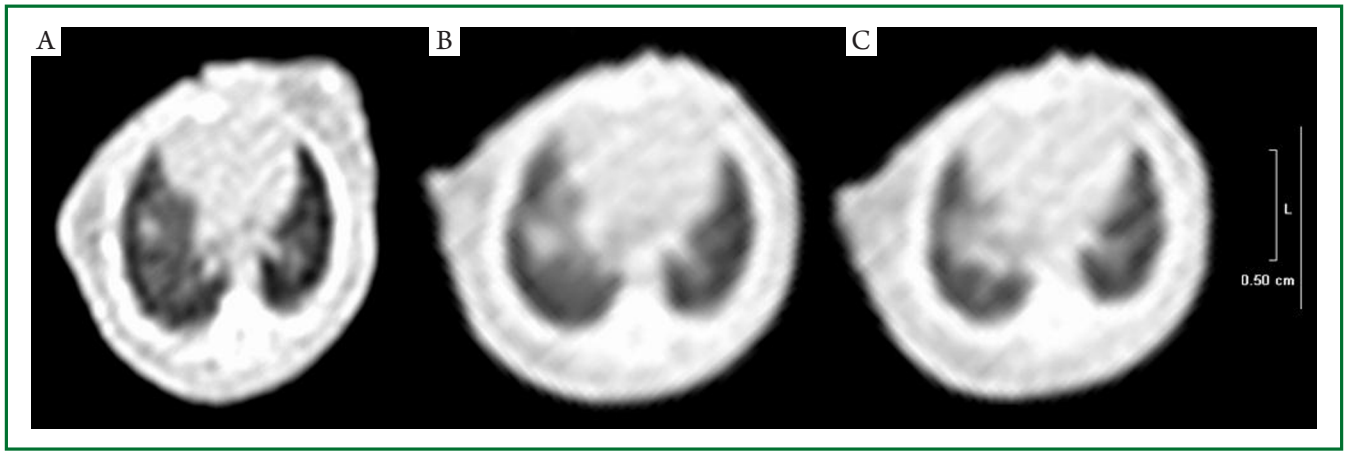

Figure 5. CT image of the orthotopic xenograft of lung cancer in nude mice. Figure A shows small right lung nodules one week after intrathoracic injection of tumor cell suspension and Figure B shows gradual growth of intrathoracic nodules; Figure C shows right lung mass markedly enlarged for several times.

inoculation of nude mice, showing solid tumor formation in right chest of mice at around one week following injection of tumor cell suspension. The trend of body weight changes in tumor-bearing nude mice in Figure 1 reflected a declining trend of mean body weight of tumor-bearing nude mice at around 10 days after inoculation of tumor cells and the time point for body weight decline of tumor-bearing nude mice was basically consistent with the emergence time of tumor formation indicated in CT results. Thus, in this model, it is critical to observe body weight changes in nude mice following orthotopic injection of tumor cells to judge the successfulness of tumor formation. Gross anatomy and pathological detection of nude mice successfully established with an orthotopic lung cancer model confirmed metastatic foci in contralateral lung tissue, mediastinal tissue and chest wall to varying degrees, with an intrathoracic tumor metastasis rate of 100%, consistent to other similar reports (2,13). This may not only be related to characteristics of tumor cell metastasis and invasion, but also be related to the short survival time of nude mice following tumor formation and short observation time.

In the preliminary experiment of this study, two nude mice rapidly died of intrathoracic massive hemorrhage and respiratory tract symptoms of hemoptysis after withdrawal of the puncture needle. This can mainly be explained as follows: the point of puncture was located besides the posterior axillary line of the sixth intercostal space of right chest wall, which was close to the hilar vessels after insertion of the needle and could pierce into hilar vessels to induce intrathoracic massive hemorrhage and rapid death, as also confirmed by subsequent gross anatomy. In order to avoid injury to hilar vessels, the location adjacent to the median or anterior axillary line of the sixth intercostal space was chosen as the point of puncture and the depth of needle insertion was strictly

controlled at around 5 mm. The subsequent puncture process was successful without occurrence of aforementioned phenomena.

The orthotopic animal model of lung cancer can well mimic the real environment of tumor growth, but due to tumor location within the chest, it is not conducive to real-time observation of the implanted tumor mass in the experimental process. In some orthotopic lung cancer model studies, pathological anatomy is performed after execution of a certain number of nude mice regularly or at the end point of observation to monitor tumor growth and dissemination, but this method requires a big number of nude mice and a great deal of time or energy (12,13). In this experiment, spiral CT scan was employed to conduct dynamic observation of intrapulmonary tumor growth within mice, which can not only clearly observe the successfulness of intrathoracic tumor implantation, but also observe the gradual tumor growth *in vivo* over time, to judge the occurrence of mediastinal tumor metastasis. Some studies also put forward using micro-MRI to assess the lung tumor model in small animals (14), but some scholars have pointed out that among lung tumor images, compared to micro-MRI, micro-CT behaves better in contrast imaging of air and soft tissue (15), so more studies apply micro-CT to observe the lung cancer model of rodents (15-17). Indeed, micro-CT is superior to conventional spiral CT in resolution of *in vivo* imaging of small animals, but micro-CT equipment is not liable to be obtained for many research units and ordinary spiral CT can achieve the slice thickness of 1mm, which can observe orthotopic intrathoracic tumor growth and development of nude mice. Thus, as an objective and feasible method, dynamic spiral CT scanning can be further used for efficacy observation during antitumor drug experiments.

To sum up, construction of orthotopic human lung cancer model via direct intrathoracic injection of human non-small cell lung cancer cell suspension is convenient, has a high tumor-forming rate and can better mimic the occurrence and development of clinical non-small cell lung cancer. Spiral CT scanning can perform dynamic observation and evaluation of the whole process of modeling.

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