Establishment of Metaasis Lung Cancer Model by Injection Of EMT-6 Cells in BALB/c Mice
Qimei Wan
Zhengzhou University Shijiazhuang, Hebei Province, 050100

Abstract: Objective To establish a metaasis lung cancer model with parent Clinic, Stable and repeatable charac-Carcinoma at logarithmic growth phase were harvest by 0. 25% trypsin digestion, And equal rated at 3 dens of 1x106.Cells per ml (High Concentration), 5x105.Cells per ml (Medium concentration), And 1x105.Cells/mL (Low Concentration). Phosphate-buffered saline, then 0. 2 mL EMT-6 tumor cells, intravenously injected. evalua-tion indicators, biological characteristics, As clinic appearance. Time. tumor formation Survival time. Tumor growth, pathological characteristics. metastasis lung models. different concentrations. then studied. screen. best metastasis lung cancer model. Finally. Repeatability, stability. model. determined by 3 repeated exper-iments. successfully establish. metastasis lung cancer model. BALB/c mice. Results BALB/c mice. anatomeed after EMT-6 tumor cells Injection,; Showed, group. high concentration treatment Tumor nodes. Lung surface began. appear at day 7, all animals died 18 d; In the group of medium concentration, Tumor nodes on Lung surface begin to appreciate at day 7 and all animals divided in 28 d; In the group of Low Concentration, Tumor nodes on Lung surface appeared partially at day 14, All mice lung surface had tumors at day 21, The number of tumors reach the highest level (An average of 12-15/mouse), The volume of tumors' became much bigger at day 35, Mice began to die at day 24, And all mice divided in 42 d. Conclusion we excluded that the establishment of mice models using low EMT-6 cell concen-tration can provide convenient 4-week time window for treatment, Observation and test from tumor formation to dead, The histo-pathology of models lung was consistent with clinical features of Lung Cancer, The clinic appearance was easy to identify, And the repeatability and stability tests the models were consistent along the 3 times.

Keywords: EMT-6 Cell; Animal Model of metaasis lung cancer; BALB/c mouse

At present, lung cancer is the most common malignant tumor with the highest morbidity and mortality in the world. (Kuper et al., 2002; Ministry of Health of the People's Republic of China, 2008; Chen Wanqing and others, 2010). Because of the abundant capillaries in the lungs, it is located in the center of the whole circulation system, and the pulmonary circulation is prone to lung metastasis due to the dual arteries. Therefore, the lung is the most common organ for malignant tumor metastasis. According to the autopsy statistics of extrapulmonary malignant tumor patients

A hot spot in cancer research (JEMAL, 2011). The main reason why the curative effect of lung cancer is not improved is because of its complicated clinical manifestations, which is difficult to be found in the early stage.

Human Lung Cancer, Pathogenesis, Animal Models with similar development process are the focus of Lung Cancer Research. Therefore, establishing multiple stability is good,

High Repeatability, Significant Clinical Features, Objective Experimental Animal models for evaluating indicators.
are the basis for studying metastatic lung cancer. Reliable Experimental Animal models can simulate the process in vivo, which is helpful for accurate observation of tumor growth characteristics. Evaluation of drug efficacy is critical, and it can also improve the clinical treatment.

Theoretical Basis. Because the lung tissue in Mice, Molecular characteristics are similar to human, and almost all human genes can be found in mice homologous genes (Gitton et al., 2002; Dragani, 2003). While mice have a short reproductive cycle, because of its rapid growth, it is widely used in the field of Tumor Research (Zhang Yongjiang and others, 2002). With the rise of research in the field of tumor immunotherapy, more and more experiments are using mice with complete immune function to establish metastatic lung cancer models that meet the research needs. Although the method of establishing metastatic lung cancer model by injecting cancer cells into the tail vein of mice is often used, there are many differences in the evaluation index of this lung cancer model. A systematic discussion on its suitable window period and model Repeatability. Currently, in addition to melanoma cells (B16) Characteristics of lung cancer model in mice with tail vein injection, in addition to more applications, the mice model of metastatic lung cancer with normal immune function is still relatively small.

EMT-6 Cell Establishment

There are few reports of metastatic lung cancer Models. So build more immune system integrity, functional, stable Clinical Features, The Experimental Animal Model of metastatic lung cancer with clear evaluation index is the basis for improving the research level of Lung Cancer Immunotherapy and the guarantee for determining the universality of the research results.

In this study, the tail vein injection, China, Low 3. A concentration EMT-6 Stable Cell Line, Reliable metastatic lung cancer BALB/c To explore the relationship between the injection concentration of cancer cells and the Development of tumor, and give a suitable window period for the study. And through 3. Repetitive experiments and detailed clinical observation indicators to evaluate EMT-6 Metastatic lung cancer BALB/c Stability of mouse model, a standardized model evaluation index system is put forward.

1. Materials and Methods

1.1 Material

1.1.1 Experimental Animals. 5~7. Week-old female BALB/c Mice, body mass 16~22g. The production license number of the Laboratory Animal Center of Sichuan University is: Scxk (Chuan) 2013-026. SPF Keep your Ventilation Under Conditions, Temperature, Suitable for humidity, normal diet, license number: Syxk (Chuan) 2013-185. All animal experiment procedures are evaluated and approved by the Animal Experiment ethics committee of the Experimental Animal Center of Sichuan University.

1.1.2 Cell Line. Murine breast cancer EMT-6 Cell lines provided by Key Laboratory of transplantation engineering and immunology, Ministry of Health, West China Hospital, Sichuan University.

1.1.3 Main Reagent. RPMI 1640 Medium, Fetal bovine serum, Trypsin and phosphate buffer (PBS) Purchased in USAGibco Company, green-Chain Double Anti-purchase in USAHy-clone Company.

1.2 Method

1.2.1 EMT-6 Cell culture and suspension preparation EMT-6 Fine

Cell in RPMI 1640 Pei, Yang Ye (Han10% Fetal, bovine, serum, serum, digestion, 1 000 r. Min 1 Centrifugal 5 min Go to the supernatant and reuse RPMI 1640 Medium Cleaning, 000 r. Min 1 Centrifugal 5 min Supernatant, cell count, PBS Dilution 1x10^6/1ml (High concentration), 5x10^5/1ml (Medium concentration), 1x10^5/1ml (Low Concentration) 3. Preparation of different concentrations, EMT-6 Cell Suspension.

1.2.2 The EMT-6 Metastatic lung cancer BALB/c Establishment of Mouse Model Will75 Only female BALB/c Mice in SPF Feeding under conditions. 1. After peripheral adaptation, the concentration of cell suspension was divided into five groups. Group, high concentration group, and low concentration groups, each group 25 Mice, each injection 0. 2
At the default point in time (7 d, 14 d, 28 d), each anatomy was taken to observe the pulmonary surface tumor and calculate the tumor rate. And then use the lung tissue 10% Poly formaldehyde fixation, Hematoxylin-Yi Hong (He) Staining and histopathological analysis. To evaluate the best injection cell suspension concentration EMT-6 Metastatic lung cancer BALB/c Mouse model, and then repeat the experiment 3. Time. The tumor number and survival time of each time point of the repeat experiment were compared to verify the repeatability of the model. The consistency of tumor formation and survival time in mice.

1.2.3 Observation index

Vaccination EMT-6 General shape after cell Condition: feed intake of Mice, Condition of clinical symptoms such as appearance and hair, as well as mental state, and at a preset point in time (Before the experiment 7 d, 14 d, 28 d, 35 d) Measuring body mass of Mice. The number of tumor foci: At the default point in time (7 d, 14 d, 21 d, 28 d, 35 d) Random fetch. Mice were randomly selected and dissected.

1.2.4 Histopathological examination

Lung Tissue of mice in each group

10% Paraformaldehyde was fixed and paraffin sections were made by conventional methods. He Observed under optical microscope after staining.

1.3 Statistical Analysis

Use SPSS 19. 0 Analysis, data to average ± Standard deviation, single factor analysis of variance and T inspection comparison, P < 0.05 That the difference was statistically significant.

2. Results and Analysis

2.1 Different Concentrations

EMT-6 Metastatic lung cancer BALB/c Xiao

Survival time of rat model BALB/c Intravenous Injection of mouse tail EMT-6 After cells, the high concentration group 10 dBegan to die, 18 dBAll deaths; Medium concentration group 11 dBegan to die, 28 dBAll deaths; Low concentration group DBegan to die, 42 dBAll deaths; According 3. Survival of mice in the group, drawn Km Survival curve (Figure 1.) The survival time of mice EMT-6 Decreased cell concentration but prolonged. Among them, the high concentration group 10 dB, Medium concentration group 11 dB, Low concentration group 21 dB The mice were found to have white tumor foci on the lung surface.

100%. Therefore, compared with the high concentration group and the middle concentration group, the low concentration group had a higher tumor rate. Early low mortality, 42 d The survival time provided a suitable lung cancer model for the experimental window period.

2.2 EMT-6 Metastatic lung cancer BALB/c Evaluation of Mouse Model

EMT-6 Metastatic lung cancer BALB/c The body mass of mouse model gradually decreased after tumor formation (Figure 2.) The main clinical symptoms before death were loss of appetite, Wasting shape, Hair erect, Untidy appearance, Sometimes humpback, Unresponsive to external stimuli, Poor performance and dull eyes, Poor mental state. The earliest death occurred after the cancer cells were inoculated. 21 d, 42 dBAll the mice died.

After injecting cancer cells 14 d The anatomy revealed that white began to appear on the lung surface of some mice. A round tumor, 21 dB It was found that there were various tumor foci on the lung surface of all mice. 100%, DI Increased number of white tumors on the surface of the lungs (Figure 3.) No tumor was found in other organs. Vaccination EMT-6

Cell Hind 3 Weeks, mice began to appear feeding decline; After inoculation 4. Individual weight loss in Mice, Hair erect, Appearance is not neat, and there is a slow reaction to external stimuli before death, Inactive and dull eyes, Poor mental state, even a bow and back state (Table 1.).

There is a strong correlation between death and the occurrence of each observation index.
Generally observed hair erect, untidy appearance, the appearance is thin. The mice died within weeks and sustained slow response was observed. Inactive and dull eyes, flagging, mice in 2 D inside will die.

2.3 EMT-6 Metastatic lung cancer BALB/c Mouse Model repetitive experiment

Ji and Di3. The second experiment and the second1. There was no significant difference in the number of tumor foci on the lung of mice at each time point.

(P > 0.05) Mice survival time was basically the same, the difference was not statistically significant (P > 0.05) (Table 2.).

3. Discussion

Quasi-in tumor of Mechanism Research and Treatment drug screening and field play the important role. Tumor transfer is refers to malignant tumor cells from primary tumor transfer to secondary organization or organ after to continue to proliferation growth formation and primary tumor pathological histological same of secondary tumor of whole process (Chaffer & Weinberg 2010). And lung metastasis animal model divided into spontaneous and Experimental lung metastasis animal model will EMT-6 cells by tail vein injection BALB/c Mice in vivo and formation of lung metastasis for Experimental lung metastasis (Price 1996). Experimental lung metastasis in tail vein inoculation cancer cells after cancer cells with blood cycle can faster to in distal proliferation can in lung formation obvious of transfer tumor (ROse & Connolly 1997).

This study by conventional modeling of Methods Selection BALB/c Small tail vein respectively Injection0. 2 mL, High, In, Low 3 A concentration EMT-6 Cells suspension again according to model into tumor time, Into tumor rate and survival final select 0. 2 mL Cell concentration \(1 \times 10^5\) A/ML into Line injection established a line with study time window period requirements have appropriate time processing, Observe, Evaluation of metastatic lung cancer Animal Model. This study further on the model of clinical symptoms, Lung Tumor quantity, Lung Tissue Pathology the research found this a kind of metastatic lung cancer BALB/c Mice Model into tumor after performance for loss of appetite, Body Quality decreased, Shape thin, The outside stimulation reaction slow, Activities reduce, Eyes dull, Depressed until death and clinical symptoms and clinical on cancer patient similar; Lung Tumor foci easy to identify count made

14 d After part mice lung surface began to appear naked eye visible of white tumor model 21 d After all mice lung surface were visible of white tumor into tumor rate 100%; Model 21 d

After mice began to appear death and lung surface tumor increased, Volume larger model 42 dln mice all death. This experimental model for study metastatic lung cancer development of each stage and the experimental drug treatment left the time length for the window period and clear and simple of clinical determination Index. Pathology detection results show that model of Lung Cancer histological characteristics typical obvious. Metastatic lung cancer BALB/c Mice Model 3 Times repeatability experimental results show that the model clinical characteristics obvious pathological with lung cancer of histological characteristics evaluation index clear system and model of biological characteristics stability repeatability good lung tumor number, Survival time consistent difference no statistical significance also not appear other organ of transfer and lung in situ Inoculation Methods of lung cancer model pathological results similar (Liu xin and, 2010) For metastatic lung cancer treatment drug of Set

Of, To Drug Programme of determine, Evaluation System Specification of provide the reliable of Experimental Research Foundation.

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References