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Establishment of Metaasis Lung Cancer Model by Injection Of EMT-6 Cells in BALB/c Mice

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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 formationSurvival 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. anatomized after EMT-6 tumor cells Injection,: Showed,. group. high concentration treatmentTumor 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-6Cell 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,Low3.A concentrationEMT-6Stable Cell Line,Reliable metastatic lung cancerBALB/cTo 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 through3.Repetitive experiments and detailed clinical observation indicators to evaluateEMT-6Metastatic lung cancerBALB/cStability of mouse model, a standardized model evaluation index system is put forward..

1. Materials and Methods

1.1 Material

1.1.1 Experimental Animals5.~7.Week-old femaleBALB/cMice, body mass16~22gThe production license number of the Laboratory Animal Center of Sichuan University is:Scxk(Chuan)2013-026.SPFKeep 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 LineMurine breast cancerEMT-6Cell lines provided by Key Laboratory of transplantation engineering and immunology, Ministry of Health, West China Hospital, Sichuan University.

1.1.3 Main ReagentRPMI 1640Medium,Fetal bovine serum,

Trypsin and phosphate buffer (PBS)Purchased in USAGibcoCompany, green-Chain Double Anti-purchase in USAHy-cloneCompany.

1.2 Method

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

Cell inRPMI 1640Pei, Yang Ye(Han10%Fetal, bovine, serum,

Foster.Collecting rat sources in exponential growth periodEMT-6Cells, firstPBSCleaning2.Time,0. 25%Trypsin digestion,1 000 r. Min^{-1}·Centrifugal5 minGo to the supernatant and reuseRPMI 1640Medium Cleaning, 000 r. Min^{-1}·Centrifugal5 minSupernatant, cell count,PBSDilution$1x10^{6}$·I/Ml(High concentration),$5x10^{5}$·I/Ml(Medium concentration),$1x10^{5}$·I/Ml(Low Concentration)3.Preparation of different concentrations.EMT-6Cell Suspension.

1.2.2 The EMT-6Metastatic lung cancerBALB/cEstablishment of Mouse ModelWill75Only femaleBALB/cMice inSPFFeeding under conditions1.After peripheral adaptation, the concentration of cell suspension was divided

Group, high concentration group,Medium and low concentration groups, each group25Mice, each injection0. 2

mL.At the default point in time (7 d,14 d,

D,28 d,35 d)Each anatomy5.Only, the lung was taken to observe the pulmonary surface tumor and calculate the tumor rate;And then use the lung tissue10%Poly formaldehyde fixation, Hematoxylin-Yi Hong (He)Staining and histopathological analysis.To evaluate the best injection cell suspension concentration EMT-6Metastatic lung cancerBALB/cMouse model, and then repeat the experiment3.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 VaccinationEMT-6General 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 experiment7 d,7 d,14 d,

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,

D)Random fetch5.Mice were randomly selected and dissected..

1.2.4 Histopathological examinationLung Tissue of mice in each group

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

1.3 Statistical Analysis

UseSPSS 19. 0Analysis, data to average±Standard deviation, single factor analysis of variance andTInspection comparison,$P<0.05$That the difference was statistically significant.

2. Results and Analysis

2.1 Different ConcentrationsEMT-6Metastatic lung cancerBALB/cXiao

Survival time of rat model BALB/cIntravenous Injection of mouse tailEMT-6After cells, the high concentration group10 dBegan to die,18 dAll deaths;Medium concentration group11 dBegan to die,28 dAll deaths;Low concentration group DBegan to die,42 dAll deaths;According3.Survival of mice in the group, drawnKmSurvival curve (Figure1.)The survival time of miceEMT-6Decreased cell concentration but prolonged.Among them, the high concentration group10 d,Medium concentration group11 d,Low concentration group21 dThe 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 dThe survival time provided a suitable lung cancer model for the experimental window period..

2.2 EMT-6Metastatic lung cancerBALB/cEvaluation of Mouse Model

EMT-6Metastatic lung cancerBALB/cThe body mass of mouse model gradually decreased after tumor formation (Figure2.)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 dAll the mice died.

After injecting cancer cells14 dThe anatomy revealed that white began to appear on the lung surface of some mice,A round tumor,21 dIt was found that there were various tumor foci on the lung surface of all mice.100%,DIncreased number of white tumors on the surface of the lungs(Figure3.)No tumor was found in other organs..VaccinationEMT-6

Cell Hind3.Weeks, mice began to appear feeding decline;After inoculation4.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 (Table1.).His

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 in2 DInside will die.

2.3 EMT-6Metastatic lung cancerBALB/cMouse 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)(Table2.).

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&Weinberg2010).And lung metastasis animal model divided into spontaneous and Experimental lung metastasis animal model will EMT-6Cells by tail vein injectionBALB/cMice in vivo and formation of lung metastasis for Experimental lung metastasis (Price1996).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&Connolly1997).

This study by conventional modeling of Methods SelectionBALB/cSmall tail vein respectively Injection0. 2 mLHigh,In,Low3A concentrationEMT-6Cells suspension again according to model into tumor time,Into tumor rate and survival final select0. 2 mLCell concentration1 × 10^5A/MLInto

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 cancerBALB/cMice 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 dAfter part mice lung surface began to appear naked eye visible of white tumor model21 dAfter all mice lung surface were visible of white tumor into tumor rate100%;Model21 d

After mice began to appear death and lung surface tumor increased,Volume larger model42 dIn 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 cancerBALB/cMice Model3Times 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

1. Chen wan qing bed thinking Zou small farmers and. 2010., China lung cancer of the disease and the death of estimation and popular trend research [J]., China lung cancer Journal,13(5):488-491.

2. Liu xin Wu zhiping left Dawn and. 2010.MiceLewisLung Cancer in situ Model Of Construction [J]., China lung cancer Journal,1(1):42-47.
3. Shao less, dry spring single Ben and. 2011.Esophageal Cancer lung metastasis42CasesXLine and CTDiagnosis [J].Shanxi Medicine Magazine,40(1):45-46.
4. Yongjiang Chen Hong rock xia you and. 2002.Experimental Study in experimental animal of select and its related interference factors excuse [J]., China Experimental zoology magazine,12(5):316-319.
5. Ministry of Health of the People's Republic of China. 2008.The third times national cause of death investigation the main [J]., China tumor,17(5):344-345.
6. Chaffer CLWeinbergRA. 2011. A Perspective. cancer cell metastasis[J]. Science331(604):1559-1564.
7. Dragani ta. 2003. 10 years of mouse cancer modifier OCI:Hu-Man-Levy[J]. CancerRSearch,63(12):3011-3018.
8. Gitton y,Dahmane n,Baid s,Et al. 2002. A gene expression map of Human Chromosome 21 orthologues in the mouse[J]. Nature,420(6915):586-590.
9. JEMAL,Bray F,Center mm,Et al. 2011. Global Cancer sta-Statistics[J]. CA-A cancer Journal for clinic,61(2.):69-90.
10. Kuper H,Adami Ho,Boffetta P,Et al. 2002. Tobacco Use,Cancer employment and public health impact[J]. Journal of In-ternational medicalRSearch,251:455-466.
11. Price je. 1996. metaasis from human breast cancer cell lines[J]. Breast CancerRSearch and Treatment,39(1.):93 to 102.
12. ROse DP,Connolly JM. 1997. Diary fat and breast cancer me-tastasis by Human Tumor[J]. Breast CancerRE-search and Treatment,46(2-3):225-237.