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Journal of Nanjing Medical University, 2009, 23(1):1–5
JNMU
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Review

Establishing of the Transplanted Animal Models for Human Lung Cancer

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Received 7 October 2008

Abstract

Lung cancer is the leading cause of cancer mortality worldwide.Even with the applications of excision, radiotherapy, chemotherapy, and gene therapy, the 5 year survival rate is only 15% in the USA. Clinically relevant laboratory animal models of the disease could greatly facilitate understanding of the pathogenesis of lung cancer, its progression, invasion and metastasis. Transplanted lung cancer models are of special interest and are widely used today. Such models are essential tools in accelerating development of new therapies for lung cancer. In this communication we will present a brief overview of the hosts, sites and pathways used to establish transplanted animal lung tumor models.

Key words: lung cancer; transplanted animal models; establish animal models

INTRODUCTION

Lung cancer is the leading cause of cancer mortality worldwide, with approximately 170,000 new cases diagnosed in the United States alone each year[1]. Despite advances in cytotoxic drug development, radiotherapy, and patient management, the cure rate for lung cancer remains dismal. The exceptionally high mortality and relatively modest level of lung cancer research are due in part to the lack of appropriate animal models[2]. Currently, several types of animal models are widely used for experimental lung cancer research. These can be broadly divided into spontaneous or induced tumors, and transplanted tumors. The latter group includes the heterograft and xenograft models.

Transplanted animal lung cancer models are those homologous or heterogenetic malignant cells or tissue directly inoculated into the host animals which then develop neoplasms that can be studied experimentally. As efficient, convenient, and cost-effective models, they are widely utilized in lung neoplasm research. This review will describe the transplanted tumor models. Although we have attempted to be as comprehensive as possible, our list is likely to be incomplete.

HOSTS OF CHOICE

Murine models

The ability to easily manipulate the mouse genome, along with the anatomical and physiological similarity of the mouse to human, and the genetic homology, make these rodents the natural choice as disease model organisms[3]. A variety of mouse models are available for lung cancer research[4]. Hairless nude mouse mutants and severe combined immunodeficient(SCID) mice are both common hosts[5], but they are not only expensive but also difficult to breed. Irradiation, thymectomy, splenectomy, and corticosteroids can initially be used to blunt acquired immunity. To establish a mouse xenograft tumor model, Xia *et al.*[6] inoculated A549 cells, derived from a human pulmonary carcinoma, into Kunming mice. The animals were then irradiated with a high-energy electron beam, The incidence rate of xenograft tumors and mortality rates of these mice were dependent on the dose of irradiation with the high-energy electron beam, with 6 Gy being optimal for

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tumor takes. Because the mouse immune system recovers, the model is only applicable for short-term experiments.

The murine lung cancer models are diverse and well developed, but they are not suitable for probing such areas as tumor vascularization. Moreover, the models are mouse models, and whatever approach the models suggest as being useful will need to be tested in a well-controlled human study[7], which is true of all animal models.

Rabbit models

Rabbit tumor models are currently in wide use, because of their many advantages, including extensive background in understanding their lung physiology, as well as the animals being easy to handle, readily available, and large enough to study lung mechanics, using the animals as their own controls. In addition, their respiratory system is similar to that of humans. The usefulness of the rabbit as a species to study lung biology in order to understand human lung conditions has been highlighted[8]. The paucity of available tools and reagents for use in rabbits is a main hindrance, however.

Sheep models

Sheep pulmonary adenomatosis(SPA), also known as ovine pulmonary carcinoma, is one of the major infectious diseases of sheep[9], and shares many similarities with some forms of human lung adenocarcinomas[10]. Both tumors have the same clinical, macroscopic, histopathologic, and ultrastructural features. The common characteristics suggest that SPA could be a unique experimental model and could offer novel insights into pulmonary carcinogenesis. But sheep have a high rate of primary lung cancer, which limits their use as xenograft models, compared to rats and other mammals.

Canine models

As a companion animal, the dog closely shares the human environment and conceivably is influenced by many of the same environmental carcinogens that induce tumorigenesis in humans. Naturally occurring lung cancer in the dog provides a model of lung cancer in an outbred animal population[11]. The canine bronchial artery resembles that of the human, which makes the dog a good choice for bronchial arteriography and interventional therapy. Cao and co-workers[12] established a model of canine lung cancer, and used the model in a bronchial arteriography study, which further facilitated diagnosis and treatment of lung cancer. Nevertheless, large and domestic animals are more difficult and generally more expensive to manage than mice or rats.

TRANSPLANTED SITES

Subcutaneous implantation

Transplanting human tumor material is the most common method of establishing a cancer model. The procedure is straightforward and the site, depending on the requirements of the experiments, could be axillary fossa, dorsal lateral flank, posterior limb, and so on. In general, the nearer the transplantation site is to the head, the higher the take-rate. There are many variables when conducting xenograft experiments which impact the outcome, viz. site of implantation, growth properties of the xenograft, together with graft size when treatment is initiated, agent formulation, scheduling, route of administration and dose, and the selected endpoint for assessing activity[13]. The axillary fossa is the most common inoculated location, because the capillary bed is extensive and the mass does not hamper the host's mobility. A posterior limb is usually chosen in radiotherapy research, as it is distant to the important organs, such as heart and lung. Arenberg and colleagues[14] selected 6-week-old female CB17-SCID mice, and inoculated 1×10^6 A549 human lung cancer cells into the axillary fossa, to achieve a take-rate of 100%. Tumor volume changes are simple and easily reproducible parameters in these models, as the size of the tumor under the skin can be directly measured. These models have a long history in the pharmaceutical industry because of their utility, ease of use, and economy. Though studies have shown that subcutaneous xenograft models can predict clinical efficacy, they have several disadvantages:① tumor growth in an unusual tissue compartment, the microenvironment of which might influence study results, and some authors have reported that there are differences between the animal models and human lung neoplasms[15], and ② rarely expressed invasive and metastatic phenotypes. Invasion and metastasis cannot usually be evaluated, which hinders the further utilization of these models.

Orthotopic implantation

Xenotransplanted models have been developed with human cancer cells being introduced through various routes into more anatomically correct positions, that is, an orthotopic model. An organ-specific site presumably provides tumor cells with the most appropriate milieu for local growth and metastasis. Orthotopic lung cancer models have been described using endobronchial, intrathoracic, or intravenous injections of tumor cell suspensions[16,17], and by surgical implantation of fresh tumor tissue. Advantages include improved tumor take and enhanced invasive and metastatic properties, with higher frequencies than when grown subcutaneously. Howard *et al*[18] described a systemic metastatic model

using endobronchial implantation of tumor fragments derived from orthotopic lung tumors grown from the H460 cell line. This H460 nude rat model had a 100% primary tumor take-rate in the lung with a rapid and reproducible growth rate to about four grams over a 32-35 day period. Since orthotopic animal lung tumors mimic biological aspects of clinical cancer, they are likely to provide more relevant pharmacokinetic and pharmacodynamic information than subcutaneous tumors. Kondo *et al*[19] applied their procedure to six lung cancer cell lines, by injecting suspensions of 2×10^4 cancer cells into the left lung of SCID mice to mimic the metastatic forms of human lung cancer. They reported that the model was similar to the metastatic form in patients with lung cancer, and the similar expression of proteins in each tumor cell line in vitro and an implanted tumor in vivo is an advantage when evaluating the effects of molecular-targeted drugs, and the relationship between specific genes and tumor potential in preclinical studies. The main downside of this model is that tumor size and tumor weight or volume changes are more difficult to continuously monitor reproducibly, except at necropsy. In addition, it requires more sophisticated techniques and has a higher incidence of trauma and mortality.

Intrathoracic implantation

Du and co-workers[20] established the thoracic cavity implant model and the subcutaneous implant model of the C57BL/6 mice bearing Lewis lung cancer. They compared parameters in the two models. The survival time of the thoracic cavity implant group was 13.5 days and the subcutaneous implant group was 45 days respectively. The variation of the thoracic cavity implant group was smaller than the subcutaneous implant group. The thoracic cavity implant group exhibited cachexia more readily than the other group. As the tumor progressed, the thoracic cavity group gradually began to exhibit micrometastases, while these were seldom seen in the subcutaneous implant group. Thus, the thoracic cavity implant model in C57BL/6 mice developed lung cancer with a high tumor take-rate, reproducible growth, and a mortality endpoint that resulted from local disease progression. This was better than the subcutaneous implant model, and may be used as an ideal model in experimental studies of advanced lung cancer. However, since cancer cells are seeded into the pleural space rather than within the lung tissue or bronchi, its comparison to human lung cancer may be suspect[21].

Vasculature injection

Malignant tumors often contain heterogeneous clones of cells with different potential for invasion and metastasis[22], and the lung is one of the frequently encroached upon organs. Although clinical observations have suggested that carcinomas frequently metastasize and grow via the lymphatic system, malignant tumors of mesenchymal origin more often spread by the hematogenous route. Researchers utilizing this biological characteristic, injected tumor cells suspensions into blood vessels or cardiac ventricles. The tumor cells are released into the circulation, arrested in the capillary beds of the lung, where oxygen and nourishment are abundant, and then extensive vascularization, penetration and extravasation take place, and proliferation within the lung parenchyma completes the metastatic process[23]. Namikawa and Shtivelman[24] surgically transplanted two or more small pieces of human fetal lung at 18~22 weeks gestational age into the fourth mammary fat pads of mice under anesthesia(SCID-hu-FL) firstly, after 7~8weeks intravenously injected $1\sim3 \times 10^6$ cells from thirty human cancer cell lines including adenocarcinoma and small cell lung cancer, into SCID-hu-FL mice. Of those, fifty-three percent generated tumors in human lung grafts.Wu and co-workers[25] described experiments in which 20 New Zealand white rabbits were intravenously implanted with VX2 single cell suspensions acquired from a previous tumor-bearing rabbit. VX2 carcinoma was successfully implanted in 19 rabbits. Because of the biological heterogeneity, most tumor cells that enter the blood stream are rapidly eliminated, and only a few cells can give rise to a metastatic focus[23]. The complexity of the pathogenesis of metastasis may partly explain why the process is often inefficient.

CONFIGURATION OF GRAFTS

Suspensions

Following a series of preparative steps, suspensions of cancer cells can be directly injected into the host animal. Goldberg *et al*[26] created pulmonary tumors in 11 New Zealand white rabbits by using CT-guided injections of a VX2 sarcoma cell suspension into the lower portion of the right lung. The subcultured Lewis lung cancer cells were inoculated subcutaneously into the axillae of C57BL/6 mice to establish an animal model of lung carcinomas. The rate of forming tumors was 80%, and the optimum cell number was 1×10^6, Li *et al*. reported[27]. Generally speaking, fresh and vigorous tumor cells should be the first choice and collagenase is essential for dissolving the tumor interstitial tissue, and might also contribute to tumor infiltration. Because of the complicated preparation protocols required to produce tumor cell suspensions, the take-rates and overall results are not very stable. With tumor-cell suspension injections[17,28], simultaneous tumor cell migration through the blood and lymph circulation, or

tumor seeding through the needle track to the pleura cannot be avoided. Though the molecular characteristic of lung cancer cell lines has been shown to closely match their original human tumor, the capacity of a cell line to maintain clonal heterogeneity has been questioned.

Tissue blocks

Fidler, based on decades of pioneering work underscoring the roles of tissue environments in cancer biology, concluded that the ideal in vivo model for studying human cancer should allow the interaction of tumor cell with their relevant organ environment[23]. To reproduce the complicated interactions that occur in human patients is, however, difficult. Fresh human lung cancer tissue or tissue from metastatic lesions can be implanted orthotopically. The implanted human tissues maintain their normal architecture and function. Therefore implanted human tissues will provide relevant microenvironments for the growth and metastasis of human cancer cells[24]. There are several routes that can be used to establish these models.

Thoracotomic route or lung-exposed inoculation

Xue *et al*[28] established a rabbit lung tumor model via the thoracotomic route. VX2 tumor tissue was cut into small strips(approximately 1.0 mm in diameter) and stored in sodium chloride solution. After being anesthetized, the rabbit was secured and a right intercostal incision was made between the fourth and fifth ribs. The ribs were cut down, the inferior lobe of the right lung exposed, an incision made in the lung lobe, and the tumor strip was implanted into the parenchyma. The take-rate is 100%. Another model involved the direct implantation through a thoracotomy into the lung parenchyma of Lewis lung carcinoma cells suspended in Matrigel. This model resulted in isolated lung tumor nodules[29]. Advantages of these models include rapid and reliable development of intrathoracic tumors with predictable metastasis. Disadvantages include complications associated with surgery and anesthesia, an increase in technical complexity, and difficulties monitoring both the development of the cancer and the effect of interventions.

Percutaneous puncture-inoculation

In order to diminish the complications of a thoracotomy, investigators attempted to produce a pulmo-nary carcinoma model by adopting a percutaneous puncture-inoculation method. Liu *et al*[30] compared the differences of VX2 lung carcinoma models in rabbits established via the thoracotomic route and the CT-guided percutaneous puncture-inoculation method. Although the take-rates of both kinds of transplanted carcinomas were 100%, the operation time of the latter method was much shorter, while the survival period was longer. Ma and colleagues[31] reproduced a lung tumor model by injecting VX2 tumor tissue blocks into rabbit lung using a percutaneous, X-ray-guided puncture-inoculation method, and compared the results with those of a model in which aVX2 tumor cell suspension was injected. The ratio of successful transplantation was 100% in the tissue block group, while it was 48% in the cell suspension group. The difference was statistically significance. All these studies prove that the percutaneous puncture-inoculation method is simpler, highly successful and less damaging when establishing a tumor model.

Modified intrapulmonary tumor implantation (IPTI) technique

Recently, Tu and colleagues[32] reported on a new technique to establish a rabbit VX2 lung cancer model that yields a solitary and localized intrapulmonary tumor, which overcame the shortcomings of lung-exposed inoculation and the percutaneous puncture-inoculation methods. They exposed the right lung of the rabbit by making an incision between the fifth and sixth ribs without inserting a tracheal tube, followed by the implantation of a narrow strip of VX2 tumor tissue into the inferior lobe of the right lung. In contrast to IPTI techniques that expose and make incisions in lung lobes[28] or require CT or X-ray to guide tumor delivery[17,30,31], the IPTI-based technique is simple and effective.

SUMMARY

In animal models tumors develop uniformly following cancer cell inoculation or tissue implantation with predictable growth and metastatic patterns. With such models areas, such as tumor growth, invasion, and metastasis become amenable to investigation. Such models are also particularly well suited to testing new therapeutic approaches and screening strategies[33]. By using advanced preclinical models, we can not only preselect the most promising drug combinations and scheduling parameters, but also obtain information on potential toxic side effects of drugs[34]. Although these models have all been informative and have further propelled our understanding of human lung cancer, they still do not fully recapitulate the complexities of human lung cancer. For example, transplanted animal tumor models are not well suited for studying early events in carcinogenesis. With the development of new theories and technologies in genomics, proteomics, and animal imaging, more sophisticated lung cancer models will be developed and these will prove useful in our battle against this unrelenting foe.

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