The Current Status of Prostate Cancer Animal Models

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Abstract
Prostate Cancer (PCA) is the most common malignant tumor in urinary system of men in Europe and the United States. It ranks second in male cancer mortality, only behind to lung cancer. Animal model is very important for researching carcinogenesis and testing treatments of prostate cancer. The ideal animal model should be able to effectively simulate the occurrence, development, metastasis and pathophysiological changes of human prostate cancer. At present, the commonly used animal models include mouse model, rat model and dog model and so on. In this review, we analyzed how widely used animal models of prostate cancer can simulate the occurrence, development and pathological changes of human prostate cancer, and compared the characteristics of these models, which laid the foundation for the study of human prostate cancer.

Introduction

The latest statistics show that the incidence of prostate cancer ranks second and the mortality ranks fifth among male malignant tumors, especially elderly male patients. As the early symptoms of prostate cancer are mild and easy to be ignored, most of the diseases are advanced or have a higher possibility of metastasis at the time of diagnosis [1,2]. The development of prostate cancer in human body is a multi-step process (Figure 1) that has multiple stages and finally metastasizes to bone, lung, liver, etc. Therefore, the establishment of an animal model of prostate cancer is crucial for studying prostate carcinogenesis and testing new anticancer drugs [3]. Widely used animal models of prostate cancer include mouse models, rat models and dog models than were established by transgene, gene knockout and xenotransplantation. They have been proved to be very valuable in expanding our knowledge of the disease [4-6]. Although animal models contribute greatly to study the prostate carcinogenesis, existing animal models do not simulate all the characteristics of human prostate cancer [7]. This paper expounds the functions, characteristics, advantages and disadvantages of these models; introduces the application of this model in prostate cancer, and points out the key problems still need to be solved.

Development of prostate cancer model

The prostate, like all other glandular organs, consists of epithelium and stromal chambers with a variety of cell types [8]. Important information about prostate development and homeostasis was obtained by studying the development of animal, especially mice, although the mice prostates are different from human. The rodent prostate contains four different segments: dorsal lobe, ventral lobe, lateral lobe and anterior lobe, which surrounds the urethra and is named according to its anatomical position. The dorsolateral lobe has the highest correlation with the peripheral zone of the human prostate. Unlike the rodent prostate, the human prostate does not have obvious lobular tissue, however, it has a similar regional structure and separation as rodent prostate, and prostate tumors usually originate in the peripheral area [9-12].

Mouse model

The genome similarity between mouse and human is more than 99%, the cost of establishing mouse model is low and the genetic modification technology is easier to achieve on mouse, so the mouse model has been well applied in the study of prostate cancer [13]. In the following, we introduced prostate cancer mice models established by different methods.

Orthotopic transplantation

There are two ways to establish orthotopic transplantation model. In the first way, BALB/C-nu mice or C57BL/6 mice are usually selected to expose the prostate through surgery, and the prostate cancer cell suspension is injected into the prostate capsule to observe the tumorigenesis. In the second way, the prostate cancer cells are inoculated into the animal subcutaneously to form a tumor, and then the tumor is removed, cut into equal volume pieces, and inoculated into the dorsal lobe of the prostate of homologous animals. At present, the commonly used cancer cell lines are PC3, LNCaP, DU145, CWR22Rv1. Two months after orthotopic transplantation of PC-3M (derived from PC3) cells into nude mice, Chu et al., [14] isolated primary tumor cells (PC-3M-PRO) and lymph node metastatic tumor cells (PC-3M-LN). In vitro experiments showed that PC-3M-LN had stronger infiltration and adhesion ability. Gene chip and RT-PCR analysis showed that the content and transcription level of metastasis-related genes of PC-3M-LN increased, suggesting that lymph node metastatic cells were cell subsets with genetic changes that different from primary tumor, and their survival and proliferation ability were stronger than primary tumor cells.

Although the orthotopic transplantation model has many advantages, it also has obvious disadvantages, such as complex surgical process, time-consuming, and postoperative complications [15]. Krbel et al., used three-dimensional ultrasound to conduct percutaneous puncture and non-invasive inoculation in nude mice, and successfully obtained the orthotopic prostate cancer transplantation model [16]. The developing of new surgical method avoids the shortcomings of open surgery and makes the orthotopic mouse model more widely used, for example, the way of injecting PC-3 CaP cells into the ventral lobe of the mice prostate by Ni and monitoring tumorigenesis by 3D MRI is very popular [17]. The results show that MRI combined with 3D reconstruction is a feasible method to evaluate the tumor growth of PC-3 in situ CaP mouse model. This monitoring method is expected to observe the efficacy of anticancer therapy after transplantation in vivo. In addition, the orthotopic transplantation model is also very suitable for examining the effects of drug therapy on tumor growth and lymph node metastasis [18]. However, the orthotopic transplantation model failed to achieve bone metastasis, which may be due to the fact that the mouse died of urinary tract obstruction before any bone metastasis occurred, or because the mouse microenvironment failed to restate the human microenvironment, which failed to develop into bone metastasis [19]. Nevertheless, the model does restate the early events of pre-embolization metastatic cascades and the entry of tumor cells into circulation, so it is a valuable tool for the study of early process of metastasis and transformation and the preclinical evaluation of new treatment strategies [20].

Gene modification

There are two kinds of gene modification models, one is gene knockout model, at present, many tumor suppressor genes knockout models have been established, such as Pten/Mmacl and Mxi1, Nkx3.1, or c-Myc knockout model [21-24]. They were knocking out conventionally or conditionally. Conditional gene knockout is more popular because conventional gene knockout may cause embryonic death or other gene expression disorders. Conditional gene knockout is mainly achieved by chromosome locus-specific recombinase systems, such as Cre-LoxP system, FLP-Frt system and Dre-Rox system, among which, Cre-LoxP system is the most commonly used, such as Pten<sup>lox/lox</sup>/PB-Cre [25] mouse model. In order to significantly shorten the latent period of PIN formation and leads to prostate cancer progression to metastatic stage, researchers established compound mutant mouse model, such as Pten<sup>lox/lox</sup>;Nkx3.1<sup>−/−</sup> [26] mice which show the incidence of high-grade PIN; Pten<sup>−/−</sup>; p27<sup>−/−</sup> [27] mice that developed completely explicit prostate cancer within three months; Pten<sup>−/−</sup>; Ink4a/Arf<sup>−/−</sup> [28] mice that have shortened latency of PIN.

The other is the transgenic mice model. Gingrich introduced the SV40 fragment carrying Probasin (PB) protein promoter into the fertilized egg cells of mice, the Transgenic Adenocarcinoma Mouse Prostate (TRAMP) model was successfully cultivated [29]. These models (TRAMP and LADY (LPB-Tag) and their derivatives) showed excessive proliferation of prostatic epithelial and stromal cells which led to a sharp increase in the size of the prostate [29,30]. The LPB-Tag/PB-Hep model also developed into metastatic prostate cancer, including bone metastasis [31]. Of note, metastatic lesions in these animals showed significant neuroendocrine differentiation, similar to human AR and PSA negative small cell carcinoma [31-33].

Inducement

Induced tumor models were established by treating animals with hormones, chemical drugs and physical factors. The commonly used chemical carcinogens were DMAB (3-dimethyl-4-
aminobiphenyl) and MNU (azomethine nitrosourea). Hormone inducers include androgen combined with estradiol induction and testosterone combined with β-estradiol [34].

Subcutaneous inoculation

C57BL/6/J mice and BALB/C nude mice were usually used to observe the tumorigenesis by subcutaneous injection of prostate cancer cell suspension or tumor mass. The characteristics of subcutaneous transplanted tumors are complete capsule formation, clear boundary with surrounding fascia and muscle, less distant metastasis [35].

In addition, there are also mice models which were established by renal subcapsular implantation, intravascular inoculation and bone metastasis.

Rat model

Compared with mouse, the advantage of rat is that the volume of prostate is significant large. The widely used rat models are spontaneous model, chemically or hormonally-induced model, tumor cell line implantation and genetic engineering model [36].

Spontaneous model

In 1963, Dr. W.F. Dunning detected a non-metastatic adenocarcinoma in a 22-month-old Copenhagen rat and identified it as a prostate tumor. This was the first reported spontaneous prostate tumor model and later on, spontaneous prostate tumor was found on Wistar, AxC and ACI/Seg rats. Because the development of the tumor requires a long incubation period (about 2-3 years) and the incidence of spontaneous tumor is rare, these tumor animal models have no obvious advantage [36-40]. However, these spontaneous models can be used to study the progression of prostate cancer. For example, the results of Campolina-Silva’s group confirmed that aged Wistar rat is a suitable clinical model for the study of prostate lesions. Their data showed that the development of prostate cancer in Wistar rats is a multi-step process involving the initiation and progression of precancerous lesions. They also confirmed that Wistar rats have an advantage over other experimental animals that they have a small number of spontaneous adenocarcinomas in a short period of time, about 18 months, which is similar to the malignant tumors in elderly men [41].

Chemical or hormone-inducement

Male hormones, such as testosterone and genotoxic compounds can induce prostate cancer in rats, the commonly used chemical reagents are BOP, MNU, DMAB and PhIP [42]. At present, the most prevalent prostate cancer model is rat induced by MNU or DMAB combined with testosterone. The tumor origin of these two models is in the dorsal and middle lobe, which is same as human prostate cancer. The malignant of these tumors is high and they are easy to metastasis [43].

Tumor cell line implantation

Prostate cancer cell lines can be implanted into rats to promote tumor formation. The most widely used cancer cell lines are PA-III and PLS10 which from Lobund-Wistar rats and male F344 rats induced by DMAB combined with testosterone, respectively. These two models have high bone metastases and are widely used to study the mechanism of bone metastasis and to evaluate the efficacy of drug therapy [44-46]. Suzuki S injected PLS10 and its derived cell lines into F344 rats to establish a rat model of orthotopic xenotransplantation for drug therapy [47]. Previous researches showed that cell line implantation tumor model has similar tumor microenvironment as human and is suitable for evaluating the efficacy of anticancer therapies, especially immunotherapy.

Genetic engineering model

In 2001, Shirai and his colleagues established a transgenic rat prostate cancer model called “Transgenic Rat With Prostate Cancer (TRAP)” [48,49]. This model was established by using the progenitor gene promoter and Smur40T (SV40-T) antigen in the genetic background of SimianVirus Sprague-Dawley rats. This model is characterized by complete androgen dependence and can be used to study the carcinogenic mechanisms such as androgen dependence, degeneration and apoptosis. Using this TRAP rat model, Suzuki S found that Pioglitazone (PGZ) can inhibit the occurrence of prostate cancer and the underlying mechanism is to regulate cell proliferation through NFκB pathway. This result confirmed that PGZ is a promising chemoprophylaxis drug for prostate cancer [49]. Another transgenic model was established in Lewis rats. Sprague-Dawley SV40-T rats were hybridized with Lewis strain to test the immunotherapy of prostate cancer and this model was 100% androgen sensitive [50].

Although the rat model of prostate cancer has many advantages, they also have disadvantages: the time period of establishing this kind of model is relatively long and the tumor often invades the seminal vesicle gland which is not happen in human. In addition, there are fewer analytical reagents for rats than for mice.

Canine model

Canine prostate cancer is a malignant epithelial tumor, often characterized by glandular or acinar structure, with human-like Benign Prostatic Hyperplasia (BPH) foci, cystic gonadal dilatation, and obvious suppurrative and lymphocytic inflammation, so it is usually classified as adenocarcinoma [51]. Due to the high metastasis of canine prostate cancer, most dogs develop advanced diseases with obvious local invasion and extensive visceral metastasis [52]. At present, the two commonly used models are spontaneous and tumor cell line transplantation in dogs.

Spontaneous model

There are many similarities in shape and function between canine prostate and human prostate, and dogs are the only non-human large mammals with spontaneous prostate cancer. Dogs with prostate cancer are usually accompanied by advanced disease and do not respond to androgen deprivation treatment [53]. Similar to humans, dogs with spontaneous prostate cancer often develop osteoblast bone metastasis in the pelvis and lumbar vertebrae, accompanied by associated pain and neurological defects. Other clinical symptoms include weight loss, drowsiness, urination and abnormal defecation. It is clear that canine prostate cancer and male advanced prostate cancer need better early detection and more effective treatment, and this model may be a useful model system for studying the mechanism of tumor progression in humans and animals, used to detect preclinical new chemotherapeutic drugs and study initially induced multiple drug resistance. For example, Winkler [54] successfully used this model to detect the therapeutic effect of reduced expression of High-Mobility Group Protein-A2 Gene (HMGA2) on tumor transformation. But as the incidence
of spontaneous prostate cancer in dogs is much lower and the exact origin of tumor is not clear, the practical application of this model is limited.

Tumor cell line orthotopic transplantation

The main cancer cell lines that used to establish orthotopic transplantation model are DPC-1, Ace-1, Leo and Probasco cells [55,56]. There are many kinds of these models, such as after suppressing the immune function of dogs, Keller et al., transplanted Ace-1 cells into the dog prostate capsule or prostate parenchyma and established prostate parenchyma tumors in 80% dogs. About 50% of these dogs have local lymph node and lung metastasis [57]. In 2018, Tweedle transplanted Ace-1 huGRPr (human Gastrin Releasing Peptide Receptor, huGRPr) cells into the parenchyma of dog prostate in situ after suppressing the immune function of dogs [58]. This model expresses an effective human growth receptor GRPr, which maintains activity against known ligands and metastases to local lymph nodes. Their study represents the first time that human cancer growth factor receptor has been expressed and grown in an experimental dog model of prostate cancer.

There are also subcutaneous transplantation dog prostate cancer model. The characteristics of subcutaneous tumors in dogs were similar to those formed in the prostate, they both can invade to skeletal muscle and adipose tissue. Mucous infiltrating prostate cancer could be seen around the tumor.

In a word, the dog model is one of the good animal models for the study of human prostate cancer. It is a favorable tool for studying the occurrence, development, metastasis and pathophysiological changes of human prostate cancer.

Conclusions

To sum up, this paper mainly reviews several commonly used animal models of prostate cancer. Prostate cancer animal models, especially models established by surgical or genetic ways, is one of the important platforms for prostate cancer research. Proper animal model of prostate cancer will help researchers to reveal the pathogenesis, disease progression and uncover the mystery of invasion and metastasis of prostate cancer. But each specific animal model may only represents a specific pathological event and has its own advantages and disadvantages. No single model can fully contain all the molecule changes and pathological events in the progression of human disease. So, researchers should choose the most suitable model according to the needs of their research. We summarized the advantages, disadvantages, applications and progressive stages of different prostate cancer animal model in (Table 1).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Model</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Application</th>
<th>Progressive stage</th>
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<tbody>
<tr>
<td>Mouse</td>
<td>Orthotopic transplantation</td>
<td>Grow fast. High rate of lymphatic and pulmonary metastasis. Can simulate microenvironment. The model of cancer metastasis is easy to be formed.</td>
<td>Many postoperative complications. Larger tumor mass is not easy to be inoculated. Complex operation. Difficult to observe.</td>
<td>Suitable for examination of drug treatment. Study on tumor growth and lymph node metastasis. To observe the development of anticancer therapy after CaP transplantation in vivo.</td>
<td>Failed to achieve bone metastasis.</td>
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<tr>
<td>Mouse</td>
<td>Subcutaneous inoculation</td>
<td>Easy to operate and observe the growth of the tumor. Can be inoculated with large tissue blocks. Easy to measure.</td>
<td>Low success rate. Less transfer. A small range of applications. Can not simulate the microenvironment of tumor growth. Fewer distant metastases occur.</td>
<td>Study on the inhibitory effect of antineoplastic drugs on human tumor.</td>
<td>Easy to form tumor. Bone metastasis is difficult to occur.</td>
</tr>
<tr>
<td>Rat</td>
<td>Model Spontaneous Model</td>
<td>High security. Low cost.</td>
<td>Long incubation period. Relatively low incidence.</td>
<td>It is rarely used in the study of prostate cancer, and it is not an ideal model to study bone metastasis of prostate cancer.</td>
<td>Prostate cancer occurs in the dorsolateral and anterior lobe of the prostate.</td>
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### Table: Characteristics of Human Prostate Cancer Models

<table>
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<tr>
<th>Model Type</th>
<th>Characteristics</th>
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<tr>
<td>Chemically or Hormonally-induced</td>
<td>High morbidity. Metastatic. It can simulate the clinical characteristics of human prostate cancer. Long preparation period. Low success rate. Multiple influencing factors. Study on carcinogenic and anticaner substances and chemical prophylaxis. The origin site is the dorsal lobe and middle lobe, which is the same as that of human prostate cancer.</td>
</tr>
<tr>
<td>Tumor Cell Line Implantation</td>
<td>Slow tumor growth. Non-metastatic. Androgen response. No need to come into contact with compounds. Reduce health risks, environmental impacts and costs for investigators. Complex and time-consuming operation. Postoperative complications. It is not suitable to inoculate larger tumor mass. Difficult to observe. Used to detect new drugs for the treatment of hormone-dependent prostate cancer. Studies on carcinogenic and anticiancer substances and chemoprevention. Tumorigenicity. The histological appearance and biochemical characteristics of the dorsal prostate of rats were maintained.</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>It is easy to occur in old age. Easy to transfer. Hormone independent transformation is easy to occur in the late stage. It has the advantage of bone metastasis from the cancer focus to the pelvis and spine. Low tumorigenesis rate. Low bone metastasis rate. High cost. Multiple influencing factors. The tumor model takes a long time. It provides a potential natural model for the study of the mechanism of prostate cancer. Occurrence of prostatic intraepithelial neoplastic hyperplasia. It can form obvious osteogenic bone metastasis.</td>
</tr>
<tr>
<td>Canine Model</td>
<td>Slow tumor growth. High rate of lymphatic. Androgen response. No need to come into contact with compounds. Long cycle. Low success rate. Many influencing factors. Difficult to control experiment. To study the occurrence, development, metastasis and pathophysiological changes of prostate cancer. Local lymph node and lung metastasis.</td>
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### References


