Status Quo and Prospect of Colorectal Animal Models: Application of Transgenic Technology

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Abstract
Colorectal cancer (CRC) is one of the most common malignant diseases and brings about serious damage to the human body. Presently, the incidence of CRC ranks third among the malignant tumors, and its 5-year survival rate is only about 50%. In recent years, more and more studies have focused on the application of transgenic technology in CRC research in vivo, which involves a great number of genes significantly related to the occurrence and development of CRC. The core of transgenic technology refers to the host chromosome gene accepting exogenous genes, which integrate into the host chromosome expressing it stably, followed by transmission to the next generations. Here, we will present a brief review of the related technologies used in transgenic animal models and provide an overview of the application of transgenic animal models in CRC research.

Introduction

In China, the incidence of colorectal cancer (CRC) is increasing year by year, with an increase by 104% in men and by 99% in women in the past 12 years in Shanghai [1]. The formation of CRC involves the activation of multiple proto-oncogenes and the inactivation of tumor suppressor genes, whose occurrence and development is a complex process.
comprising multiple stages and multiple genes [2]. With the development of genetic engineering technology, CRC transgenic animal models play an important role in the study of CRC in vivo.

The common CRC animal models include spontaneous animal models, induced animal models and the homograft or xenograft tumor animal models. The spontaneous animal models are largely dependent on feed and environment, and the incubation periods of most of these tumors are so long that the established models cannot reflect the in-house characteristics of human tumors. Secondly, the induced animal models are built by way of inducing chemical compounds, which has been gradually abandoned because of the very low incidence of tumors. Thirdly, the homograft or xenograft tumor animal models refer to the transplantation of a portable tumor to the congenere or heterogenic nude mice in vivo, including the following methods: cell cultivation method, organization cultivation method, and the renal subcapsular grafting method. Due to the congenital immunodeficiency of nude mice, experimental animal models have a high success rate, keeping the structure and function of original tumors. However, nude mice still have humoral immunity function, and, thus, they do not represent optimal experimental CRC animal models. At present, transgenic technology is invading many research fields, with broad application prospects in species transformation and animal trait changes. It is gratifying to note that transgenic technology is responsible for multiple breakthroughs in CRC research. The established CRC animal model using transgenic technology has advantages over breeding, and the etiology of this model is closer to the naturally occurring process of CRC. In general, transgenic technology can provide ideal animal models for CRC research on pathology, diagnosis and treatment [3]. Therefore, we next present a brief review of the related technologies used in transgenic animal models and provide an overview of the application of transgenic animal models in CRC research.

Related Technologies of Transgenic Animal Models

Currently, there are various types of transgenic technologies, including the microinjection method, sperm carrier method, somatic cell cloning method, retrovirus method, embryonic stem cell-mediated method and oocyte carrier method [4, 5]. The microinjection technology microinjects exogenous DNA into the former nucleus of a fertilized egg cell by a microscopic instrument [4]. The sperm carrier method incubates the exogenous gene with the live sperm, and then, the live sperm will take up the exogenous genes and be fertilized in vitro; finally, the transgenic mice model will be acquired [6, 7]. The basic principle of the somatic cell cloning method is the same as that of the sperm carrier method. The retrovirus method is implemented through a variety of retrovirus vectors, which help genes transfer to germ cells [7]. The embryonic stem cell-mediated method is described in detail as follows: the exogenous gene is firstly imported into embryonic stem cells by transfection, then the screened-out embryonic stem cells which carry the exogenous genes stably are operated in series to obtain chimeric mice, and, finally, the gene knockout homozygous mice will be produced through mutual mating. With the oocyte carrier method, the exogenous genes are imported into former oocytes of the fertilized egg. After the exogenous genes have entered the oocytes, sperm chromatin gets into the egg cells. As long as the exogenous genes integrate, each cell will carry the exogenous genes from the division to the maturation process of the fertilized egg, which avoids generating the chimeric animals. In addition, the gene knockout mice model was established based on transgenic technology, whose core technology is to artificially delete tumor suppressor genes using the homologous recombination method and the Cre/loxP-induced conditional gene knockout method [8].
Application of the Transgenic Animal Model in CRC Research

APC\(^{\text{Min/}+}\) Transgenic Animals

The APC\(^{\text{Min/}+}\) (C57BL/6J-APC\(^{\text{Min/}+}\)) mouse model is the classical animal model to study the development of CRC [9, 10]. APC is a tumor suppressor gene, which is directly involved in the Wnt signaling pathways, controlling the conservative evolution mechanism of cells in the division process and organization of the mature embryo. Eighty-five percent of colon cancers have APC gene deletion or inactivation. APC mutation or deletion will lead to a β-catenin deposit in the nuclear, activated Wnt signaling pathways, stimulate downstream proto-oncogene cyclin D1 and, finally, cause cancer [11]. Li and colleagues [12] found that the adenomas of APC\(^{\text{Min/}+}\) mice began to occur at 9 weeks in the intestinal tract, and, in the following 9–24 weeks, the glands continued to increase until the mice died. With the emergence of the APC\(^{\text{Min/}+}\) mouse model, Hong et al. [13] prepared various kinds of animal models based on the mutation of the APC gene, including APC\(^{\Delta 716}\), APC\(^{\Delta 1309}\), APC\(^{1638N}\) and others. The researchers also found that the locations of the intestinal tumors of the APC mice were different. The tumor of the APC\(^{\text{Min/}+}\) mice can be located in the colon and small intestine. The tumor of APC\(^{\Delta 716}\) was mainly concentrated in the small intestine. The polyps of APC\(^{\Delta 716}\) Cdx2\(^{+/–}\) based on APC\(^{\text{Min/}+}\) were mainly located in the colon, but the reason remains unclear.

APC\(^{\text{Min/}+}\) animal models have been widely used in intestinal cancer research; especially, their application for the study of drug effects and mechanisms has increased. With the development of various new research, Sohn et al. [14] have established the Min (APC\(^{+/–}\)-Msh2\(^{–/–}\)) mouse model based on the APC\(^{\text{Min/}+}\) mouse model and showed that Msh2 gene mutation or deletion can promote mutation of the APC and the p53 gene, which confirms that the Msh2 gene plays an important role in the development of intestinal cancer and provides the foundation for further research on the relationship between related genes and CRC. Similarly, Yamada et al. [15] established the Min (APC\(^{\text{Min/}+}\)-Mdr1a/b\(^{–/–}\)) mouse model based on the APC\(^{\text{Min/}+}\) mouse model and preliminarily elaborated the relationship between Mdr1 gene mutations and CRC. The mutation of the Mdr1 gene inhibited the occurrence of intestinal polyposis. The Mdr1 gene can make a β-catenin deposit in the nuclear, activated Wnt signaling pathways, leading to a tumor. Thus, Mdr1 gene deletion of the Min (APC\(^{\text{Min/}+}\)-Mdr1a/b\(^{–/–}\)) mouse model can reduce the incidence of tumor in mice significantly. Niho et al. [16] have used the APC\(^{1309}\) mouse model to confirm that APC gene mutations can inhibit the formation of small intestinal adenoma tumors when in a state of hyperlipidemia. Moreover, Akeus et al. [17] have successfully set up an APC\(^{\text{Min/}+}\) knockout gene mouse model and identified the relationship between cancer-associated lymphocytes and the accumulation of gastrointestinal adenoma. They draw a preliminary conclusion that mutations and the deletion of APC\(^{\text{Min/}+}\) in Wnt signaling pathways may affect the development of lymphatic system tumors, the number of T cells and the accumulation of adenoma.

From the reviews mentioned above, we know that the APC\(^{\text{Min/}+}\) mouse model has been widely used in intestinal cancer research. At the same time, some studies suggest that APC\(^{\text{Min/}+}\) mouse models also include the obvious pathological changes in the digestive system, reproductive system, endocrine system, immune system and hematopoietic system. Therefore, the APC\(^{\text{Min/}+}\) mouse models can be improved to adapt to each system disease research with all kinds of drug intervention or transgenic technology, and the APC\(^{\text{Min/}+}\) mouse models can serve as a good model of ‘integration’ [18].

Tiam1 Transgenic Animals

The Tiam1 (T-cell lymphoma invasion and metastasis) gene is an induced factor in the invasion and metastasis of T-cell lymphoma [19]. Tiam1, with its high and positive expression in colon cancer cells, can act as a promoter in the invasion and metastasis of CRC. It was found
that the tumor cells expressing high Tiam1 have more phenotypes, a higher expression of β-catenin and vimentin, and a reduced E-cadherin expression which can increase cell adhesion [20]. In order to explore the mechanisms of the Tiam1 gene leading to cancer, Yu et al. [20] have established a Tiam1/C1199-CopGFP transgenic mouse model using microinjection to research oncogenes and the mechanisms of invasion and metastasis of CRC. Compared to wild-type mice, the tumor volume of Tiam1 transgenic mice is bigger, and the invasion is stronger. Tiam1 likely accelerated the invasion and metastasis of CRC through the activation of the Wnt/β-catenin signaling pathway. Tiam1 is associated with the differentiation and metastasis of CRC [21]. The mechanism of Tiam1 promoting CRC metastasis may also relate to epithelial mesenchymal transition. All the above results provide a reliable basis for further investigation into the mechanisms of Tiam1, which accelerated the invasion and metastasis of CRC.

**Cdc20 Transgenic Animals**

Cdc20, a cell cycle regulation factor, is indispensable to cell mitosis. Its main feature is to make the APC/C substrate degrade in an orderly manner during the process of mitosis to coordinate the process of mitosis. APC/C, also known as cell cycle enzyme, is a kind of E3 ubiquitin-ligating enzyme and can make cell cycle proteins biodegradable [22]. Nilsson et al. [23] have produced Cdc20 loxp/+ APCMin/+ gene mutation mouse models. Firstly, they prepared Cdc20 loxp/+ gene mutant mice with the help of gene-targeting technology, then let the Cdc20 loxp/+ gene mutant mice and APCMin/+ transgenic mice hybridize. The pathological type of intestinal tumor in this kind of mouse model is adenoma, whose malignant degree is higher than in the other models. A specific knockout homologous arm of the Cdc20 gene in the colon can accelerate the development of CRC. Pathological biopsy showed that the gland structure disorder has reached mucosal muscularis, with obvious cellular variation, nuclear atypia and hyperchromatism, and pathological fission visible in Cdc20 loxp/+ APCMin/+ tumor tissue. Cdc20 transgenic animal models have laid a solid foundation for exploring the relationship between Cdc20 and the development of CRC and have well prepared for research on its molecular mechanism. Current research has shown that mutation of the Cdc20 gene can affect the development of CRC through the gene instability.

**MLH1 Transgenic Animals**

MLH1, a mismatch repair gene [24], can ensure the accuracy of DNA replication. The carcinogenic effects of MLH1 are mainly caused by the methylation of its promoter, followed by the inactivation of the DNA mismatch repair gene and the point mutation of DNA which cannot be repaired in time. This mechanism is thought to be an important pathogenesis of CRC [25]. Recent studies have shown that hereditary nonpolyposis CRC (HNPCC), an autosomal dominant genetic disease syndrome, can cause gene inactivation and lead to tumorigenesis mainly by making CpG islands located in the MLH1 gene promoter region methylate [26]. MLH1 knockout mice have been successfully applied in CRC research [27]. Zeng et al. [28] have successfully built an MLH1 knockout mouse model and found that the incidence of colon cancer in mice increased significantly, but the survival of mice was significantly reduced. They have also built a Mlh1 +/- heterozygous mouse model based on the MLH1 knockout mice model and further confirmed that the expression of decline or inactivation of the tumor suppressor gene DKK1 (an important molecule antagonist of the Wnt signaling pathway) is an important index of CRC.

**hMSH2 Transgenic Animals**

The basic function of hMSH2, another mismatch repair gene, is to move insertion/deletion rings generated by the primary template in the case of repetitive DNA sequence slipping and
to check the single-base mismatch that escaped the correction of the read code in order to prevent the accumulation of spontaneous mutation and to guarantee the integrity and stability of the DNA replication [29]. The mechanism of hMSH2-induced CRC is similar to that of MLH1. Methylation of the hMSH2 gene promoter can cause the MMR gene to express failure, which leads to CRC [30–32]. The MMR, an important repair system after replication, can maintain an accurate copy and control gene mutation [33]. In order to explore the effect of the hMSH2 gene in the development of CRC, Lowsky et al. [34] have successfully built a hMSH2 knockout mouse model. They found that the incidence of intestine adenoma in the hMSH2 knockout mice was obviously higher than in normal mice. hMSH2 genes may play a driving role in every link in the development of CRC.

About 90% of HNPCC have an abnormal hMSH2 and MLH1 gene expression [35]. DNA mismatch repair gene mutation or deletion played an important role in the pathogenesis of CRC. All the models presented above provided new insight for CRC research, guiding the clinical diagnosis and targeted therapy of CRC.

Claudin-7 Transgenic Animals

The Claudin-7 family protein is one of the main structural elements of intercellular tight junction proteins, whose expression has tissue specificity. Claudin-7 played an important role in maintaining intestinal epithelial cell polarity and in closely connecting the barrier function [36]. The mutation or abnormal expression of Claudin-7, as a potential tumor suppressor gene, is closely related to the occurrence of a variety of tumors [37]. In normal state, Claudin-7 is widely expressed in the colon and small intestine. In order to confirm the tumor suppressor function of this gene, Ding et al. [38] have established a Claudin-7 knockout mouse model by knockout Claudin-7 gene. They found mucosal epithelial cell shedding, vacuolation, and inflammatory cell infiltration in the small intestines of knockout Claudin-7 gene mice. At the same time, C-fos, C-jun, and COX-2 showed significantly higher expression, and the expression of NF-κB, p65, P-p65, and P-IKBα and the phosphorylation of histone H3 in the intestinal tract of the Claudin-7 knockout mice were also significantly increased [39]. Claudin-7 knockout mice stopped growing at day 4 and showed the obvious phenomenon of dehydration. Then they died at day 7; however, the controls were normal. After dissecting the intestines of these mice, inflammation and proliferation in the intestinal tract was obviously found [40]. Thus, we can draw the conclusion that Claudin-7 is a potential tumor suppressor gene in the development of CRC. It should be applied to further research inflammation in CRC and as a new target for CRC treatment.

HLA-B27 Transgenic Animals

HLA-B27, one product of the human histocompatibility complex, is a specific diagnosing factor for ankylosing spondylitis. Recent research revealed that HLA-B27 is closely related to CRC [40]. In 1995, Hammer et al. [41] have built an F344-B27 transgenic rat model which was an inbred line of the HLA-B27 transgenic rat. This transgenic rat model showed chronic inflammation in the intestinal tract. The inflammatory sites have the tendency of developing hyperplasia. The incidence of colorectal polyps is high, with the tendency of evolution from adenoma to carcinoma. The high expression of HLA-B27 will increase the incidence of CRC, and early detection and elimination of HLA-B27 expression can reduce the risk of CRC.

Microsatellite Instability Transgenic Animals

Microsatellite instability (MSI) plays an important role in the development of CRC. It can be an important index to judge the prognosis of CRC [42]. A related study showed that the main tumorigenic mechanism is that MSI can cause microsatellite sequence mutations in the gene exon region and mutate the protein reading frame, producing abnormal peptide frag-
ments and stimulating the body to produce an antitumor immune response [43]. The main pathological features of MSI are as follows: the tumors are mainly located in the proximal colon, and mucous adenocarcinomas are often seen, with poor differentiation. However, currently, MSI animal models are still not established worldwide, which restricts research on its biological characteristics as well as on related treatments [44].

**DCC Transgenic Animals**

*DCC* is a suppressor gene of CRC, whose main functions are to increase the adhesion and contact ability among the cells [45]. The mutation or deletion of *DCC* will lead to differentiation, metastasis and lymph node metastases of the cancer. *DCC* can be an index for evaluating the prognosis of CRC. However, there is a lack of *DCC* transgenic mouse models [46]. In recent years, researchers have found that the cytoplasm of *DCC* can be merged with the *Sina* protein of drosophila. Based on this, researchers have built a *DCC/Sina* transgenic drosophila and found that these two genes are expressed in drosophila eyes. The adjustment effect of the *Sina* protein to *DCC* is achieved by the ubiquitin proteasome pathway [47, 48]. The results of this former study inspired us to build *DCC* transgenic mice models. The *DCC* gene is expected to become important in the clinical diagnosis index of the invasion and metastasis of CRC and drive on drug research in CRC.

**Discussions**

The application of transgenic animal models in the research of CRC has made certain progress. *APC*\(^{Min/+}\) mice, a typical animal model, have been widely used in the research of CRC. The newly built models based on the *APC*\(^{Min/+}\) mice model can also be applied to the research on other system cancers, so *APC*\(^{Min/+}\) mice can be seen as the 'integration' model. A host of other transgenic animal models associated with CRC genes have been established and applied in CRC research.

Although the application of transgenic technology in CRC research has made some progress, the establishment of a transgenic animal model for specific signaling pathways is still lacking. Further research is needed to knock out a specific gene to directly induce CRC. Transgenic technology in the study of CRC has a lot of advantages; however, some reports showed that transgenic animal models have a high fatality rate, and it was often seen in transgenic animals that the targeted gene is not well expressed or eventually silenced [49]. We are looking forward to build better animal models in order to guide the research on the molecular mechanisms and biology of CRC.

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References


