Recent advances in the development of transplanted colorectal cancer mouse models

YU-SHEN YANG#, CHU-YUN LIU#, DAN WEN, DA-ZHI GAO, SHU LIN, HE-FAN HE, and XUE-FENG ZHAO
QUANZHOU, AND DALIAN, CHINA; AND DARLINGHURST, SYDNEY, NSW, AUSTRALIA

Despite progress in prevention and treatment, colorectal cancer (CRC) remains the third most common malignancy worldwide and the second most common cause of cancer death in 2020. To evaluate various characteristics of human CRC, a variety of mouse models have been established. Transplant mouse models have distinct advantages in studying the clinical behavior and therapeutic progress of CRC. Host, xenograft, and transplantation routes are the basis of transplant mouse models. As the effects of the tumor microenvironment and the systemic environment on cancer cells are gradually revealed, 3 key elements of transplanted CRC mouse models have been revolutionized. This has led to the development of humanized mice, patient-derived xenografts, and orthotopic transplants that reflect the human systemic environment, patient’s tumor of origin, and tumor growth microenvironments in immunodeficient mice, respectively. These milestone events have allowed for great progress in tumor biology and the treatment of CRC. This article reviews the evolution of these events and points out their strengths and weaknesses as innovative and useful preclinical tools to study CRC progression and metastasis and to exploit novel treatment schedules by establishing a testing platform. This review article depicts the optimal transplanted CRC mouse models and emphasizes the significance of surgical models in the study of CRC behavior and treatment response.

(Translational Research 2022; 249:128–143)

Abbreviations: CDX = cell line-derived xenograft; CRC = colorectal cancer; EGFR = epidermal growth factor receptor; FGFR1 = fibroblast Growth Factor Receptor 1; HER2 = human epidermal growth factor receptor 2; IL2Rγ = interleukin 2-receptor common gamma chain gene; Jak3 = Janus kinase 3; NK cells = natural killer cells; NOD mice = nonobese diabetic mice; NOG mice = NOD/SCID/IL2Rγtm1Sug mice with complete loss of NK cells; NOJ mice = Jak3-deficient NOD/SCID/Jak3null mice; Nude R/J mice = BALB/c Nude Rag-2/Jak3 double-deficient mice;

#Co-first authors.

From the Department of Anaesthesiology, the Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China; Department of General Surgery, the Affiliated Xinhua Hospital of Dalian University, Dalian, 116021, China; Centre of Neurological and Metabolic Research, the Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China; Group of Neuroendocrinology, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW 2010, Australia.

Submitted for Publication April 10, 2022; revision submitted June 13, 2022; Accepted for Publication July 11, 2022.
Reprint requests: Shu Lin, He-fan He, No. 34 North Zhongshan Road, Quanzhou, Fujian Province, 362000, China e-mail: shulin1956@126.com.Reprint requests: Prof. Xue-Feng Zhao, No.156 Wansui Street, Shahekou District, Dalian, 116021, China e-mail: zoserbong@163.com.

1931-5244/S - see front matter
© 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/) https://doi.org/10.1016/j.trsl.2022.07.003
INTRODUCTION

Despite significant efforts to prevent and treat colorectal cancer (CRC), a total of 935,173 deaths worldwide from CRC were reported in 2020.1 The primary problem of CRC treatment is not the eradication of the primary tumor itself but rather the formation of incurable metastases. The reported 5-year incidence of stage III metastatic CRC is 28%—73%, while that of stage IV is 5%.2 Therefore, the early diagnosis, detection, and treatment of CRC patients are very important. Although CRC research has made great progress over the years, there are still many critical problems to be solved, such as early detection of micro-metastases and overcoming chemotherapy resistance. To address these issues, preclinical animal models are an indispensable tool.

In recent years, animal models of human cancer have served as important research models in gaining an in-depth understanding of the biological mechanisms of the pathogenesis and development of malignant tumors. More than any other animal model system; the mouse models have led to revolutionizing our capability to understand the molecular mechanisms of CRC initiation, progression, and treatment. Compared with other mammalian models, the mouse models have several important advantages including (1) small size, (2) inexpensive maintenance, (3) short reproductive cycle, and (4) genetic controllability.3 Currently, there are many preclinical mouse models, including carcinogen-induced models, genetically engineered mouse models, and transplanted CRC mouse models. However, the defects of the first two models, which include low invasiveness, time inefficiency, and uncertain formation sites, greatly confined their application in the aspect of CRC metastasis and treatment.4 In contrast, transplanted CRC mouse models can overcome these limitations. Therefore, these mouse models have a vital role in the research of human CRC.

Host, xenograft, and routes of transplantation are the pillars of transplant mice models. During many years of continuous development and growth, the host has undergone several changes from first generation to fourth generation (namely from remaining complete immune system of mice to rebuilding human functional immune system in mice). The first-, second-, third- and fourth-generation hosts respectively include immunocompetent mice, genetically immunodeficient mice, novel combined immunodeficient mice, and humanized mice. CRC samples derived from patients are used for the generation of tumor cell lines, patient-derived xenograft (PDX) and/or patient-derived organoid (PDO). For the establishment of the transplant mice model, patient-derived tumor materials are implanted into host mainly via subcutaneous, intrasplenic, or orthotopic routes. Transplant mice model has commonly been exploited to find novel biomarkers, create a drug testing platform, and develop surgical models (Fig 1).

This, review summarizes the recent progress of transplanted CRC mouse models and points out their advantages and disadvantages. These pieces of information can provide suitable and useful preclinical tools for studying the invasion and metastasis of CRC and developing new drugs by establishing drug test platforms. In addition, this review describes the optimal transplanted CRC mouse models and emphasizes the significance of the surgical model in the study of CRC behavior and treatment response.

HOSTS FOR HUMAN CRC XENOGRAFT

Immune rejection is the primary problem that needs to be addressed during xenograft. Rejection reaction is mainly mediated by immune cells, especially natural killer (NK) cells and T cells. Studies have demonstrated that T cell-mediated cellular immune responses can destroy malignant cells and mediate host-versus-graft responses when exotic cells are transplanted into the mice. In addition, recent evidence has shown that NK cells also play an important role in xenograft rejection by recognizing xenogeneic cells.5,6 Therefore, avoiding or eliminating these cells is necessary to overcome the immune rejection of xenografts.

Immunodeficient mice: the first attack of the mouse immune system. Early attempts in achieving xenograft included: (1) finding sites of blunted or delayed immune responses (eg, anterior chamber of the eye,7 brain,8 and cheek pouch in the hamster9); (2) using immunosuppressive agents and immune-incompetent animals (new-born mice and fetuses which are naturally immune-incompetent)10-12; and (3) using antilymphocyte13 or antithymocyte serum,14 or thymectomy15 in different combinations. However, these strategies are limited by their innate drawbacks (Table 1). The emergence of genetically immunosuppressed mice is undoubtedly a milestone in the development of immunosuppressive measures for xenografts.

First proposed by Flanagan in 1966, nude mice are the oldest immunodeficient mice, which are characterized...
by significant hair loss due to a novel recessive gene. The most important feature of nude mice used for cancer research is the lack of thymus and T lymphocytes, which ensures the survival and biological behavior of tumor cells after implantation. Furthermore, the natural lack of hair in nude mice made subcutaneously transplanted tumors easy to observe. Therefore, following the first successful transplantation of patient-derived CRC tissue into nude mice, engraftment of human colorectal tumors into these rodents has been reported by many investigators. However, although only a small number of T lymphocytes are present in the peripheral blood of nude mice, they still carry intact innate immune system components, especially NK cells and B cells, thus limiting the take rate and subsequent biological process of engrafted tumors.

In 1983, another genetic immunodeficiency mouse appeared. Due to the lack of DNA-dependent protein kinase, it was called severe combined immunodeficiency (SCID) mouse, which largely prevents the development of T and B lymphocytes. Because of the lack of functional T and B cells, SCID mice were first used to transplant human hematopoietic stem cells and peripheral blood mononuclear cells. Subsequent studies further showed that the rate of human tumor engraftment into SCID mice was higher than that in nude mice. Therefore, SCID mice are mainly used as recipients of human tumors. However, recent studies have come to the opposite conclusion that the engraftment efficiency of human gastrointestinal tumors in SCID mice is lower than that in nude mice. The exact mechanism behind this finding is unclear. Although the SCID mouse model holds great potential, it also has certain limitations. For example, its DNA repair mechanisms are defective and vulnerable to radiation. Furthermore, like nude mice, SCID mice exhibit leakage of functional T and B cells later in life, as well as functional macrophages and high levels of NK cell activity. These shortcomings limit the efficiency of engrafting human tumors in this model.

**Novel combined immunocompromised mice: the second attack to the mouse immune system.** As mentioned earlier, immunodeficient mice provide an excellent platform for the study of human tumors, but remnant NK cells prevent homing and maintenance of the tumor cells. Thus, the engraftment efficiencies of human tumor cells in these mice are not as high as expected. To abolish NK cell activity, the novel combined immunocompromised mice were created by crossing SCID mice or nude mice with other breeds.

NOD/SCID mice were generated by crossing nonobesity diabetes (NOD) mice with SCID mice. Due to...
to multiple deficiencies in both innate and adaptive immunity, NOD/SCID mice proved to be well-suited for transplantation of human tumors including CRC. However, NOD/SCID mice have some lethal defects, such as short lifespan, immune leakiness of T and B cells, and residual NK activities. The development of immunodeficient mice with targeted mutations in the interleukin 2 receptor common gamma chain gene (IL2Rg) represents a dramatic advancement in the elimination of immune function in mice. As a common receptor complex for 6 different IL receptors, IL2Rg plays a key role in the development of NK cells and lymphocytes by interacting with the Janus kinase 3 (Jak3) nonreceptor tyrosine kinase. Hence, IL2Rg- and Jak3-deficient mice exhibit common phenotypes including NK deficiency and reduced T and B cells. To completely overcome NK cell function, IL2Rg/Jak3-deficient mice were used to establish the NOG/NSG/NOJ mice (NOG, NOD/SCID/IL2Rg tm1-Sug; NSG, NOD/SCID/IL2Rg tm1Wjl; NOJ, NOD/SCID/Jak3null) by crossbreeding with NOD/SCID. The NOG and NSG mice show a similar genetic background with partial or complete deletion of IL2Rg, which makes them better recipients for human CRC.

In addition to SCID, some scholars have also used nude mice to generate new types of combined immunocompromised mice. For example, Rag-2null and Jak3null mice with a BALB/c background were crossed with nude mice to establish BALB/c Nude Rag-2/Jak3 double-deficient (Nude R/J) mice. Nude R/J mice preserved the characteristic of no fur and had a higher immune-deficient level (no NK, T and B cells) than nude mice.

Taken together, the novel combined immunocompromised mice provide a better model for overcoming immune rejection by selectively and completely eliminating immune cells in the mice (Table 2).

**Humanized mice: from destroying the mouse immune system to rebuilding the human immune system in mice.** Immune cells that mediate tumor-associated inflammation and immune destruction have been demonstrated to play a role in the formation and progression of human tumors. In 2022, Douglas Hanahan published a sensational review that incorporated tumor-promoting inflammation and avoidance of immune destruction into the hallmarks of cancer, which were segregated as “core hallmarks.” Consequently, interest in tumor-immune system interactions and immunotherapy has

---

**Table 1.** The characteristics of animal transplant model for studies of colorectal cancer

<table>
<thead>
<tr>
<th>Animal</th>
<th>Measures to overcome immune rejection</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>Intraorbital engraftment</td>
<td>1. Immunological unresponsiveness</td>
<td>1. Difficult operation</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Intracranial engraftment</td>
<td>1. Immunological unresponsiveness</td>
<td>1. Inaccessible inspection</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Engraftment into the cheek pouch</td>
<td>1. Easy to inspect and follow 2. Immunologically delayed responsiveness</td>
<td>1. Immune response eventually develops; 2. Very difficult to evaluate many therapeutic results obtained; 3. Low take rate; 4. Difficult serial passages</td>
<td>9</td>
</tr>
<tr>
<td>Newborn mice and fetuses</td>
<td>Undeveloped immune system</td>
<td>1. Immunosuppression</td>
<td>1. Eventually acquisition of immunocompetence; 2. Higher rate of abortion; 3. A higher rate of destruction of the newborns through maternal cannibalism</td>
<td>10–12</td>
</tr>
<tr>
<td>Immunocompetent animals</td>
<td>Whole-body irradiation with or without corticosteroids</td>
<td>1. Immunosuppression</td>
<td>1. Very toxic to the animals; 2. Severe damage to the guts and bone marrow; 3. High incidence of infections; 4. A shortened life span</td>
<td>106</td>
</tr>
<tr>
<td>Immunocompetent animals</td>
<td>The use of thymectomy, ALS, or ATS in various combinations</td>
<td>1. Immunosuppression</td>
<td>1. Difficulty for complete removal of all the thymic tissue; 2. Maternal cannibalism; 3. Irreconcilable titration standard; 4. Toxic and allergic serum reaction; 5. Difficult reconstitution with syngeneic bone marrow</td>
<td>13–15</td>
</tr>
</tbody>
</table>

ALS, antilymphocyte serum; ATS, antithymocyte serum.
grown over the past few years. However, there are significant differences in the immune system between humans and mice, which lead to the inability of mice to mimic the immune properties of actual cancer patients. The development of humanized mice addressed this major limitation.

Humanized mice are referred to as immunodeficient mice engrafted with a functioning human immune system. The immunodeficient mice can be considered humanized mice after engrafting human immune systems into them. Currently, there are 3 methods used to establish humanized mice for tumor growth and tumor immunology studies according to different engraftment combinations: (1) combined engraftment of immune cells or tissues with tumor cells; (2) single patient-derived xenograft (PDX) engraftment; and (3) combined engraftment of immune cells or tissues with PDX. An ideal humanized mice model not only replicates the complex human immune system and tumor but also can be immediately available for an experiment (Table 2).

Thus, much literature reported the application of humanized mice in the research of human CRC immunotherapy (relevant research has been summarized and presented in Table 3). The humanization model also has some limitations, such as high cost, low success rates, and long experiment period, which impede its spread and development. There is little doubt, however, that the discovery of "humanized" mice provides an optimal human CRC xenograft host for uncovering tumor-immune interactions, monitoring immunotherapy, and evaluating efficacy.

### TUMOR MATERIALS FOR HUMAN CRC XENOGRAFT

Distant metastasis is the main cause of high mortality in CRC patients. An applicable preclinical model to mimic this behavior plays a crucial role in oncology research. Existing models cannot replicate human CRC as an entity, but available transplant mouse models

---

**Table 2. The characteristics of transplant mouse models for studies of colorectal cancer (CRC)**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Genetic alterations</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athymic nude mice16</td>
<td>Spontaneous mutation of Foxn1 causing a lack of thymic tissue</td>
<td>1. No T cells; 2. High take rate of human tumors; 3. No coat of hair; 4. Easy to detect subcutaneous tumor</td>
<td>1. No deficiency in B cells or myeloid cells; 2. T-cell functionality increases with age; 3. Not suitable for primary cell transplantation</td>
</tr>
<tr>
<td>SCID mice22</td>
<td>Defect in DNA protein kinase, no functional rearrangement of antigen-specific receptors</td>
<td>1. No mature T and B cells; 2. Better engraftment compared with nude mice</td>
<td>1. Short lifespan (&lt;12 months); 2. Functional NK cell; 3. Leakage of T cells; 4. Thymic lymphoma development; 5. Radiosensitive</td>
</tr>
<tr>
<td>NOG/NSG/NOJ mice108–110</td>
<td>1. NOG: NOD.Cg-Pkdac&lt;sup&gt;sci&lt;/sup&gt;Il2rg&lt;sup&gt;tm1Sug/Jic&lt;/sup&gt; SCID, IL-2/γ- deficiency; 2. NSG: NOD.Cg-Pkdac&lt;i&gt;sci&lt;/i&gt;Il2rg&lt;sup&gt;tm1Wjl/Jsz&lt;/sup&gt;; NOD-SCID, IL-2γ&lt;sup&gt;Rγ&lt;/sup&gt; Complete deficiency; 3. NOJ: NOD.Cg-Pkdac&lt;i&gt;sci&lt;/i&gt;Jak3&lt;sup&gt;tm1card&lt;/sup&gt; SCID, Jak3 deficiency</td>
<td>1. No mature T and B cells; 2. No NK cells; 3. Impaired macrophage and dendritic cell; 4. High engraftment</td>
<td>1. Need strict SPF conditions; 2. Difficult and expensive breeding</td>
</tr>
<tr>
<td>Nude R/J mice37,38</td>
<td>Rag-2/Jak3 double-deficiency</td>
<td>1. No mature T and B cells; 2. No NK cells; 3. High engraftment; 4. Resistant to stress; 5. Easy breeding; 6. Radio resistant</td>
<td>-</td>
</tr>
<tr>
<td>Humanized mice 111–114</td>
<td>Immunodeficient mouse engrafted with human immune systems</td>
<td>1. Immediately available for experiment; 2. The complex human immune system and human CRC can be replicated</td>
<td>1. Time-consuming; 2. Difficult to set up; 3. High in cost</td>
</tr>
</tbody>
</table>

IL2RG, IL-2 receptor γ-deficient; NK cell, natural killer cell; NOD, nonobese diabetic; NOG, NOD/SCID/IL2RG<sup>nu</sup> mice with complete loss of NK cells; NOJ mice, Jak3-deficient NOD/SCID/Jak3<sup>nu</sup> mice; Nude R/J mice, BALB/c Nude Rag-2/Jak3 double-deficient mice; NSG mice, NOD/SCID/IL2RG<sup>nu</sup> mice with complete loss of NK cells; Pkdac, protein kinase, DNA activated, catalytic polypeptide; SCID, severe combined immunodeficiency mice; SPF, specified pathogen free.
approximate many features of colonic invasion and metastasis. The metastatic capacity of transplanted tumors in immunodeficient mice depends on the experimental technique, the routes of transplantation, the origin of tumor materials, and the source and conditions of the mice used. Among these, the origin of tumor materials is one of the most critical factors affecting the biological phenotype of xenografts in immunodeficient mice. There are 3 sources of xenografts for colorectal tumors, including cell line-derived xenografts (CDX), PDX, and PDO. Fig 2 summarizes the characteristics of these xenografts.

CDX. The culture of colorectal tumors provides useful experimental material for various studies. Human CRC cells propagated in immunodeficient mice have been shown to resemble tumor origins. Other important
features, including morphological characteristics, the extent of tumor necrosis, isoenzyme mobility patterns, and the capacity to produce cellular products such as mucin, were also well-preserved in xenografts. The high degree of identity between CDXs and human tumors makes it a useful tool in studying human CRC.43

Aside from the transplantation route, the invasiveness and metastasis of CDXs in immunodeficient mice are mainly related to their primary tumors. Giavazzi et al.44 investigated the growth characteristics and metastatic behavior of several tumor lines derived from primary CRC and hepatic metastases. They found that models infused with metastatic cells grew and metastasized faster than primary tumor cells. Subsequent studies have yielded similar results.45,46 These findings confirm that: (1) tumor lines of different origins differ in biological phenotypes after transplantation into nude mice and (2) the metastatic potential of human malignant tumors in nude mice increases with the degree of malignancy of the original tumors. Another interesting finding is that it is indeed possible to select highly metastatic cells from low metastatic CRC by in vivo passages.47 Additional advantages of the CDX model are easy operation, high take rate, and short tumor-forming time (2–8 weeks).48

However, some reports have found that orthotopic injection of cell suspensions may not express the full metastatic potential and therapeutic response of the primary tumor. This is due to the deterioration of 3-dimensional tissue architecture and the loss of tumor heterogeneity.49 Therefore, CDXs have limited predictive value for human CRC research.

**PDX.** Nowadays, there is growing interest in PDXs as a more advanced preclinical cancer model. PDXs are usually implanted subcutaneously or orthotopically into immunodeficient mice and more accurately reflect human CRC than CDXs. Indeed, PDXs not only preserve the 3-dimensional tissue structures and cell-to-cell interactions within tumor tissues, but even in chromosomally unstable early-stage human CRC tumors, they also retain chromosomal instability, intratumor heterogeneity, and histology of the parent tumor for over 14 passages.50 Although human microvascular and stromal compounds are eventually replaced by their murine counterparts, tumor tissue remains intact in most cases.51 Furthermore, these mouse mesenchymal cells have also been shown to have a metabolic phenotype similar to that of humans.52 These characteristics suggest that the PDX model is a good and valuable platform that can be used to study human tumors.

CRC tumors have a higher tumor collection rate (> 60%) in the PDX model compared with other solid tumors, including breast cancer (10%–37%) and prostate cancer (<5%).53 However, the success rate of PDX varies in different pieces of literature, and is influenced by several factors including (1) characteristics of the tumor, such as sample type, tumor subtype, and tumor stage; (2) PDX preservation and incubation mode; (3) number and size of the implanted tumor tissue; (4) implant location; and (5) recipient strain.58 In fact, the PDX model of human CRC was originally developed to mimic various clinical behaviors in human subjects, including local invasion and

---

**Fig 2.** Schematic diagram for the summary of the characteristics of implantation routes and xenografts. (Color version of figure is available online.)
been reported to establish a liver seeding model.\textsuperscript{67} Mouse flanks, spleen, and kidney sub-capsules has also been used in development, and improved engraftment rates in CRC PDX models.\textsuperscript{58,61} Only a few pieces of literatures report in detail the clinical behavioral outcomes of PDX in mice.\textsuperscript{62}

PDX also has some drawbacks, including long establishment time (around 2–4 months), high cost, and the possibility that a low percentage of mutated variants occur through the passages. Nonetheless, PDXs appear to be more suitable for studying cancer biology and therapeutic development than CDXs due to the former’s high fidelity to the original tumor at the genomic and transcriptomic levels.

PDOs. Although the development of the PDX model is insightful, it is a relatively expensive and time-consuming task. The emergence of PDOs provides a possible solution to these problems. PDOs are clusters of 3-dimensional cultured multicellular aggregates grown from patient stem cells or isolated organ progenitor cells. It has been demonstrated that PDOs not only retain the characteristics of the parent matrix but also preserve their tissue functions.\textsuperscript{63} Moreover, PDOs can be easily maintained and genetically manipulated in vitro and dutifully exhibit features of in vivo tissues during homeostasis and disease such as CRC.\textsuperscript{64} Another major advantage of PDOs is the high success rate of generation from primary CRC tissue (\~90% for primary cancer).\textsuperscript{63} The high tumor formation rates upon the transplantation of PODs into the cecal (100%) or colon wall (60%) make it a good platform for establishing more efficient CRC mice models.\textsuperscript{65}

It has been reported that PDOs with multiple cancer-related mutations were transplanted into mouse renal capsule and spleen to establish CRC and liver metastasis models.\textsuperscript{66} In addition, transplantation of PDOs into mouse flanks, spleen, and kidney sub-capsules has also been reported to establish a liver seeding model.\textsuperscript{67} However, these ectopic transplantation models do not allow the study of tumor invasion into the muscularis propria or extravasation into the circulation through the colon serosa. Therefore, some research groups orthotopically transplanted PDOs into colonic or rectal mucosa to study primary cancers and liver metastases.\textsuperscript{58} PDOs can also be engineered for desired mutations using gene-editing techniques,\textsuperscript{66} which is much faster than generating germline genetically engineered mouse models. Fluorescent labels and other desired features can also be easily inserted into PDOs.

There are some inadequacies in PDOs, such as long culture cycle, single source of cell, and unstable culture conditions. Yet, as the latest source of xenografts with the terrific fidelity and maneuverability in the CRC model, we still consider PDOs as another breakthrough in cancer research after the PDX model, holding great promise in the study of the biological behaviors and drug response of CRC.

**Routes of Human CRC Xenograft**

In addition to the origin of tumor materials, another crucial factor determining the malignant behavior of transplanted tumor is the routes of transplantation. Human CRC transplant mouse models can be divided into 2 types: heterotopic transplantation and orthotopic transplantation. The former mainly consists of subcutaneous transplantation and intrasplenic transplantation. On the other hand, the latter was involved in intramural transplantation of the mouse colon or rectum. The characteristics of different routes are presented in Fig 2.

**Subcutaneous transplantation.** In 1969, Rygaard et al.\textsuperscript{19} reported the first case of subcutaneous transplantation of human CRC tissue, indicating that nude mice were more tumorigenic than immune. Subsequently, Kyriazis et al.\textsuperscript{69} presented data involving the growth pattern and metastatic capacity of human colon cancer cells grown subcutaneously in nude mice. While tumor growth was seen in all subcutaneous models, they showed that none developed metastases. This is mainly due to poor angiogenesis in subcutaneously transplanted tumors caused by the fibrous capsule. Local vascular supply is a key factor affecting the biological behavior of xenografts. However, the fibrous capsule can interfere with the vascularization of the graft, resulting in tumor cell necrosis, which in turn, affects tumor growth and prevents distant metastasis.

The reason why subcutaneous transplantation is widely used can be attributed to its unique advantages, namely, nominal surgical skills, short time, and easy observation. Due to these characteristics, subcutaneous tumor models are usually used to evaluate therapeutic effects in human cancers. Some of these models have been reported to correctly predict clinical responses in specific cancers.\textsuperscript{70} Nevertheless, this model suffers from a major disadvantage: the subcutaneous microenvironment is greatly different from that of the colon. Interactions between the tumor microenvironment and tumor grafts have been shown to determine the molecular properties and biological behavior of tumors. Subcutaneous tumor models cannot replicate the characteristics of primary CRC due to differences in the microenvironment. Extensive drug screening has

\[\text{Translation of Research} \quad Volume \, 249 \quad \text{Yang et al} \quad 135\]
shown that subcutaneous transplant models have rather limited value in predicting clinical response in humans.71

**Intrasplenic transplantation.** The most common site of CRC metastasis is the liver. Hence, there is an urgent need to find a suitable CRC liver metastasis model for research. The intrasplenic transplantation mouse model is an ideal platform for studying hepatic metastasis of human tumors. The injection of human tumor cell lines into the spleen of nude mice allows for the most dramatic overall expression of metastatic potential in these cell lines compared to other transplantation routes.72 After intrasplenic injection, tumor cells directly enter the bloodstream and then reach the liver, where they proliferate towards secondary lesions, which prevents them from undergoing the initial stage of metastasis. These characteristics make the intrasplenic transplantation model irreplaceable in the study of human tumor liver metastasis.73

However, the traditional intrasplenic transplantation model, also known as the whole spleen model, still has some disadvantages, such as tumor growth in the spleen and the inability to repeatedly administer drugs. To overcome these drawbacks, researchers established a mouse subcutaneous hemi-spleen depot model to treat liver malignancies through repeated portal vein injections.74 Unlike humans, mice have 2 splenic pedicles, which are located at opposite ends of the spleen. Therefore, this model divides the spleen into 2 hemi-spleens with vascular pedicles: one inoculated with tumor cells and removed after 10 minutes, and the other one transposed subcutaneously for multiple intrasplenic administrations. The hemi-spleen model solves the aforementioned problems well and is widely used in the study of the treatment of liver tumors.75

Circumventing the initial steps of metastasis helps to improve the efficiency of liver metastasis but cannot truly reflect the metastatic process from the primary tumor to the metastatic tumor. This limits the clinical value of the intrasplenic transplantation model.

**Orthotopic transplantation.** Tumor dissemination is considered to begin when the tumor graft invades the surrounding host tissues. This initial step is very important in the metastatic cascade, as tumor cells need to undergo a series of functional and morphological changes from a nonmetastatic to a metastatic state. However, ectopic models usually bypass this initial process and lack an appropriate tumor microenvironment. Therefore, orthotopic CRC models were established to overcome the drawbacks of heterotopic models.

The establishment of an orthotopic colon cancer model in mice after intramural cell injection was first described by Tan et al. in 1977. In their report, Tan et al. not only demonstrated the feasibility of intramural cell injection but also showed that the take rate (90%) of the cecum was higher than that of other intestinal areas.76 Subsequently, intercecal injection of human CRC cells in immunodeficient mice was also successful and resulted in liver metastases.47 Morikata et al. even proposed that human colon cancer cell lines must be directly injected into the cecum wall of nude mice for sustained metastasis.47 Since then, to better reflect the original nature of human cancer and avoid spilling over of cells, many improvement strategies have been adopted. These strategies include the addition of Matrigel to the cell suspension to avoid cell extravasation77; suturing of histologically intact tumor tissue to the cecum wall78; formation and suturing of subserosa “pockets”79 between the mucosa and the extracecal muscularis for tumor seeding, or *in situ* cell microinjection.46

Despite the clear advantages of intercecal transplantation, there remain some aspects that require careful consideration, including the requirement for laparotomy and limited relevance with rectal cancer. Hence, a novel animal model of rectal cancer was developed.80 Rectal anatomy and lymph node drainage patterns in mice are similar to those in humans.81 The growth pattern of tumor cells in the rectal wall of mice is similar to that of clinical CRC patients,80 suggesting the clinical relevance of this model. Furthermore, Hite et al.82 compared 3 orthotopic models, namely, intercecal injection, transanal submucosal injection, and acid enema. They found that intrarectal injection was the safest, most reproducible, and most successful orthotopic model for primary tumor growth and spontaneous metastasis in human CRC. Therefore, mouse models of rectal cancer have received increasing attention and interest. In practical applications, some modifications are made to meet the needs of specific research. Enquist et al.83 used the prolapse technique to suture human CRC cell lines to the rectal mucosa to reveal the origin of liver metastases. In a recent mouse model of rectal cancer, clinical-grade titanium fiducial clips were placed on opposite sides of a rectal tumor to enable targeted delivery of short-term radiation therapy.84 Interestingly, to avoid laparotomy and reduce the damage caused by surgery, some nonsurgical orthotopic CRC mouse models have also been established using mouse colonoscopy or other instruments.85 For example, through a p200 pipette enema or colonoscopy, O’Rourke et al.86 and Roper et al.87 established distinct experimental systems for the orthotopic transplantation of CRC organoids into mice to model the adenoma-carcinoma-metastasis sequence. These approaches make it possible to rapidly characterize the cancer-associated genes and reproduce the entire spectrum of tumor progression and metastasis.
Although the optimal site (cecum or rectum) for orthotopic transplantation remains controversial, orthotopic transplantation model mimics the environment in which human tumors grow in mice. Thus, we regard it as another milestone in cancer research with great potential in the development of precision medicine in CRC.

APPLICATION OF TRANSPLANT MOUSE MODEL IN CRC

As a bridge to clinical application, transplant mouse models have been widely used in various aspects of CRC therapy. Different transplant mouse models have their unique characteristics, and likewise, each model has its drawbacks. For example, CDX models do not adequately reflect patient drug responses, resulting in very low rates of clinical approval of cancer drugs (approximately <15%). Subcutaneous transplantation models have limited predictive value for human clinical response through large drug screening. In contrast, the orthotopic PDX models maintain the highest concordance of drug responses between patients and mouse models, supporting their use as an optimal screening platform for anticancer drug evaluation.

Currently, the applications of transplant mouse models mainly involve the development of biomarkers, drug testing, and surgical modeling.

Development of biomarkers. Transplant mouse models can reveal novel biomarkers, thus providing reliable evidence for individualized treatment of CRC patients. Wang et al. showed that tumor-transplanted mice developed severe colonic tissue damage after upregulation of ring finger protein 2 (RNF2), suggesting that RNF2 may be a potential therapeutic target for CRC. Furthermore, the correlation between drug efficacy and molecular properties can be easily studied using transplant mouse models. In a recent study, amplification of the human epidermal growth factor receptor 2 (HER2) gene was shown to promote cetuximab resistance in a KRAS/NRAS/BRAF/PI3KCA wild-type PDX model with metastatic CRC and was found to predict responses to antiepidermal growth factor receptor (EGFR) and anti-HER2 antibodies. Subsequently, these findings were further translated into successful clinical studies. Other molecular targets have also been shown to be biomarkers of cetuximab resistance, such as the MET proto-oncogene and fibroblast growth factor receptor 1 amplification, through candidate gene or comprehensive genomic analysis.

The application of transplant mouse models has advanced the understanding of adaptive escape mechanisms that maintain residual lesions during maximal drug response. Lupo et al. recently found that in the PDX model, EGFR-inhibited surviving metastatic CRC cells exhibited reduced EGFR ligand expression, enhanced HER2/HER3 signaling pathway activity, and sustained activation of the phosphatidylinositol 3-kinase (PI3K) pathway. They further demonstrated that Pan-HER antibodies minimized residual disease, blunted PI3K signaling, and induced long-term tumor control after treatment discontinuation in a preclinical trial. Another interesting finding was that chemotherapy preferentially eradicated actively proliferating cells and promoted the dominance of previously minor or dormant lineages in the CRC PDX model. Undoubtedly, these findings provide opportunities to preemptively target residual disease.

In summary, the discovery of novel tumor biomarkers with the help of transplant mouse models is essential in promoting translational research in clinical and basic sciences. Moreover, it can facilitate individualized treatment based on tumor molecular classification.

Drug testing. Transplant mouse models can be used in investigating the efficacy of controversial or novel anti-tumor agents against CRC. Using the CDX model, Liu et al. demonstrated that a novel andrographolide derivative AGS-30 could induce apoptosis in CRC cells by activating the ros-dependent JNK signaling pathway.

Through the CDX model, our group found that the traditional Chinese medicine sophocarpine can enhance the inhibitory effect of oxaliplatin on metastatic CRC. Additionally, in the KRAS-mutated metastatic CRC xenografted model, metformin was reported to inhibit tumor growth and cell viability.

Another application of transplant mice models is the coclinical trial, which is defined as a clinical trial conducted in parallel with a preclinical trial. Currently, coclinical trials in CRC have already generated many hopeful outcomes. For example, in BRAF-mutated CRCs, PDX models were identified to faithfully replicate clinical results and permit further study of acquired resistance mechanisms. Julien and colleagues reported that by using the PDX models, they were able to reproduce the results observed in clinical trials of cetuximab in KRAS. Therefore, by establishing PDX models of patients enrolled in clinical trials and treating them with new drugs, prognostic biomarkers can be screened, and potential drug response mechanisms can be studied. Although not widely attempted so far, the co-clinical trial may be further utilized in the future as it can shorten the time to drug development and demonstrate individualized medicines in a preclinical setting.

Beyond coclinical trials, transplant mouse models are also considered useful tools for studying immunotherapy. Hollandsworth et al. verified that near-infrared photoimmunotherapy is an effective method for the
treatment of CRC using humanized mice. To overcome the limitations of antibody therapy for immune checkpoint blockade, Lee et al. explored a novel blockade in traditional oriental medicine, called Sangsiurbae Radix extract (SRE). They demonstrated that SRE alone has anticancer effects via immune checkpoint blockade and that the combination therapy of SRE and pembrolizumab has enhanced immuno-oncologic effects. Using transplant mouse models, many new immunotherapeutic strategies are being developed, of which have received regulatory approval or are being studied in clinical trials.

**Surgical model.** The standard treatment modality for CRC is a surgery-based holistic therapy. Although transplant mice models are a valuable tool for biomarker development and drug screening, their predictive power is limited by several factors. Among them, surgery is extremely important but often overlooked. Preclinical studies have demonstrated that resection of the primary tumor activates proliferative and metastatic pathways that accelerate the growth of microscopic or macroscopic residual tumors. Wang et al. further demonstrated that colorectal surgery enhances tumor cell adhesion and invasion through endotoxin/LPS-related and/or β1 integrin-dependent mechanisms. A similar phenomenon was observed in mice. Therefore, surgery has an important impact on the biological behavior of postoperative residual tumors and treatment response. Evaluating this impact requires establishing a surgical model that reflects more realistic clinical practice through a combination of drug trials and surgery.

Previously, some simple surgical models have been established for certain scientific purposes. In 1986, Giavazzi et al. found that CRC growth after thigh resection did not increase the incidence of metastases. In 1989, Schackert et al. found that after resection of the tumor in the mouse cecum, most models had tumor recurrence at the resection site and mesenteric regional lymph nodes. In 1995, Allendorf et al. reported that compared with laparotomy, tumors were less likely to form after laparoscopic surgery, and the tumor was less aggressive. Although the above surgical models facilitated the in-depth understanding of tumor growth and recurrence, none of them can reflect real-life clinical situations. Interestingly, Pang et al. recently reported a robust and reproducible technique for a mouse pancreatic cancer surgical model and showed that this model could be useful in testing both preoperative and postoperative adjuvant treatments. Thus, establishing the surgical mouse model of CRC perhaps contributes to the development of future surgery-based combination therapy in the treatment of CRC.

**CONCLUSION AND FUTURE DIRECTIONS**

Due to the high failure rate of new therapeutic strategies in clinical studies, establishing good preclinical models is crucial in translational cancer research. However, differences between species lead to biologically inadequate preclinical models that do not accurately reflect the considerable genetic and phenotypic heterogeneity of tumors. Cancer has been shown to be a systemic disease rather than an isolated disease, and it is thus influenced by both systemic (such as hormone, immunity and metabolism) and local factors (such as tumor growth environment and intra-tumoral microenvironment). Therefore, the factors with the ability to influence tumor progression, metastasis, and treatment response can be roughly divided into 3 points: systemic factors, local growth conditions of tumor cells, and intra-tumoral microenvironment. An ideal model is expected to fully replicate the impact of the above factors on tumor cells.

The development of humanized mice, PDXs/PODs, and orthotopic transplantation provides a promising solution to the above issues. Since orthotopic transplantation approximates the environment for tumor growth and PDXs/PODs maintain the intact tumor tissue structure and intra-tumoral microenvironment as much as possible, humanized mice make it possible to re-establish the human system environment in mice. Thus, the orthotopic transplantation of PDXs/PODs into humanized mice is potentially an ideal CRC transplant model to anastomize the multifactorial etiology and progression of the tumor (Fig 3). However, there is less literature providing clear evidence demonstrating the superiority of this model to date.

On the other hand, humanized mice were generally used to reconstitute the human immune system in mice and then study the impacts of immunity on disease. As previously mentioned, other systemic factors, such as metabolism and endocrine, also exert a regulatory effect on the tumor. However, establishing a rodent animal model that can reflect these factors is still in groping. In addition, our team have preliminarily established an early-stage CRC resection model (Supplementary Fig 1) and demonstrated the technical viability of this model. However, much work is still needed to establish the surgical model of stage II-IV CRC to evaluate the efficacy of surgery-based combination therapy. Further studies are required to address these gaps.

In conclusion, this is the first review of CRC transplant models to provide a comprehensive assessment of their characteristics in the research of human CRC. We believe the CRC transplant model is an essential preclinical tool to broaden the personalized medicine strategy in the future. More studies for improving the
graft success rate and generating humanized mice with a similar systemic environment as patients will accelerate the usage of this preclinical model.

FUNDING

This work was supported by Liaoning Provincial Natural Science Foundations of China (LJKZ1192), Natural Science Foundation of Fujian Province (2020J01227), Medical Innovation Science and Technology Project of Fujian Province (2020CXA047), and Science and Technology Bureau of Quanzhou (2020CT003).

AUTHOR CONTRIBUTIONS

Yu-Shen Yang, Xue-Feng Zhao, He-fan He, and Shu Lin: conceptualization, writing-original draft preparation, writing-editing and reviewing. Yu-Shen Yang and Chu-Yun Liu: writing-original draft preparation, writing-editing and reviewing. Dan Wen: writing-editing and review. Da-Gao Zhi: writing-editing and review. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

All authors have read the journal's authorship agreement. We are thankful to Dalian University Affiliated Xinhua Hospital and The Second Affiliated Hospital of Fujian Medical University for providing infrastructure facilities. We would like to thank Editage (www.editage.cn) for English language editing.

Conflict of Interest: All authors have read the journal's policy on conflicts of interest and declare that they have no competing interests.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.trsl.2022.07.003.

REFERENCES


Fig 3. The ideal transplant mice model of colorectal cancer. The development of PDXs/PDOs, orthotopic transplantation, and humanized mice can accurately reflect the intact intra-tumoral microenvironment, actual context of tumor growth, and human systemic environment in immunodeficiency mice. Thus, the orthotopic transplantation of PDXs/PDOs into humanized mice is potentially the ideal CRC transplant model to anatomize the multifactorial etiology and progression of the tumor. CRC, colorectal cancer; PDXs, patients-derived xenografts; PDOs, patients-derived organoids. (Color version of figure is available online.)


57. Gock M, K...


44. Giavazzi R, Campbell DE, Jessup JM, et al. Metastatic behav-

43. Prasetyanti PR, van Hooff SR, van Herwaarden T, et al. Captur-

46. C...


47. Morikawa K, Walker SM, Jessup JM, et al. In vivo selection of


