

Animal Models of Transplantable Bladder Cancer: A Review

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Abstract: Bladder cancer (BC) is one of the most common cancers worldwide. At present, numerous treatments of BC are available, but the response to treatment varies from patient to patient. Hence, there is a need to re-explore the mechanisms and biology of BC development and develop robust and effective therapies against BC. Animal models are the powerful tools which have been and are still used in studies that link the knowledge gap between fundamental and clinical research. Animal models of transplantable BC is the most frequently used animal models that are established by transplanting BC cell lines or BC tissues into the animal to form a tumor. Recapitulation of human cancer condition in these animal models enables the scientists to further explore BC development, progression, and metastasis. In addition, the use of animal models of transplantable BC facilitates the identification of drug targets that are the primary part of an attempt to reverse therapeutic resistance and improve clinical outcomes. Overall, animal models present themselves as critical tools which help address the complex issues in BC research. This review summarizes the classification of animal models of transplantable BC, discusses advanced methods of establishing and monitoring tumor models, and compares the advantages and disadvantages of each establishment method.

Keywords: Bladder cancer, Animal model, Therapy, Tumor development

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1. Introduction

Affecting about 430,000 patients worldwide, bladder cancer (BC) has become the fourth and ninth most common cancer among men and women, respectively^[1]. The development of BC arises through dual-track pathway^[2]. About 80% of BC consists of the superficial papillary lesion which is also known as non-muscle invasive BC (NMIBC), whereas the remaining 20% of BC is non-papillary bladder tumors which are also known as muscle-invasive BC (MIBC). NMIBC does not invade the bladder wall or metastasize to other tissues. In contrast, MIBC commonly leads to metastasis and worsen the prognosis of BC patients^[3].

The treatment of BC is determined depending on the clinical stages and the associated risk factors. To date, the standard treatment for BC is the transurethral resection of bladder tumors, followed by intravesical therapy with the administration of Bacillus Calmette-Guérin^[4]. Since the treatment response varies among the patients and there has been no improvement in treatment regimens, the investigation on the pathobiology of BC and the identification of novel therapeutic targets are worth exploring.

Animal models are powerful tools in providing fundamental and translational relevance knowledge in BC research. Attributed by its recapitulation of biological and

physiological characteristics of human, animal models are always used to mimic human cancers. Thus, this enables mechanistic studies on BC development, progression, and metastasis^[5]. The study of BC in animal models not only allows studying the behavior of tumor cells but also enables the exploration of interactions between bladder tumor cells and other cell types such as stromal cells and immune cells in the tumor microenvironment^[6].

Furthermore, the use of animal models enables the modeling of cancer metastasis. Accompanied by the accelerated pace of the development of sequencing technologies and bioinformatic analyses, scientists can identify the main factors contributing to BC metastasis. Besides, real-time monitoring of tumor growth with various imaging techniques promotes a deeper understanding of the metastatic properties of BC. Next, animal models are the potential candidates in the modeling of therapeutic resistance. In short, these ideal features make the animal models suitable for the identification of prognostic biomarkers and the development of therapeutic targets besides improving our knowledge in the pathobiology of BC^[7].

This review focuses on the animal models of transplantable BC which is established through the injection of human or animal BC cell lines into their body. Besides, the methods of the transplantation and monitoring of BC in animal models are also discussed. This review further explains the pros and cons of using animal models in BC research. These animal models have provided unprecedented knowledge on BC biology and facilitated the development of targeted therapies, although extensive research is warranted to translate the findings into clinical settings.

2. Immunodeficient animals

Immunodeficient animals are appropriate target for establishing animal models of transplantable BC. An immunodeficient animal is an animal that is defective in one or more immune system components by means of congenital genetic mutations or artificial manipulation^[7]. Lacking thymus, nude mice cannot generate mature T lymphocytes and are unable to mount the majority of types of immune response, while the strong rejection response in immunocompetent mice would lead to either the death of the animal or the cancer cells. The most frequently used animals for the establishment of animal models of transplantable BC is nude mice and severe combined immunodeficiency (SCID) mice.

In 1962, Scottish researcher Grist first discovered the hairless nude mice that were unable to generate mature T lymphocytes due to the lack of thymus, resulting in the impairment of immune system^[8]. In 1969, Danish researcher Rygaard first transplanted human malignant tumor into nude mice. In 1975, Weldon *et al.* established the BC animal model through the transplantation of BC

cells into the bladder cavity. They reported that the tumor formation rate in normal nude mice was only 13% after transplantation. Furthermore, bladder inflammation was induced in nude mice using N-methyl-N-nitrosourea before transplantation, and tumor formation rate was 60%^[9]. In the late 1970s, nude mice were introduced in China for experimental research.

Characterized by congenital athymia and complete hairlessness, nude mice are lacking in T lymphocyte, thus defective in cellular immune function. Furthermore, it shows no rejection response to allograft and is susceptible to the transplantation of allogeneic tumor. It is the most well-known immunodeficient animal for the establishment of animal model of BC. Its main feature is that the *in vivo* immunodeficiency mechanism of a nude mouse is relatively conserved, but the genetic conservation is affected by individual difference to some extent. Besides, the occurrence and development of tumor are easy to control using this animal model. Due to the similar structural and pathological features of tumor tissues in nude mice to human tumor tissues, these characteristics are beneficial in the understanding of *in vivo* tumor growth, identification of tumor cell lines, and the assessment of the relationship between tumor and host immunity^[10].

In contrast, SCID mice acquire the single recessive mutant gene (*Scid* gene) which was originally discovered in syngeneic C.B-17/ICR mice of BALB/c mice cohort. Due to the defects in the joining of non-homologous ends of double-stranded DNA, the mice have impaired production of T and B lymphocytes, low natural killer cell activities, no circulating complement, and impaired function of macrophage and antigen-presenting cell^[11,12]. The mice are widely applied in human immunology and virology, oncology, physiology, hematology, and pathology research. SCID mice can be transplanted with human peripheral blood lymphocytes or human normal tissues into chimeric mice such as the SCID-hu model, for research on reconstruction of human immune function and oncology studies. Mainly occurring in the thymus, the incidence of spontaneous T-cell lymphoma in SCID mice is about 15%. At present, the relationship between *Scid* mutant gene and high incidence of T-cell lymphoma has not been elucidated. According to Katano *et al.*, SCID mice without T and B lymphocytes can be a result of defective gene recombination^[13]. These mice present two characteristics, i.e., high incidence of thymic lymphoma and inability to produce some functional B and T cells. Therefore, these characteristics limit the widespread use of SCID mice^[13].

3. Classification of animal models of transplantable BC

Based on the source and the host of transplantation, the tumor graft can be divided into allograft and xenograft (**Table 1**). The allograft animal model of BC is established by transplanting BC cell line into an animal of the

same species but different genetic makeup. In contrast, the xenograft animal model of BC is established by transplanting human BC cell line into an immunodeficient animal.

3.1. Allograft animal models of BC

Allograft animal models of BC include the animal models constructed by transplantation of established BC cell line into the animal of the same species, or the transplant of an organ or tissue from one individual to another of the same species with a different genotype^[14]. The widely used murine BC cell lines for allograft transplantation include MBT-2, BTT-739, MB49, and AY-27 cell lines^[15-18].

Smith *et al.* infused 7.5×10^4 MB49 cells into the bladder of C57BL/6 syngeneic mice and 1×10^5 MBT-2 cells into the bladder of C3H/He mice^[19]. These successfully established allograft animal models of BC were applied for evaluating the potential of immunotherapy of BC^[19]. Furthermore, the anti-cancer effects of recombinant human interleukin-6 (rhIL-6) on an allograft animal model of BC inoculated subcutaneously with 1×10^6 cells/mL BTT-739 cells into the flank of T739 inbred mice were also investigated^[17]. The allograft animal models of BC were established successfully to study the anti-BC effects and drug toxicity of rhIL-6. The results demonstrated that rhIL-6 showed prominent anti-tumor effects in the

treatment of BC of mice, which resulted from the activation of FAS signaling pathway that elicits the apoptosis of BC cells. Thus, this study shows that allograft animal model of BC is suitable for studying the effects of anti-cancer drug.

Conclusively, allograft models offer a more realistic environment to reconstruct aspects of the associated stromal and immune features. These models are versatile tools that recapitulate the interplay between the tumor microenvironment and cancer cells in disease progression^[20] (Table 1).

3.2. Xenograft animal models of BC

Xenograft animal models of BC are established by transplanting an organ, tissue, or cells to an individual of another species. Animal model constructed by transplantation of the primary human BC cells or established human BC cell lines into an immunodeficient animal is the most often used model for drug evaluation of human BC cells^[21]. There are two types of models of transplantable tumors: Orthotopic and heterotopic models. The human BC cell lines, including EJ, T24, BIU-87, 5637, RT4, HT1197, and HT1376, were widely used for the xenograft transplantation of BC animal models^[22-24].

Kuwada *et al.* inoculated BC cell T24 into scapular region subcutaneous tissue of BALB/c nude mouse ($1 \times$

Table 1. Characteristics of animal models of transplantable bladder cancer

Animal models	Host	Advantages	Disadvantages	References
Allograft animal model	Immunocompetent	<ul style="list-style-type: none"> • Time- and cost-effective • Reproducible characteristics of the model • Able to recapitulate of the interplay between tumor microenvironment and cancer cells depicted in disease progression 	<ul style="list-style-type: none"> • Inherent characteristics including tumor growth, latency, growth rate, invasion and metastasis may be different from those of human BC • The findings from allograft animal model are less likely to be translated compared to xenograft animal model 	[14,20]
Xenograft animal model	Immunocompromised and severely immunodeficient	<ul style="list-style-type: none"> • Time- and cost-effective • Reproducible characteristics of the model • Able to recapitulate the biological characteristics of the disease of origin • Can be used to evaluate a cancer chemosensitivity of a patient • Can be used to study tumor biology and behavior, and to evaluate new anticancer drugs before the initiation of human clinical trials 	Not suitable for studies on interactions between the host immune system and the tumor	[21,60]

10^7 cells) and tail vein of the mouse (1×10^6 cells), and established BC heterotopic and lung metastasis animal model, respectively^[25]. Cui *et al.* used 5 – 6-week-old female athymic nude mice and subcutaneously injected RT4 cell suspension containing 3×10^6 cells into each of the left and right flanks of the mice. When the tumor diameter was >3 mm after 28 days, *in vivo* therapeutic test was performed^[26].

Compared to allograft, the molecular properties of xenograft models are similar to clinical features of human cancer. Xenograft models enable analyses of clinical responses based on the unique characteristics of clinical patients. However, it is not suitable for studies on interactions between the host immune system and the tumor (Table 1).

Based on the inoculation site of tumor cell transplantation, the animal model can be divided into orthotopic transplantation model and heterotopic transplantation model^[27] (Table 2). Orthotopic transplantation model refers to the animal model established by injecting or implanting BC cells into the bladder cavity. This allows the investigation of tumor behavior in the organ-specific microenvironment. In contrast, the heterotopic transplantation model is the animal model established by implanting BC cells or tissues in an ectopic site other than the tissue of origin. This model is suitable to study different aspects of tumor growth, metastasis process, and tumor-stromal interactions^[28,29].

3.3. Animal models of orthotopic BC

The histological pathology and metastasis pattern in nude mouse with orthotopically transplanted BC can more accurately simulate these features of human BC than in heterotopic transplantation BC model^[30]. In recent years, the literature reported that orthotopic model established by infusion of cell suspension into the bladder cavity following injuring bladder mucosa by transurethral electrocautery or mechanical damage gives rise to higher tumor formation rate. For example, under ultrasonography guidance, Jäger *et al.* injected phosphate-buffered saline into bladder mucosa first in 50 nude mice^[31]. Next, the BC cell lines UM-UC1, UM-UC3, and UM-UC13 were percutaneously injected into the established spaces under bladder mucosa with guidance of ultrasound. Fluorescence imaging and ultrasound were used for regular surveillance of tumor growth and orthotopic transplantation models were successfully established in 50 nude mice^[31].

Using various imaging methods (such as transabdominal micro-ultrasound imaging [MUI], intravesical ultrasonography, fluorescence imaging, and so on), the results demonstrated that orthotopic transplantation tumor formation rate in immunodeficient mice was 93%^[32]. Tumors could be histologically detected 7 – 9 days after infusion and the transplantation tumor specimens were histopathologically confirmed as II – III grade bladder

transitional cell carcinomas. For example, Miyake *et al.* reported that CXCL1 promoted tumor progression in human BC by mediating interaction of cancer cells with tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs)^[33]. The orthotopic BC models were constructed by injecting BC cells UMUC3 (2×10^6) and TAMs or CAFs (1×10^6) into the bladder cavity of SCID mice. Two weeks after cell injection, the bladders of the mice were resected. The results revealed that CXCL1 production in TAMs/CAFs supported tumor implantation in the murine bladder wall, which was confirmed by clinical data of patients with BC. Thus, CXCL1 signaling in the tumor microenvironment is highly responsible for disease progression and drug resistance through enhanced invasion ability. Furthermore, 15 – 20 days after the inoculation of T24 cells into the bladder cavity of BALB/c nude mice, orthotopic animal model bearing a human bladder tumor was established with a tumor formation rate of 100%^[34].

Together, the establishment of the orthotopic mouse tumor model not only possesses high tumor formation rate and reproducibility but also provides ideal animal model for preclinical experimental research of BC. However, since the operation cannot be directly observed, it is difficult to control the area of injury^[35] (Table 2).

3.4. Animal models of heterotopic BC

Heterotopic transplantable models are established by injecting or transplanting human BC cells or tissues in an ectopic site instead of tissue of origin. These models are adopted to study the molecular signatures and pathomechanisms of BC, especially when the *in situ* inoculation is unavailable. This type of animal model is cost-effective, easy to establish and manage, and widely used in mechanistic studies as well as efficacy evaluation of novel therapeutic agents^[36].

In an experiment, Wu *et al.* anesthetized 4-week-old female BALB/c nude mice and injected 1×10^6 EJ cell suspensions into the sub-epithelial layers of nude mice bladders with an appearance of “semi-transparent bubbles”^[37]. When the tumors reached a diameter of 3 – 5 mm after 1 – 2 weeks, the inoculation process is considered successful^[37,38]. The established model was used to study the clinical value of intravesical resveratrol instillation for the treatment of BC^[37]. Tao *et al.* established xenograft mouse models of BC by injecting T24 cells (stably expressing luciferase reporter gene) in the left sub-renal capsule of the mice^[39]. This study showed that the mice which received T24 and HH cells displayed stronger metastatic luminescent signals located at distant organs compared with a group of mice that received T24 and HH cells as well as ASC-J9 treatment, and another group that received only T24 cells^[40]. In summary, heterotopic transplantable models are efficient, convenient, and easy to establish by injecting human BC cells for studying BC.

Table 2. The description of two types of xenograft bladder cancer models.

Animal models	Advantages	Disadvantages	References
Orthotopic animal model	<ul style="list-style-type: none"> • Able to preserve natural microenvironment • Allows the development of local and distant metastases • Representative of human characteristics in clinical settings 	<ul style="list-style-type: none"> • Requires more animals • Requires more detection devices to assess established tumors 	[62,63]
Heterotopic animal model	<ul style="list-style-type: none"> • Technically simple • Allows tumor detection via non-invasive means • Can be used to assess the efficacy of novel therapeutic agents 	<ul style="list-style-type: none"> • May lead to alterations of tumor microenvironment and the efficacy of anti-proliferative agents since different inoculation sites may affect the growth, metastasis, genetic expression of BC • Rarely metastasize and has a relatively limited blood supply 	[41,64,65]

Taken together, the sizes of tumor in animal models of heterotopic BC are easy to measure but the tumors may have different responses to drugs and different tumor microenvironment compared to the orthotopic location^[41]. Furthermore, it is not suitable for the study of metastasis, because it rarely metastasizes and has a relatively limited blood supply (Table 2).

4. Establishment methods of animal models of transplantable BC

4.1. Tissue implantation method

Tissue implantation method refers to transplanting human BC tissue into animal through subcutaneous injection to establish animal BC model. Typically, this injection targets the flank or hind leg of immunodeficient mice. Park *et al.* transplanted BC tissues from 65 patients into immunodeficient mice (BALB/C-nu) subcutaneously and 15.4% of the mice successfully developed tumors^[42]. Histological analysis showed that transplantation tumor and original tumor specimen possessed similar histological features. This was verified by gene sequencing analysis confirming that the gene sequences of transplantation models are identical to those of original tumor tissue specimens. Furthermore, the mutation analysis revealed that four transplantation and original tumor tissue specimens had the mutations in *TP53*, *HRAS*, *BRAF*, and *CTNNB1* genes^[42]. Since patient tumor tissue possesses higher value in the establishment of a BC animal model, it is useful for the elucidation of occurrence and development of tumor and exploration of new therapeutic methods. Besides, this method is easy and cost-effective. Nevertheless, the tissue implantation method is limited by tissue damage and infection in mice. In addition, the inoculation amount is difficult to control, and athymic animals which usually have a relatively short life can seldom survive and proceed to next stage of experiments. Another shortcoming is the

insufficient blood supply in the transplanted tissue that slows down tumor growth, resulting in lower transplant rate^[43].

4.2. Cell suspension injection method

4.2.1. Subcutaneous tumor model

Cell suspension injection method is a method of injecting cell suspension consisting of human BC cell line cultured *in vitro* into an animal to establish animal bladder model. Gong *et al.* established an animal model of transplantable BC by irradiating CB-17 SCID mice with a radiation dose of 3.5 Gy and each mouse was injected with 3×10^6 BC cells BIU-87^[44]. After 6 weeks of transplantation, human CD3⁺ cells, with an average ratio of 19%, and human immunoglobulins IgG, with an average concentration of 532.4 µg/mL, appeared in the peripheral blood of mice injected with 5×10^7 human lymphocytes. This result demonstrated that cell suspension injection not only could establish animal model of BC but also help investigate human immune responses and evaluate immunotherapeutic approaches. The successful engraftment of the human BC xenografts and the establishment of the human immune system *in vivo* model may provide a useful tool for the development of novel therapeutic strategies targeting BC. However, this method causes minor injury to the animal model. Moreover, the cell suspensions can easily extravasate through the needle lumen, thus it is difficult to control the exact number of BC cells inoculated. In addition, the tumor growth rate at different inoculation site also differs due to different blood supply at different sites^[45].

4.2.2. Orthotopic tumor model

Intravesical infusion method is a method of infusing cell suspension made of BC cells cultured *in vitro* into the bladder cavity of an animal through catheter or urethra for

the establishment of an animal BC model. The formation rate of tumor induced by intravesical infusion method can be affected by many factors, including the number of BC cells inoculated, time taken for cancer cells to come into contact with bladder mucosa, and the status of the bladder mucosa^[46]. Patel *et al.* pointed out that tumor formation rate can be increased by clamping indwelling bladder catheter of anaesthetized animal for a period of time to prevent the outflow of cancer cells, thereby increasing their contact time with bladder mucosa^[47]. Besides, Jankun *et al.* successfully established an orthotopic animal model of BC by injuring the bladder mucosa through cauterization, followed by the injection of cell suspension consisting BC cell line AY-27 cultured *in vitro* into bladder cavity^[15]. On the other hand, Lee *et al.* used pre-treatment method to evaluate the effectiveness of different approaches in tumor formation. After being subject to pre-treatment with electrocautery or hydrochloric acid (HCl), each syngeneic C3H/He mice were inoculated with 1.2×10^6 MBT-2 cells (a mouse BC cell line)^[48]. The histopathological results showed that the tumor formation rates for the mice that underwent electrocautery and HCl pre-treatment were 54% and 100%, respectively. Further pathological examinations confirmed that all transplantation tumors were high-grade papillary urothelial carcinoma. The orthotopic murine BC model is ideal for the evaluation of novel intravesical therapy. However, on account of the low tumor take rate, several modifications have been proposed. HCl pre-treatment is the preferred method for establishing murine BC model which can be used to evaluate further therapeutic interventions. Other literature reported that trypsin can be used to disrupt the glycosaminoglycan of the bladder mucosa. Hence, the tumor formation rate of bladder infusion with transplantation tumor is significantly improved following the injury of bladder mucosa^[49].

5. Methods for monitoring transplanted tumor formation

5.1. Transabdominal MUI

Animal imaging for bladder tumors *in vivo* can provide valuable information, such as location, size, and stage of tumor. In addition, non-invasive imaging potentially protects mice with no bladder tumors from being hurt by receiving unnecessary treatments. Numerous noninvasive imaging modalities have been reported previously, and one of the examples is transabdominal MUI which is a non-invasive real-time modality for detecting bladder tumors in mice. MUI uses higher frequency ultrasound (40 Hz) as compared with conventional ultrasound (2 – 4 Hz). Shorter spatial pulse lengths which are caused by higher frequencies can result in images with high resolution and high clarity. Real-time, non-invasive imaging of bladder tumors can be visualized and followed up for progression and/or regression during treatment. After the injection of 5

$\times 10^5$ MBT-2 cells into the bladders of female C3H/He mice through a catheter, Patel *et al.* found that the final tumor formation rate was 45% when MUI and stereomicroscopy were used for the evaluation of the size and volume of bladder tumors^[47]. Comparison of data on tumor size monitored by abdominal MUI and stereomicroscopy suggested that measurement results were relatively similar. The results demonstrated that transabdominal MUI is equipped with a high degree of accuracy in detecting histopathologically confirmed transplanted tumors, with the smallest detectable volume of 0.52 mm^3 and an average volume of 0.95 mm^3 . Transabdominal MUI is an effective and non-invasive means for monitoring orthotopic transplantation tumor in mice as it can provide real-time and high-resolution *in vivo* imaging for bladder tumor monitoring.

5.2. Intravesical ultrasonography

Intravesical ultrasonography is based on an intravascular ultrasound system. It appears to be a promising approach for characterizing a vessel wall since the technique enables visualization of the transverse planes of vessels with a spatial resolution at a level of sub-millimeter. Hence, the catheter-based system has been used as evaluation method in an animal model of vascular disease^[50]. Satoh *et al.* pre-treated the bladders of Fisher 344 rats with HCl and injected AY-27 cell suspension into bladder cavities of the rats to establish orthotopic rat models of transplanted BC^[51]. After 7 – 10 days of transplantation, the sizes and invasion depths of transplantation tumors were measured transurethrally using ultra-thin (2.5 Fr) intravesical ultrasound catheter, which accurately determined the size of bladder tumor in which the smallest observable diameter was 0.5 mm, and the depth of tumor invasion. Intravesical ultrasonography allows clear observation of the layer structure of bladder wall, in addition to accurately illustrate determination of bladder tumor size and invasion depth. In addition, intravesical ultrasonography could achieve a positive predictive value of almost 85% in the assessment of tumor stage, and the examination results obtained from this technique were reproducible. However, to monitor tumor formation, this imaging system requires special equipment, such as intravascular ultrasound devices.

5.3. Fluorescence imaging

Fluorescence agents which are used as the contrast agents can be administered intravesically or intravenously without engendering high toxicity^[52]. Confocal laser endomicroscopy (CLE) is an optical technology that provides high resolution microscopy of mucosal lesions. Given high spatial resolution sufficient to resolve tumor microenvironment and cellular features, CLE is capable of showing the characters of BC based on fluorescence agents^[53]. Fazel *et al.* pre-treated bladder cavities of nude mice with electrocautery, and injected luciferase-transfected

human BC cells EJ28 into bladder cavities of nude mice^[54]. Bioluminescence imaging technique was used to monitor tumor growth. Model constructed was used to evaluate the potential of new radioimmunotherapy for the treatment of BC^[54]. After transfecting highly invasive BC cell line EJ with green fluorescent protein (GFP) plasmid, Yang *et al.* screened for cell clones that could stably express an enhanced GFP (EGFP)^[55]. The cell clones were cultured and infused into bladders of nude mice. The whole-body fluorescence imaging system was used to monitor growth, invasion, and metastasis of tumor^[55]. Since transfected EJ cells could stably and efficiently express EGFP in nude mice, the growth, invasion, and metastasis of transplantation tumors could be accurately observed by fluorescence microscopy. Taken together, fluorescence imaging is a feasible and simple means to investigate *in vivo* metastasis mechanisms of transplanted bladder tumor based on fluorescence expression.

5.4. Nanoparticles-based magnetic resonance imaging (MRI)

MRI is a cross-sectional imaging technique that has been proven to be useful for detecting malignant tumors^[56]. The introduction of iron-oxide particles that improves imaging resolution facilitates examination of BC easily. Cho *et al.* conjugated VEGF121/rGel with MnFe_2O_4 nanoparticles (MNPs) to form VEGF121/rGel-MNPs complexes; they were dissolved in biological medium and used as a contrast agent in MRI examination for inspection of tumor formation in orthotopic mouse BC model^[57]. Results showed that VEGF121/rGel-MNPs could provide accurate positioning of tumor besides displaying vascular distribution and anatomical details of tumor tissue in clear image. Immunohistochemistry studies showed that VEGF121/rGel-MNPs could specifically target blood vessel of the tumor, indicating that a contrast agent containing VEGF121/rGel-MNPs complexes can effectively improve the sensitivity of MRI for the examination of BC in mice.

5.5. Ultrathin cystoscopy

Orthotopic bladder tumor models are invaluable experimental tool for assessing potential therapeutic effects on BC. However, the therapeutic effects of anti-tumor drugs could only be evaluated when laparotomy or cystectomy is performed. To solve this limitation, non-invasive diagnosis for animal superficial bladder tumor should be applied. Besides MRI, ultrathin cystoscopy has been reported for its application in noninvasive diagnosis of bladder tumor^[58]. In an experiment, ultrathin cystoscopy was used to transurethrally observed tumor growth in the bladder cavities of Fisher 344 rats that had been pre-treated with a weak acid and infused with AY-27 cell suspension^[59]. Through the use of ultrathin cystoscopy on the orthotopic transplantation rat model of BC, the tumor grew to a diameter of 0.75 mm after 5 – 14 days of transplantation.

Tumors were successfully formed in all rats 7 – 10 days after transplantation, most of which were superficial. Ultrathin cystoscopy could examine urethra and bladder in all directions, thus providing accurate positions and observation of tumor, including papillary protrusion with a diameter of 1 mm.

6. Advantages and disadvantages of animal models of transplantable BC

The animal models of transplantable BC could not only be utilized in the study of tumor proliferation process but also applied in revealing the mechanisms of occurrence and metastasis. In short, careful consideration and planning are required to generate stable and uniform animal models of transplantable BC to ensure successful generation of *in vivo* model for the experimental purposes.

The animal models of transplantable BC have high tumor formation rate and short experimental cycle. Besides, this animal model can be generated by batch and the heterogeneity of tumor formation between each batch is little^[60]. Despite the transplanted tumor retains most biological characteristics of human BC, this model has a more rapid growth rate and shorter tumor volume doubling time relative to human BC^[60].

Despite the advantages, it is important to understand the challenges of using animal models of transplantable BC so that researchers can make better informed decision before beginning an experiment. Researchers should be aware that the breeding of immunodeficient animals requires a clean and sterile environment. Therefore, the maintenance cost of a pathogen-free animal room is high. At the molecular level, it is noteworthy that the interstitial spaces of BC tissue may carry components of immunodeficient animal which may potentially affect the validity of experiment results^[61].

7. Conclusion

The development in transplantation technologies and the generation of immunodeficient mice has broadened the application of cancer cell transplantation in animal models. This has facilitated the research on multiple aspects of BC development, metastasis, and recurrence. Furthermore, the transplanted animal models can be used in the investigation of the cancer cell regulations in tumor microenvironment, shedding light on the interactions between tumor cells and other cell types to a greater extent. However, several limitations such as the heterogeneity in tumor formation and the variation in transplantation efficiency rate should be noted and optimization is therefore required. Nevertheless, with the successful generation of animal model of stably transplanted BC, researchers can carry out more extensive experiments encompassing the discovery of targeted therapy, tumor-drugs interaction studies, and drug response prediction.

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Conflicts of interest

The authors declare no potential conflicts of interest.

Author contributions

Z.Y. and C.L. conceived the idea of this review. Z.Y. and Z.S. wrote the paper. F.Z. and L.W. revised the paper.

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