Cancer+

REVIEW ARTICLE

Spontaneous and Chemically-induced Bladder Cancer Animal Model

Zhao Yang[1†], Zongyi Shen[1†], Nan Zhang[1], Suhang Bai[1], Luyao Wang[1], Faiza Naz[1], Manxia Chang[1], Tiantong Chen[1], Changyuan Yu[1]*, Chong Li[2]*

[1]College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China

[2]Core Facility for Protein Research, Institute of Biophysics, Chinese Academy of Sciences, Beijing 10010, China

[†]These authors contributed equally to this work.

Abstract: Investigations using animal model systems have been enlightening us with the biology underpinning the development of bladder cancer besides strengthening the existing therapy to improve the clinical outcome in patients. To date, spontaneous and chemical inductions are the classical methods in the generation of animal models of bladder cancer. Attributed to many benefits such as simple protocols and lower maintenance cost, these animal models are widely applied in the investigations of bladder cancer pathogenesis and screening of therapeutic drug. In this review, we give an overview of spontaneous- and chemically-induced bladder cancer animal models accompanying by the pros and cons of these two types of models. Furthermore, various chemical carcinogens used in the induction are discussed with the potential benefits and pitfalls in the establishment of animal models. This review will provide insightful information about the selection of the correct method in establishing the animal models of bladder cancer which are instrumental for studying potential therapeutic agents that target bladder cancer.

Keywords: Bladder cancer, Animal model, Pathogenesis, Drug screening, Chemical carcinogen

Received: January 3, 2020
Accepted: March 7, 2020
Published Online: March 13, 2020

***CORRESPONDING AUTHORS**
Chong Li,
Email: lichong@moon.ibp.ac.cn
Changyuan Yu,
Email: yucy@mail.buct.edu.cn

CITATION
Yang Z, Shen Z, Zhang N, *et al*., 2020, Spontaneous and Chemically-induced Bladder Cancer Animal Model. *Cancer+*, 2(1):11-20.
DOI: 10.18063/cp.v2i1.272

1 Introduction

To mimic human diseases, animal models have been utilized in decades for the acquisition of disease mechanisms and discovery potential drug targets. This inevitably has helped the clinicians and researchers in understanding the etiology, pathogenesis, diagnosis, and treatment of many human diseases[1]. Despite the simulation, the biological characteristics and clinical characteristics, as shown in the animal models, cannot entirely represent the real picture of the disease in human. Therefore, translation of *in vivo* studies from the bench to the bedside for further validations is warranted[2].

At present, the mouse model is the most popular animal model used for modeling human diseases. Especially in oncology research, inbred mice (e.g., C57BL/6, C3H/He, BALB/c, and DBA/2J), outbred mice (e.g., National Institutes of Health, Institute of Cancer Research and Kunming mouse), mutant mice (e.g., severe combined immunodeficient

mouse and nude mouse), inbred rats (e.g., Brown Norway and Fischer 344 rats), and outbred rats (e.g., Wistar and Sprague Dawley [SD]) models are usually utilized[3-5]. The studies using this animal model have considerably contributed to the discovery of underlying mechanisms of cancer development and prediction of drug efficacy. Besides, animal model research also facilitates the discovery of biomarkers in human diseases[6].

In this review, we provide an overview of the animal models used for the study of bladder cancer. Besides, spontaneous and chemical induction which are the classical methods for establishing animal models of bladder cancer are discussed. This review highlights various chemical induction methods besides the benefits and potential pitfalls of this cancer modeling method. Therefore, this review provides insightful information for researchers who want to establish animal models of bladder cancer.

2 Significance of animal models of bladder cancer

As the most common urothelial malignancy among men, bladder cancer has implicated an estimation of 430,000 cases yearly[7]. Besides being spatially multicentric and temporally recurrent, bladder cancer is prone to local invasive metastasis and associated with a high postoperative recurrence rate. Therefore, the study on the occurrence and development mechanism of bladder cancer forms the basis for early detection and diagnosis of bladder cancer[8].

Previously, *in vitro* experiments provided invaluable knowledge in the biological characteristics of bladder cancer cells. Nevertheless, the tumor initiation, progression, and metastasis of bladder cancer in the body are not well investigated. Therefore, the elucidation of cancer cell regulation in tumor microenvironment will be a plus point through the study in animal models. The similarities between animal models and human, such as genetic constitution, anatomy and physiology, make the animal model as an ideal tool for experimental research. Thus, this will bridge the gap between *in vitro* experimental and clinical settings[9]. Of course, the results obtained from animal models are not representative of the exact human conditions due to the differences in body composition. Therefore, these results need further verifications in clinical trials[10].

3 Characteristics of an ideal animal model of bladder cancer

To establish a stable and uniform animal model of bladder cancer, careful consideration needs to be taken to simulate cancer and its biological characteristics in the animal model with a higher accuracy so that the identification of potential drug candidates can become more accurate[6].

The cancer-induced in the animal model should be bladder-specific and cause limited adverse effects on other tissues and organs. In addition to being easily available, low cost and easy maintenance, an ideal animal model should take a relatively short period for tumor formation. Most importantly, the pathological and biological characteristics of formed tumors should be similar to human bladder cancer[11]. Hence, these aforementioned factors should be considered before establishing animal models to ensure high efficiency, high reproducibility, controllable, and reliable experiments.

4 Animals commonly used for the establishment of a bladder cancer animal model

The most commonly used animal models for the establishment of bladder cancer are mice, rats, guinea pigs, rabbits, and dogs[12]. These animal models are ideal for use in research because they are widely available and sensitive to carcinogen induction. Apart from low cost and easy maintenance, these animals take a relatively short time for tumor formation.

5 Common methods for monitoring tumorigenesis in animal models of bladder cancer

For examining tumorigenesis in an animal model, the most common methods used include pathological examination, palpation of lower abdomen, acridine orange (AO) staining, and imaging techniques. These provide basic

information on the carcinogenesis of bladder cancer.

Pathological examination remains the gold standard for tumor examination. However, the animals must be sacrificed, followed by a pathological examination conducted by an experienced researcher[13]. Palpation of the lower abdomen is a method that deduces the tumor formation through hand palpation[14]. Although it is simple to perform, this technique poses a difficulty in assessing the size, stage, grade, proliferation, and metastasis of the tumor.

Apart from that, AO staining of exfoliated cells in 24-h urine specimen can be used to detect the presence of bladder cancer cells. The presence of reddish-orange fluorescence in the cells under a fluorescent microscope indicates the presence of bladder cancer cells[15]. Nevertheless, this method is limited by its low positive rate and high false-positive rate.

Imaging systems such as computed tomography (CT) scan, intravesical ultrasound, and magnetic resonance imaging (MRI) are always used in diagnosing and monitoring cancer development. Johnson *et al*. applied CT imaging to monitor the bladder cancer tumorigenesis in UPII-SV40T transgenic mice, which expressed SV40T antigen specifically in the urothelium and reliably developed noninvasive bladder cancer[16]. Exophytic bladder cancer is not detectable by CT imaging at time points earlier than 24 weeks of age, although carcinoma *in situ* is present as early as 4 weeks. Due to a small-sized sample of the animal model, CT scan is difficult to provide accurate information about the position and morphology of the lesion[17,18].

Ultrasonic wave is a kind of sound wave whose frequency is higher than 20,000 Hertz. Ultrasound imaging applies the use of ultrasonic beam scanning over the body to obtain images of internal organs through the reception and processing of reflected signals[19]. Patel *et al*. used micro-ultrasound imaging (MUI) to identify the formation of tumors in 15 of 33 C3H/He mice (45%), which were anesthetized with 70 mg/kg ketamine and 5 mg/kg xylazine, and 5×10^5 murine bladder tumor cells were transurethrally injected into the bladder with a syringe attached to the catheter[19]. The smallest confirmed tumor on MUI that was detected was only 0.52 mm^3 and the mean tumor volume was 0.95 mm^3. Measurements of tumor size by MUI and gross microscopy had a high correlation coefficient ($r = 0.97$). MUI recognized all the tumors, which were confirmed by pathological examination. Taken together, transabdominal MUI is an invaluable tool in translational studies that involve orthotopic mouse models of bladder cancer, as this imaging technique provides real-time, high resolution *in vivo* images of bladder tumors. In an experiment, Glaser *et al*. applied an MR sequence approach to monitor the growth of bladder cancer-induced by the N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen[20]. Based on the image obtained from MRI, the bladder wall and tumor area could be analyzed and calculated, and this measurement correlates with *ex vivo* bladder weight and tumor stage. Thus, MRI enables quick and reliable assessment of tumor burden before dividing animals into the control group and experimental group randomly in studies. Taken together, the combination of these examinations is essential to confirm and verify the presence of tumor cells in these animal models of bladder cancer.

6 Animal models of spontaneous bladder cancer

The term "spontaneous tumors" refers to tumors that arise in mice which have never been deliberately exposed to any carcinogenic agent and which are of strains not known to harbor any vertically or horizontally transmitted oncogenic virus; that is, the subsequent discovery of the implication of such an agent in a mouse strain would require recategorization of the tumors arising in it[21].

In animal models of spontaneous bladder cancer, cancer naturally occurs in the experimental animal population or tumor formation is preserved by genetic breeding. Some researchers have conducted in-depth and extensive research on spontaneous transitional cell carcinoma of bladder in canine model. The results showed that spontaneous transitional cell carcinoma of

bladder in dogs is highly similar to human bladder cancer in many aspects, such as the proportion of bladder cancer in malignant cases, incidence of bladder cancer, risk factors, pathological features, gene expression profile, invasiveness and metastasis of tumor, and response to the therapeutic drug[18,19,22-24]. However, the location of tumor in the bladder is different from human. Bladder cancer in dog is prone to occur in the trigone of bladder, while human bladder cancer can occur anywhere in the bladder cavity.

In addition, a spontaneously appearing bladder cancer was firstly observed in Nb rat over a 15-month period[25]. In another study, Moorselaar *et al.* carried out a cohort of 300 ACI rats and found two spontaneous bladder cancers, namely, RBT323 and RBT157[26]. The aforementioned spontaneous bladder cancer cells were serially transplantable, and they harbored different capabilities of metastasis to lung[26]. Furthermore, rat spontaneous bladder cancers were similar to human bladder cancer in histological pattern, gene expression pattern, and gene mutation profile[26], thereby making rats an ideal model of bladder cancer for treatment evaluation[25].

Taken together, the advantage of using a spontaneous animal model of bladder cancer in the experimental studies is that cancer occurs completely under natural conditions. Besides, the occurrence and development of tumor are very similar to human bladder cancer. This aids in investigating the role of environmental factors, carcinogenic factors, and genetic factors in the development of bladder cancer and the therapeutic effect of drugs. However, there are some disadvantages of this model in experimental research. For instance, the difference in growth rate between individuals is large. Besides, there is no uniformity in the occurrence and development of bladder cancer; thus, it is difficult to obtain a large quantity of tumor-bearing animals with uniform tumor growth within a specified time. Furthermore, the establishment of animal models of spontaneous cancer is associated with a long establishment cycle, complex process, low reproducibility, high animal demand, and high maintenance cost.

7 Animal models of chemically-induced bladder cancer

Animal model of chemically-induced bladder cancer is established by inducing bladder cancer in the experimental animal population using various carcinogenic factors. The bladder tumor can be induced in various animals using chemical carcinogens, physical factors, and biological factors. In 1937, Hueper was the first who reported the successful induction of bladder cancer model in a dog using β-naphthylamine. In 1964, Druckrey *et al.* successfully induced bladder tumor in an animal model by feeding the animals with water pre-mixed with BBN for 40 weeks[27]. In 1967, Ertürk reported the establishment of an animal model of bladder cancer which was induced by N-[3-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT)[28]. In 1972, Hicks *et al.* instilled N-methyl-N-nitrosourea (MNU) into the bladder cavity and induced bladder cancer[29]. During the establishment of bladder cancer-induced by chemical carcinogen, the histopathological change of bladder mucosa of the animals can be roughly divided into three phases, namely, simple proliferative phase, atypical proliferative phase, and cancerous phase. Although the majority of chemically-induced tumors are low grade[30], the chemical carcinogens have an important role in the induction of cancer in the animal model. This helps in future research in delineating the molecular pathogenesis of bladder cancer and developing novel therapeutic strategies in treating bladder cancer.

7.1 BBN-induced bladder cancer

BBN is closely associated with the occurrence of human bladder cancer as its carcinogenesis mechanism is similar to that of smoking-induced bladder cancer[5]. N-butyl-N-(3-carboxypropyl) nitrosamine, a metabolite of BBN, is excreted in the urinary system, so it can directly come in contact with urothelium. However, their encounter would implicate damage to DNA in the urothelial cells and eventually leads to bladder cancer induction. In experimental research, the conventional method to induce bladder cancer development involves feeding the nude mice with 0.05% BNN dissolved

in water. However, this method is superseded by the gavage method which offers higher accuracy in controlling the carcinogen dose. Scientists had used this method to feed the female SD rats of 6–8 weeks old with 0.05% BBN in water and used the ultrasound imaging to monitor tumor formation. Tumor growth was detected within 3 weeks with the confirmation of histopathological examination and bladder cancer was successfully induced in all experimental rats, recording a tumor formation rate of 100%[31].

In another study, He *et al.* showed that BBN caused a high level of mutagenesis, specifically in the epithelial cells of the urinary bladder in Big Blue® mice containing *lacI* gene[32]. Specifically, the mutation frequencies in urothelial cells of mice were about two orders of magnitude greater than the spontaneous mutation background, and no appreciable mutagenesis was observed in kidney, ureter, liver, or forestomach. Although there is an elevation of BBN-induced mutagenesis in urothelial cells of rats, this level of mutagenesis was not as profound as in the mice. This might explain why rats are less prone than mice to the establishment of bladder cancer-induced by BBN. These results demonstrated that BBN induction is a novel method for initiating the growth of bladder cancer because urothelial cells are highly susceptible to BBN.

To reveal whether BBN-induced model mimics human bladder cancer at the molecular and mutational level, Fantini *et al.* analyzed gene expression and mutational landscape of the BBN model by next-generation sequencing followed by a bioinformatic comparison to human bladder cancer[33]. The analysis of sequencing data indicated that BBN tumors expressed markers of basal cancer subtype such as *Cd44*, *Cdh3*, *Krt14,* and *Krt5*. In addition, *Trp53 (*80%), *Kmt2d* (70%), and *Kmt2c* (90%) were frequently mutated in BBN-induced tumors. These results revealed several similarities between human bladder cancer and the BBN-induced cancer in mouse model, providing a strong rationale for its use in molecular and drug discovery studies.

There are some benefits of utilizing BBN as a mean of bladder cancer induction in animal models. Apart from having high similarity to human bladder cancer[33], an animal model of BBN-induced bladder cancer is immunocompetent and easy to establish. Thus, this allows the investigations of immune responses to bladder cancer in this model.

7.2 FANFT-induced bladder cancer

FANFT is usually pre mixed in food before feeding to an animal. Similar to BBN, FANFT is one of the chemical carcinogens contained in tobacco. Several studies showed that this chemical agent is more likely to induce urothelial cancer in rodents[34]. With a typical concentration of 0.2% FANFT, an animal model of bladder cancer was successfully established, and researchers also found that simultaneous administration of indomethacin and FANFT by feeding could effectively enhance carcinogenicity of FANFT in rats[35]. However, reports on FANFT induction of bladder cancer are relatively scanty as BBN is a more favored carcinogen for induction.

7.3 MNU-induced bladder cancer

Being a nitrosamine of nitroso compounds, MNU acts as a direct carcinogen that induces tumor by causing DNA damage through the methylation of guanine molecules in nucleic acid. MNU is administered by intravesical instillation, and standardized operation is of great significance to ensure a high level of resemblance in animals throughout the model establishment process. Pathological and morphological features of MNU-induced bladder cancer are similar to those of human bladder cancer as they are transitional cell carcinomas originating from the mucosal epithelium.

Hicks *et al.* found that single instillation of MNU into the bladder cavity could cause extensive necrosis and ulceration of bladder mucosa, but not cancer. Following that, MNU was administered twice a week with 1.5 mg each time, resulting in the establishment of an orthotopic mouse model of bladder cancer 2 weeks later with a tumor occurrence rate of 100%[29]. Furthermore, Ferrari *et al.* successfully established an animal model of induced non-muscle invasive bladder cancer through intravesical injection of 1.5 mg/kg of

Table 1. Characteristics of animal models of bladder cancer

Mode of establishment	Host	Characteristics		References
		Advantages	**Disadvantages**	
Spontaneous	Dog	• Cancer occurs completely under natural conditions • The pathological features, gene expression profile, invasiveness and metastasis of tumor, and response to the therapeutic drug are highly similar to human bladder cancer • This establishment mode allows the development of novel drugs and disease management strategies.	Tumor development takes a long time	[22-24]
BBN-induced	Mouse, rat	• The pathological features, gene expression, and mutation profile are similar to human bladder cancer • The animal model is easy to establish and takes a short duration to develop tumor • The animal model is associated with a high rate of tumor formation (up to 100%) • The immune system of the animal model is intact after induction.	• The location of tumor growth in the bladder is different from human • Tumor growth rate varies among individuals • There is no uniformity in the occurrence and development of tumor • This establishment mode is associated with a complex process, low reproducibility, high animal demand, and high maintenance cost.	[5,33]
FANFT-induced	Rodents	The pathological features of transitional cell carcinoma are similar to human bladder cancer	• This establishment mode is associated with a low rate of tumor formation (20%) • Moderate and severe hyperplasia and hydronephrosis can be resulted • This establishment mode can lead to breast cancer and transitional cell carcinomas of renal pelvis.	[34,35,44,45]
MNU-induced	Rats	• The pathological features of tumor-induced by MNU are similar to human bladder cancer • The animal model is associated with a high rate of tumor formation (70–100%) • Tumor induction is manageable	• Intravesical instillation that involves insertion of thin catheter into bladder may damage the urethra and cause urethral infection or bleeding. • This establishment mode can result in urethral injury, urinary tract infection, and secondary stage of bladder calculi.	[29,36,37]

MNU to 7-week-old Fischer rats, once every other week for 6 consecutive weeks[36]. On the other hand, Li *et al.* found that at 18th week, tumor occurrence rate of 6-week-old female Wistar rats treated with MNU alone was 70.6%, while the tumor occurrence rate was only 22.2% in the rats

treated with MNU and fisetin (200 mg/kg)[37]. Fisetin is a type of flavonoid that can induce apoptosis of bladder cancer cells by activating p53 and inhibiting NF-κB pathways.

Induction of bladder cancer using MNU in an animal model is a favorable method because the establishment technique is straightforward and tumor occurrence rate can be manipulated as tumor formation follows a dose-dependent manner. Therefore, the control of tumor induction is manageable. Besides, the metabolic half-life of MNU in the body is relatively long, which is beneficial for tumor formation in animal models. Nonetheless, MNU is toxic and the direct administration through repetitive intubation can easily lead to urethral injury, urinary tract infection, and secondary stage of bladder calculi.

7.4 Multiple drugs-induced bladder cancer

At present, there are many chemical carcinogens that can induce bladder carcinoma *in situ*. However, most of them contribute to low tumor formation rate, and associated with long carcinogenesis cycle when the chemical is administered alone, which limits their applications to some extent. This is evident in a study conducted by Nakanishi *et al.* which found out that administration of sodium saccharin following BBN in Fischer 344 rats significantly enhanced the induction of bladder hyperplasia in comparison to administration of BBN alone, indicating the complementary potential of saccharin in the induction of early-stage bladder lesions[38]. Complementary role in carcinogenesis of auxiliary drugs was also found in another study conducted by Cohen *et al.*[39] In this study, male Fischer rats were fed a diet containing 0.2% FANFT for 6 weeks, followed by 5% sodium saccharin or 2% DL-tryptophan for another 6 weeks. Feeding FANFT followed by sodium saccharin significantly elevated the incidence of bladder tumors (83.5%) compared to feeding FANFT only (20.00%). On the other hand, incorporation of DL-tryptophan in the diet following FANFT feeding also significantly elevated the incidence of bladder tumors (51.32%) compared to feeding FANFT only (20.00%). Thus, saccharin and tryptophan might act as tumor-promoting agents during bladder carcinogenesis.

Besides, carcinogenic agents such as sodium L-ascorbate (SA)[40], sodium saccharin[41], butylated hydroxyanisole (BHA)[40,42], and epidermal growth factor (EGF)[43] can significantly shorten tumor induction time when they were used in combination with MNU. Particularly, the incidence of hyperplasia in the epithelium of bladder was higher in Fischer 344 rats treated with SA and MNU (95.0%) compared to that in the control group treated with MUN alone (64.7%)[40]. BHA enhanced the incidence of hyperplasia in the epithelium of bladder in Fischer 344 rats treated with MNU (55%–100%) relative to that in Fischer 344 rats treated with either BHA or MNU alone in the control groups (0%)[42]. Similarly, EGF could also increase the incidence of hyperplasia in the epithelium of bladder in Fischer 344 rats treated with MNU (21%) in comparison to that in Fischer 344 rats treated with MNU alone in the control group (12%)[43]. These findings suggest that the appropriate combination of multiple chemical carcinogens can effectively increase tumor formation rate in animal models.

7.5 Advantages and disadvantages of animal models of chemically-induced bladder cancer

Using carcinogen to establish animal of model bladder carcinoma *in situ* has many advantages. For instance, the tumor-induced using this method is urothelium-selective and the carcinogenesis rate is high which is up to 100%. Most importantly, the pathological features, gene expression, and mutation profile of the induced tumor are similar to human bladder cancer, which provides an ideal model to study the occurrence, progression, and drug-resistance of this cancer. Furthermore, the time taken for tumor formation is relatively short and usually can be completed within a few weeks. Thus, a large number of animal models with a tumor could be produced in a short period. Furthermore, the intact immune system after chemical induction method makes it possible to explore the function of tumor microenvironment and tumor-infiltrating lymphocytes, and the susceptibility of bladder cancer to immunotherapy (**Table 1**).

However, researchers who work animal models of carcinogen-induced tumors with still encounter several drawbacks. Due to the high mortality rate caused by carcinogens, the cost for the model establishment will be increased directly. It should also be noted that chemical carcinogen could lead to distinct histological changes in various animal types or even in different individual animals from a specific stain. For example, FANFT could be applied in the establishment of bladder cancer in rodents. However, BBN is suitable for both genders of mice and rats, although male rodents are commonly used in this regard. MNU is only suitable for rats since the urethra of mice is too thin for catheterization. In general, female rats are preferred for their anatomic structure (**Table 1**).

Taken together, the use of different induction agents and genetic backgrounds of animals can result in differences such as location, timing, and number of tumor lesions among individuals. In addition, the characteristics of most bladder cancer established by artificially defined methods are different from those of naturally occurring bladder cancer found in a clinical setting. Therefore, to perform cancer induction through chemical means, attentive measures in optimization, and close monitoring of the establishment of animal models need to be taken to generate a stable and uniform model for research. Furthermore, researchers should carefully select the suitable methods for animal model establishment according to research purpose.

8 Conclusion

Bladder cancer is a heterogeneous disease that requires intensive clinical care for the patients. With years of research and discovery, many advanced techniques have been developed for establishing animal models of bladder cancer, these include the genetically engineered animal models and animal models with patient-derived xenograft transplant. However, the spontaneous tumor growth and chemical inductions remain the classical techniques in the generation of animal models of bladder cancer attributed to their simple and well-established protocols. However, these animal models cannot utterly resemble human cancer due to the difference in genetic background and environmental factors. Bladder cancer animal models provide an avenue for carrying out an in-depth study of bladder cancer development, progression, metastasis, and recurrence, which collectively form the foundation of bladder cancer biology. It is noteworthy that the exploration of bladder cancer biology also allows more evidence-based investigations of potential therapeutic drugs. Taken together, establishing animal models of bladder cancer represent the primer that spawns investigations entailing a deeper understanding of bladder cancer pathogenesis and development of novel therapeutic strategies.

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities (No. buctrc201910), Beijing-Tianjin-Hebei Basic Research Cooperation Special Project (19JCZDJC65800(Z)) and the grant from the Ministry of Science and Technology of China (2010ZX09401-403).

Conflicts of interest

The authors declare no potential conflicts of interest.

Author contributions

C.L. and Z.Y. conceived the idea of this review. C.L., Z.Y. and Z.S. wrote the paper. N.Z., S.B., L.W. F.N., M.C. T.C. and C.Y. revised the paper.

References

1. Jackson SJ, Thomas GJ, 2017, Human Tissue Models in Cancer Research: Looking Beyond the Mouse. *Dis Model Mech*, 10:939–42. DOI: 10.1242/dmm.031260.
2. Cekanova M, Rathore K, 2014, Animal Models and Therapeutic Molecular Targets of Cancer: Utility and Limitations. *Drug Des Dev Ther*, 8:1911–21. DOI: 10.2147/dddt.s49584.
3. Braida D, Ponzoni L, Matteoli M, *et al*., 2016, Different Attentional Abilities among Inbred Mice Strains Using Virtual Object Recognition Task (VORT): SNAP25$^{+/-}$ Mice as a Model of Attentional Deficit. *Basic Clin Pharmacol*

Toxicol, 296:393–400. DOI: 10.1016/j.bbr.2015.08.016.

4. Ito H, Aizawa N, Fujita Y, *et al*., 2014, Long-Term Caloric Restriction in Rats May Prevent Age Related Impairment of *In Vitro* Bladder Function. *J Urol*, 193:2123–30. DOI: 10.1016/j.juro.2014.11.005.
5. Vasconcelos-Nóbrega C, Colaço A, Lopes C, *et al*., 2012, Review: BBN as an Urothelial Carcinogen. *In Vivo*, 26:727–39.
6. Ireson C, Alavijeh M, Palmer A, *et al*., 2019, The Role of Mouse Tumour Models in the Discovery and Development of Anticancer Drugs. *Br J Cancer*, 121:101–8. DOI: 10.1038/s41416-019-0495-5.
7. Yang Z, He L, Lin K, *et al*., 2017, The KMT1A-GATA3-STAT3 Circuit is a Novel Self-Renewal Signaling of Human Bladder Cancer Stem Cells. *Clin Cancer Res*, 23:6673–85. DOI: 10.1158/1078-0432.ccr-17-0882.
8. Marco V, Cerruto MA, D'Elia C, *et al*., 2014, Prognostic Role of Substaging in T1G3 Transitional Cell Carcinoma of the Urinary Bladder. *Mol Clin Oncol*, 2:575–80. DOI: 10.3892/mco.2014.290.
9. Katt M, Placone A, Wong A, *et al*., 2016, *In Vitro* Tumor Models: Advantages, Disadvantages, Variables, and Selecting the Right Platform. *Front Bioeng Biotechnol*, 4:12. DOI: 10.3389/fbioe.2016.00012.
10. Arantes-Rodrigues R, Colaço A, Pinto-Leite R, *et al*., 2013, *In Vitro* and *In Vivo* Experimental Models as Tools to Investigate the Efficacy of Antineoplastic Drugs on Urinary Bladder Cancer. *Anticancer Res*, 33:1273–96.
11. Rockwell S, 1980, *In Vivo In Vitro* Tumour Cell Lines: Characteristics and Limitations as Models for Human Cancer. *Br J Cancer Suppl*, 4:118–22.
12. Wancket L, 2015, Animal Models for Evaluation of Bone Implants and Devices: Comparative Bone Structure and Common Model Uses. *Vet Pathol*, 52:842–50. DOI: 10.1177/0300985815593124.
13. Degeorge K, Holt H, Hodges S, 2017, Bladder Cancer: Diagnosis and Treatment. *Am Fam Physician*, 96:507–14.
14. Siriwardena A, Samarji WN, 1993, Cutaneous Tumour Seeding from a Previously Undiagnosed Pancreatic Carcinoma after Laparoscopic Cholecystectomy. *Ann R Coll Surg Engl*, 75:199–200.
15. Li J, Zhang Z, Wang J, *et al*., 2014, Urinary Cytology with Acridine Orange Fluorescence is Highly Valuable for Predicting High-grade Upper Urinary Tract Urothelial Carcinoma. *Int J Clin Exp Pathol*, 2014;7:774–8.
16. Johnson AM, O'Connell MJ, Messing EM, *et al*., 2008, Decreased Bladder Cancer Growth in Parous Mice. *Urology*, 72:470–3. DOI: 10.1016/j.urology.2008.04.028.
17. Günther J, Frambach M, Deinert I, *et al*., 1999, Effects of Acetylic Salicylic Acid and Pentoxifylline on the Efficacy of Intravesical BCG Therapy in Orthotopic Murine Bladder Cancer (MB49). *J Urol*, 161:1702–6. DOI: 10.1016/s0022-5347(05)69008-8.
18. Hafeez S, Huddart R, 2013, Advances in Bladder Cancer Imaging. *BMC Med*, 11:104.
19. Patel AR, Chan ES, Hansel DE, *et al*, 2010, Transabdominal Micro-ultrasound Imaging of Bladder Cancer in a Mouse Model: A Validation Study. *Urology*, 75:799–804. DOI: 10.1016/j.urology.2009.06.047.
20. Glaser AP, Procissi D, Yu Y, *et al*., 2019, Magnetic Resonance Imaging Assessment of Carcinogen-induced Murine Bladder Tumors. *J Vis Exp*, 145:e59101. DOI: 10.3791/59101.
21. Hewitt HB, 1978, The Choice of Animal Tumors for Experimental Studies of Cancer Therapy. *Adv Cancer Res*, 27:149–200.
22. Nolan M, Gieger T, Vaden S, 2015, Management of Transitional Cell Carcinoma of the Urinary Bladder in Dogs: Important Challenges to Consider. *Vet J*, 205:126–7. DOI: 10.1016/j.tvjl.2015.03.022.
23. Knapp D, Ramos-Vara JA, Moore GE, *et al*., 2014, Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. *ILAR J*, 55:100–18. DOI: 10.1093/ilar/ilu018.
24. Maeda S, Tomiyasu H, Tsuboi M, *et al*., 2018, Comprehensive gene expression analysis of canine invasive urothelial bladder carcinoma by RNA-Seq. *BMC Cancer*, 18:472. DOI: 10.1186/s12885-018-4409-3.
25. Drago JR, Nesbitt JA, 1987, NB Rat Bladder Cancer Model: Evaluation of the Subrenal Capsular Assay System. *J Surg Oncol*, 36:5–7. DOI: 10.1002/jso.2930360103.
26. van Moorselaar RJ, Ichikawa T, Schaafsma HE, *et al*., 1993, The Rat Bladder Tumor Model System RBT Resembles Phenotypically and Cytogenetically Human Superficial Transitional Cell Carcinoma. *Urol Res*, 21:413–21. DOI: 10.1007/bf00300078.
27. Druckrey H, Preussmann R, Ivankovic S, *et al*.,1964, Selective Induction of Bladder Cancer in Rats by Dibutyl and n-Butyl-n-Butanol(4)-Nitrosamine. *Z Krebsforsch*, 66:280–90.
28. Ertürk E, Price JM, Morris JE, *et al*., 1967, The Production of Carcinoma of the Urinary Bladder in Rats by Feeding N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide. *Cancer Res*, 27:1998–2002.
29. Hicks R, Wakefield J, 1972, Rapid Induction of Bladder Cancer in Rats with TV-methyl-N-Nitrosurea. 1. Histology. *Chem Biol Interact*, 5:139–52.
30. Cohen S, 2002, Comparative Pathology of Proliferative

Lesions of the Urinary Bladder. *Toxicol Pathol*, 30:663–71. DOI: 10.1080/01926230290166751.

31. Gomes-Giacoia E, Miyake M, Goodison S, *et al*., 2014, Intravesical ALT-803 and BCG Treatment Reduces Tumor Burden in a Carcinogen Induced Bladder Cancer Rat Model; a Role for Cytokine Production and NK Cell Expansion. *PLoS One*, 9:e96705. DOI: 10.1371/journal.pone.0096705.
32. He Z, Kosinska W, Zhao ZL, *et al*., 2012, Tissue-specific Mutagenesis by N-butyl-N-(4-Hydroxybutyl)Nitrosamine as the Basis for Urothelial Carcinogenesis. *Mutat Res*, 742:92–5. DOI: 10.1016/j.mrgentox.2011.11.015.
33. Fantini D, Glaser AP, Rimar KJ, *et al*., 2018, A Carcinogen-induced Mouse Model Recapitulates the Molecular Alterations of Human Muscle Invasive Bladder Cancer. *Oncogene*, 37:1911–25. DOI: 10.1158/1538-7445. mousemodels17-b29.
34. Arum CJ, Kodama Y, Rolim N, *et al*., 2010, A rat model of intravesical delivery of small interfering RNA for studying urinary carcinoma. *World J Urol*, 28:479–85. DOI: 10.1007/s00345-010-0542-6.
35. Holmäng S, Cano M, Grenabo L, *et al*., 1995, Effect of Indomethacin on N-[4-(5-nitro-2-furyl)-2-thiazolyl] Formamide-Induced Urinary Tract Carcinogenesis. *Carcinogenesis*, 16:1493–8. DOI: 10.1093/carcin/16.7.1493.
36. Ferrari K, de Camargo JA, Rocha GZ, *et al*., 2014, Intravesical Bacillus Calmette-Guérin Efficiently Reduces p70S6K1 but Not 4E-BP1 Phosphorylation in Nonmuscle Invasive Bladder Cancer. *J Urol*, 193:682–9. DOI: 10.1016/j.juro.2014.08.106.
37. Li J, Qu W, Cheng Y, *et al*., 2014, The Inhibitory Effect of Intravesical Fisetin against Bladder Cancer by Induction of p53 and Down-Regulation of NF-kappa B Pathways in a Rat Bladder Carcinogenesis Model. *Basic Clin Pharmacol Toxicol*, 115:321–9. DOI: 10.1111/bcpt.12229.
38. Nakanishi K, Hagiwara A, Shibata M, *et al*., 1980, Dose Response of Saccharin in Induction of Urinary Bladder Hyperplasias in Fischer 344 Rats Pretreated with N-butyl-N-(4-Hydroxybutyl)Nitrosamine. *J Natl Cancer Inst*, 65:1005–10. DOI: 10.1093/jnci/65.5.1005.
39. Cohen SM, Aral M, Jacobs JB, *et al*., 1979, Promoting Effect of Saccharin and DL-Tryptophan in Urinary Bladder Carcinogenesis. *Cancer Res*, 39:1207.
40. Tsuda H, Sakata T, Shirai T, *et al*., 1984, Modification of N-methyl-N-Nitrosourea Initiated Carcinogenesis in the Rat by Subsequent Treatment with Antioxidants, Phenobarbital and Ethinyl Estradiol. *Cancer Lett*, 24:19–27. DOI: 10.1016/0304-3835(84)90075-2.
41. Tsuda H, Fukushima S, Imaida K, *et al*., 1983, Organ-specific Promoting Effect of Phenobarbital and Saccharin in Induction of Thyroid, Liver, and Urinary Bladder Tumors in Rats after Initiation with N-Nitrosomethylurea. *Cancer Res*, 43:3292–6.
42. Ito N, Hirose M, Shibata M, *et al*., 1989, Modifying Effects of Simultaneous Treatment with Butylated Hydroxyanisole (BHA) on Rat Tumor Induction by 3,2'-Dimethyl-4-Aminobiphenyl, 2,2'-Dihydroxy-di-n-Propylnitrosamine and N-Methylnitrosourea. *Carcinogenesis*, 10:2255–9. DOI: 10.1093/carcin/10.12.2255.
43. Kawai K, Kawamata H, Kameyama S, *et al*., 1995, Intraluminal Epidermal Growth Factor affects Growth of N-methyl-N-Nitrosourea-Initiated Rat Bladder Carcinoma. *Jpn J Cancer Res*, 86:429–34. DOI: 10.1111/j.1349-7006.1995.tb03075.x.
44. Ibrahiem EH, Nigam VN, Brailovsky CA, *et al*., 1983, Orthotopic Implantation of Primary N-[4-(5-Nitro-2-furyl)-2-thiazolyl]Formamide-Induced Bladder Cancer in Bladder Submucosa: An Animal Model for Bladder Cancer Study. *Cancer Res*, 43:617–22. DOI: 10.1016/0304-3835(94)90366-2.
45. Kiel H, Adolphs HD, Thiele J, *et al*., 1978, Biological Behavior of N-[4-(5-nitro-2-furyl)-2-thiazolyl]-Formamide Induced Carcinoma of the Transitional Epithelium in the Rat. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol*, 91:267–77. DOI: 10.1007/bf00312289.