

EXPERIMENTAL BLADDER CARCINOGENESIS-RODENT MODELS

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Several rodent models of bladder cancer development have been established. The aim of this review article is to provide a critical assessment of different animal models available for the study of bladder carcinogenesis, its chemoprevention and therapy. All, except for transgenic and knockout animals, require 8–12 months experimental periods in order to generate a high yield of neoplasias. Spontaneous bladder tumor models are extremely rare. The significance of the results from animal experiments is dependent upon the selection of a suitable animal model. There are no rules regarding the choice of a model, it is however very useful to have knowledge of relevant comparative medical aspects concerning this subject. We describe chemical carcinogens most commonly used to induce bladder cancer, pellet implantation and urinary calculi, agents that promote bladder cancer, and irradiation. We also evaluated other tools such as cell cultures, tumor implantation and transgenic models for bladder cancer, that have been developed to study the process. The review considers how several imaging techniques can be applied to study rodent bladder carcinogenesis.

Key Words: bladder cancer, mice, rat, rodent model.

The bladder is one of the most common sites of cancer in the urinary tract. Bladder tumors are manifestations of a multifocal disease whose natural history has not been completely elucidated and the response of bladder tumors to radio — and chemotherapy is unpredictable.

Urothelial carcinogenesis in the rat goes through a sequence of morphologic changes beginning as simple hyperplasia. It then progresses to nodular and papillary hyperplasia. These progress to papillomas and can eventually progress to higher-grade, noninvasive carcinomas and ultimately to invasive neoplasms (Fig. 1) [20, 72]. Many exophytic tumors induced in rats are polypoid, often pedunculated and with an inverted papillary growth pattern [72]. Nodular hyperplasia, in mice, is considerably more common than papillary proliferations and nodular hyperplasia frequently occurs with a complete absence of papillary hyperplasia [32]. Thus, the rat model strongly resembles papillary neoplasms and the mouse model resembles flat urothelial lesions, both identified in man [28].

Since the Surgeon Dr. Rehn first suggested a role for aniline dye in the etiology of bladder cancer in 1895, this has become the neoplastic disease which has been traditionally most strongly linked to occupational and environmental exposure to chemicals [83]. To study urothelial

carcinogenesis it is possible to use dogs, rabbits, guinea pigs, hamster, rats and mice. However, for investigation of chemical carcinogenesis mice and rats are generally used, for several reasons. For example, in addition to benefits related to size, there is an abundance of information concerning the biological characteristics of neoplastic development in these species. This is why we have focused this review on urothelial carcinogenesis in rats and mice. However, other animals are also mentioned.

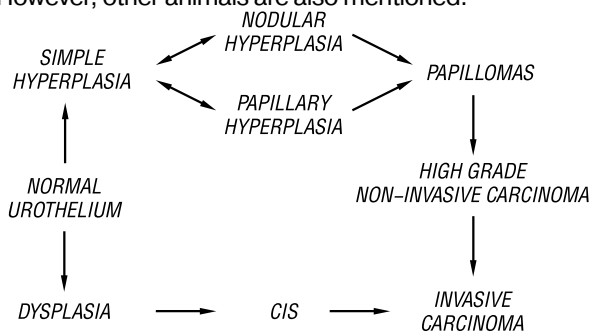


Fig. 1. Pathogenic pathways of rat and mice urinary bladder carcinogenesis

This review focuses on several *in vivo* and *in vitro* rodent urinary bladder cancers that have been established over the past 40 years. In Fig. 2 we present the potential applications of rodent bladder specimens obtained following chemical carcinogenesis of the urothelium.

THE RODENT BLADDER

The structure and function of rodents' lower urinary tract is remarkably similar to that of humans [72]. It extends from the renal pelvis through the ureters, urinary bladder, and into the urethra [90]. Excluding the urethra, the urinary tract consists of four layers: mucosa, lamina propria, muscular and serosa [21, 24, 25]. The mucosa of the lower urinary tract has been referred to as a transitional cell epithelium or as an urothelium. In the urinary bladder it is made up of three cell layers: super-

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Abbreviations used: AAF – 2-acetylaminofluorene; BBN – N-butyl-N-(4-hydroxybutyl) nitrosamine; BCPN – N-butyl-N-(3-carboxybutyl) nitrosamine; BW – body weight; CIS – carcinoma *in situ*; DBN – dibutyl nitrosamine; EHBN – N-Ethyl-N-(4-hydroxybutyl) nitrosamine; ENS – 4-ethylsulfonylnaphthalene-1-sulfonamide; FANFT – N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; MNU – N-Methyl-N-nitrosourea; SCC – squamous cell carcinoma; TCC – transitional cell carcinoma; X – times; W – weeks; ♀ – female; ♂ – male.

ficial, intermediate and basal [21, 72]. Since the entire urinary tract is lined with urothelium, theoretically the urethra and ureters could develop similar proliferative lesions. However, induced ureter and urethral proliferative lesions are rare in the rat. The reason for this may be explained by the quick passage of urine containing toxicants, compared to the more lengthy contact taking place in the urinary bladder [30].

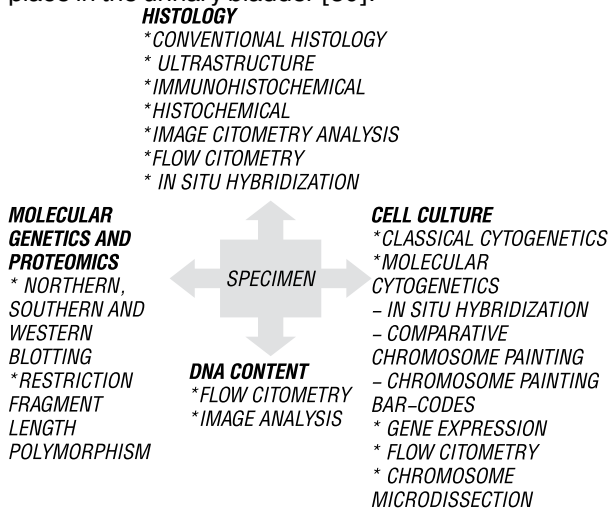


Fig. 2. Potential applications of bladder samples

SPONTANEOUS TUMORS

The incidence of spontaneous tumors in rodents plays an important practical role in the design and interpretation of carcinogenicity bioassays. The effect of a weak carcinogen may be more readily detected if it is not obscured by a background incidence of spontaneous bladder tumors, so the choice of test strain is important [51]. However, most naturally occurring strains of rodent do not develop spontaneous bladder cancer, more than 99% are predominantly associated with advancing age [11, 16, 18]. An exceptionally high incidence of urothelial and ureteric neoplasms have been reported in two rat strains, Brown/Norway (BN/RijHsd) and Dark Agouti (DA/OlaHsd), which were associated with the presence of calculi [10, 79]. Lesions identified in those animals were classified as papillary and multifocal, with simultaneous carcinoma *in situ* (CIS) [24, 77]. Other spontaneous non-neoplastic changes such as inflammation and epithelial hyperplasia occur infrequently in the bladder. Some spontaneous bladder tumors in rats can generally be explained by infection by the bladder parasite *Trichosomoides crassicauda* [19]. The complications induced by this parasite in experiments on rat bladder carcinogenesis are uncommon, since noninfected rats maintained under optimal conditions do not become infected [14]. There are no known reports of parasitic infection of the mouse bladder. Spontaneous bladder tumors in other rodent species including hamsters, guinea pigs, and rabbits appear equally as rarely as in mice [18].

REQUIREMENTS FOR APPROPRIATE ANIMAL MODELS OF URINARY BLADDER CARCINOGENESIS

Appropriate and valid animal models for urinary bladder carcinogenesis must be similar to human blad-

der cancer in their histology, biochemical properties, molecular and genetic characteristics, natural history, and biological behaviour [96]. A simple and natural method for the administration of the carcinogen is required; the carcinogen ought to be not toxic and should affect only the urothelium. The incidence of tumors should be reliable and high and the tumor induction time should be relatively short [69]. The investigator should be able to monitor the experiment or be able to predict results. Some of the requirements to animal models are listed in Table 1.

Table 1. Requirements for appropriate animal models to study experimental bladder carcinogenesis

Histology	The tumor should be of urothelial origin, with different stages of disease progression. We should identify the flat urothelial lesions and the papillary tumor growth
Biologic behaviour	The tumor should grow intravesical, so that the tumor can be directly exposed to intravesical anticancer drugs in its natural environment
Natural history	Mimic pathogenesis of human urinary bladder cancer, with identifiable precursor lesions
Genetic alterations	Stable molecular and genetic alterations similar to those found in human bladder cancer
Feasibility	Simple induction procedure, predictable and adjustable incidence

There are several advantages to using rodents in medical research, their physiology and genetics are well understood, they are relatively easy and cheap to maintain, and like humans, they are mammals. Rodents develop cancer relatively easily in response to chemical carcinogens. Their tumors also develop rather quickly, over months rather than years [69]. A major difference may be a lower rate of distant metastases in rats than in humans, although this is not certain [90]. Metastases and invasions of bladder cancer are more common in mice than in rats. The lack of metastasis in experimental animals may be due to the time factor. Animals are killed at the end of experimental period, or even earlier, to prevent suffering and for optimal tissue collection, which may be too soon for metastases development [11]. Concerning the selection of animals, we may choose between inbred or outbred strains of rodents. An important benefit of the use of inbred animals is that many can be supplied which develops virtually identical tumors at the same stage of growth. However, an important concern is the degree to which tumor heterogeneity may be lost [77]. Since the aim of laboratory animal experiments is to elucidate the pathological process in humans as well as to test chemicals for carcinogenic potential, classification of animal tumors should agree with the nomenclature used for human neoplasms [21]. However, the differences in their histological appearance, growth pattern and biological behaviour need to be emphasized [72].

INDUCED TUMORS

Yamagiwa and Ichikawa in 1918 were the first to prove that cancer could be induced in experimental animals by chemical means [98]. The induction of bladder cancer in dogs by 2-naphtylamine, reported by Hueper in 1938, established the experimental basis of bladder carcinogenesis [40]. Early attempts to induce tumors in mice bladders by means of chemicals were unsuccessful until Armstrong and Bronser (1944)

induced papillomas and carcinomas through the oral administration of 2-acetylaminofluorene (AAF) in CBA strain mice [4].

In the 1960s and early 1970s, organospecific chemically-defined bladder carcinogens were discovered for rodents. These chemicals and their application provided the readily-available reproducible models necessary for detailed studies of the biochemical, pathobiological and immunological mechanisms involved in the pathogenesis of bladder cancer [18].

There are several methods of inducing urothelial tumors: oral administration of a chemical carcinogen in diet, water or gastric intubation (gavage), by injection or intravesical instillation; the application of the stimulus directly by implanting in the bladder a pellet either by itself or with a chemical incorporated in it; and exposure to X-rays.

Chemical carcinogens. As mentioned before, the first compound applied in experimental bladder carcinogenesis was AAF. Although of considerable usefulness in experimental bladder carcinogenesis research, the AAF is a pluripotential carcinogen, inducing tumors of several tissues in addition to the urothelium particularly the liver, pancreas, breast, skin, forestomach and ear duct [4, 18, 21]. Hepatic tumors may cause early death of the animals and confusion in the interpretation of the experimental results [79].

Over the past few decades, research efforts have focused on the development of rodent models that permit the reproducible induction of bladder cancer with minimal or no induction of tumors in other organs. Three chemicals have been proved to be particularly effective, in that, when administered via the appropriate route, at the appropriate dose and in the appropriate strain of animal, all produce 100% incidence of bladder tumors; these chemicals are N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT), N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) and N-Methyl-N-nitrosurea (MNU) [30]. These compounds are complete carcinogens, the total dose has a greater effect when administered as several fractions, i.e. the effect of the fractions is synergistic rather than additive. The grade of cellular atypia and the extent of invasion increase as the dose of carcinogen increases as well as when the experimental period is extended [31, 51].

The nitrofurantoin FANFT is highly specific to the urinary bladder in the rat, mouse, hamster and dog. It is a genotoxic compound and can act as an initiator or as a promoter. It is metabolically activated into reactive electrophiles, produces DNA adducts and ultimately produces mutation [6, 21, 51]. FANFT is deformed in 2-amino-4-(5-nitro-2-furyl) thiazole by liver and kidney enzymes before excretion [89]. Tumors induced by this compound are predominantly transitional cell carcinoma (TCC), with a large proportion exhibiting squamous cell differentiation. However, hyperplasia, dysplasia and CIS have also been observed [6, 51, 77]. FANFT is incorporated in the diet and induction of bladder cancer requires 8 to 11 months [6, 29]. Transplantable cell lines have been developed from

these FANFT-induced tumors, the most commonly used are labelled MBT-2 and AY-27 [96]. However, the use of this compound presents safety concerns for the researchers involved and the environment.

BBN is one of the most suitable urinary bladder carcinogens for animal models, since its carcinogenic potential is essentially limited to this organ and is probably the most commonly-referenced experimental bladder carcinogen [55, 70]. Bladder tumors induced by BBN in rats and mice resemble their human counterparts [55]. BBN is a metabolite of the symmetric dibutyl nitrosamine (DBN) [46]. In rats, both were demonstrated to be urinary bladder carcinogens, with BBN being specific to the urinary bladder, because DBN also induced tumors of the liver, lung, kidney and oesophagus [17, 39]. In mice, similar aspects have been found [9, 21]. The difference in lesions histopathology identified between different rodents exposed to BBN can be attributed to dosage, lag time until tumor development, species, and strain. BBN is bladder specific not only in rats but also in mice and dogs. However, it is a weak bladder carcinogen in hamsters and pigs [51, 69]. A 100% incidence of tumors can be induced by continuous and prolonged administration of BBN in drinking water. This compound can also be administered by oral gavage [51]. Subcutaneous injections generally result in a lower incidence of tumors [21]. BBN can also be introduced into the bladder by intravesical instillation [95]. BBN is a yellow oily liquid not very volatile but is soluble due to terminal hydroxylation. When dissolved in water BBN is easy to apply. However, it is photosensitive and therefore an opaque feeding-bottle is necessary [55]. BBN is a genotoxic compound. The alcoholic group of administered BBN is rapidly oxidized to a carboxyl group by the liver enzymatic system alcohol/aldehyde dehydrogenase; the metabolite formed by N-butyl-N-(3-carboxybutyl) nitrosamine (BCPN) is also a bladder carcinogen and comes in contact with the urothelium via the urine. BCPN is a stable compound that binds covalently to cellular macromolecules and is ultimately responsible for the initiation of the carcinogenic process [1]. BCPN produces neoplastic transformation of rat urothelial cells cultured *in vitro* [9]. In dogs, the dosage of BBN is related to the grade of bladder cancer [2]. In low dosages, BBN causes low-grade papillary carcinoma after long periods of exposure [77].

N-Ethyl-N-(4-hydroxybutyl) nitrosamine (EHBN) is a genotoxic compound even more potent than BBN. It targets the urothelium of mice, rats and dogs [93]. Hamsters are however less sensitive to this carcinogen and guinea pigs appear to be resistant [21, 39]. EHBN is metabolized by hepatic enzymes in N-ethyl-N-(3-carboxypropyl) nitrosamine and excreted in urine. In mice this compound increased the incidence of squamous cell carcinoma and is a good model for the investigation of nonpapillary invasive bladder carcinoma. The carcinomas that develop demonstrate invasion of the surrounding tissues and are associated with hematogenous metastatic spread to distant organs [93]. Other nitroso compounds such as N, N-dibutyl-

nitrosamine and N-methyl-N-dodecyl nitrosamine are carcinogenic to the urinary bladder in rats [51].

MNU is the only carcinogen known to act directly on the urothelium following spontaneous pH-dependent decomposition without requiring metabolic activation. At present MNU is the only urothelial carcinogen known to produce bladder cancer at a single dose [51]. MNU is a fine yellowish crystalline powder stabilized by addition of 5% acetic acid [52]. Because MNU is intrinsically unstable, variations in carcinogenic potency can arise unless care is taken during its storage, preparation and use [51]. It is a genotoxic compound that can act as an initiator or as a promoter and cause persistent methylation of the DNA [53, 57]. The MNU model of bladder cancer has particular advantages for the experimental analysis of complete carcinogenesis, since the carcinogen can be administered directly in quantifiable pulse doses, via intravesical instillation [76]. The disadvantage of this procedure is that in some animals bladder concretions and/or urocystitis may develop [53, 86]. Bladders treated with intravesical MNU develop progressive neoplastic changes, and the tumors become progressively less differentiated with time. These lesions progress from hyperplasia, atypia, CIS, and papillary carcinoma to large bulky muscle invasive tumors that completely fill the bladder lumen, obstruct the ureters and kill the animal [53, 91]. These tumors have a relatively low potential for metastasis with deposits being found in abdominal lymph nodes and within the abdominal cavity [7]. MNU also causes local massive inflammation of the bladder mucosa and submucosa and infiltration of the bladder wall with acute inflammatory cells and exudates [42].

4-Ethylsulfonynaphthalene-1-sulfonamide (ENS) is a carbonic anhydrase inhibitor that produces alkaline, hypoosmolar urine with crystalluria and calculi formation. Urinary tract hyperplasia occurred in mice fed ENS at a dose of 0.1% of their diet [21].

Benzidine, 3, 3'-dichlorobenzidine, 2-naphthylamine, 4-aminobiphenyl, 2-acetylaminofluorene, phenacetin, and sodium o-phenylphenate are additional compounds which are carcinogenic for the urinary bladder [86].

Bracken fern (*Pteridium aquilinum*) induces upper alimentary tract and bladder cancer in a number of species including rats, guinea pigs and cows [21, 81]. The major carcinogenic compound of bracken fern is ptaquiloside, a non-sesquiterpene that alkylates DNA when metabolized into an unstable dienone under alkaline conditions [87]. Ptaquiloside accumulates in the body of rats artificially fed bracken fern and is eliminated in urine where its carcinogenic activity is preserved [75].

In Table 2, we show the results of several studies with different carcinogens, treatments, animals used and lesions identified.

Using bladder cancer models, several compounds have been evaluated as suitable for intravesical therapy (chemotherapy and immunotherapy) and systemic chemotherapy [64, 66, 88, 91]. More recently photodynamic therapy with hypericin and protoporphyrin IX was also investigated [97].

Pellet implantation and urinary calculi. The presence of foreign bodies within the lumen of the bladder can cause irritation or trauma to the urothelium, both stimulate mitotic activity, thereby causing nodular and papillary hyperplasia [24, 27]. Mitotic activity predisposes cells to the action of both initiating and promoting substances [24]. If there is extensive ulceration of the bladder surface, diffuse papillomatosis is produced [20]. Although direct implantation of pellets containing suspected carcinogens was attempted in 1924, it wasn't until 1951 when Jull successfully induced bladder tumors by this method [47]. This technique involves surgical implantation of pellets into the lumen of the rodent bladder. The materials used as the basis for the pellets were paraffin or cholesterol and it was thought that these materials remained biologically inert in the bladder lumen. Various chemicals were incorporated into these pellets for implantation into the bladder lumen. It was assumed that the bladder epithelium was incapable of metabolizing these chemicals, so that the introduced chemical represented the ultimate form of the carcinogen [15]. It was also assumed that the pellet itself did not pose a carcinogenic risk to the urothelium [47]. In 1979 Jull demonstrated in an experiment that the pellets inserted into the mouse bladder itself represented a carcinogenic risk [48]. In addition, it became apparent that the urothelium is metabolically active, through evidence of the activities of enzymes involved in the metabolism of exogenous and endogenous chemicals [23]. Urine was required as a co-factor for the carcinogenic effect of the pellet [13]. The rapidity with which the chemical was leached from the pellet varied considerably, depending on the solubility. For highly water-soluble compounds such as saccharin and cyclamate, the chemical was rapidly leached from the pellet [12]. In addition, the surgical procedure, to implant the pellet, produced nodular and papillary hyperplasia [32]. This method has since been abandoned, because these problems made the interpretation of the results utilizing the pellet implantation technique difficult [20].

During the past three decades, numerous chemicals have been identified as producing bladder tumors in rodents and are associated with the appearance of urinary calculi [16]. Such chemicals include uric acid, calcium oxalate, uracil, thymine, melamine, and others [34, 85]. Of these compounds, uracil is the most widely applied [67]. In the rat urinary bladder, uracil is reported to induce calculi and papillomatosis when administered in the diet [56, 84]. The proliferative effects of calculi are commonly sustained in rodents since these species are normally horizontally positioned allowing the object to remain within the lumen of the urinary bladder, with less chance of elimination [24]. If the calculus is removed before a neoplasm is produced, the proliferative changes are rapidly reversed. Urinary tract calculi represent foreign bodies, similar to pellets, but do not require surgical implantation into the bladder lumen [16, 20, 23]. The calculi can arise from the administered chemical itself, from one of its metabolites or from an endogenous metabolic product that is caused by the administration of the chemical. In general, calculi form more

Table 2. Chemically induced bladder tumors

Agent	Treatment	Host	Tumor type
Bracken Fern	Dietary [75]	Albino rats (♂ and ♀)	Hyperplasia, TCC, invasion.
BBN	0.05% in water, 40 w (weeks) [61] 0.05%; 0.01% and 0.005% in water, 4, 8, 12, 16, 20, 26 w [68]	ACI rats ♂ (6w) C3H/He ♀ mice (5w)	1 w no lesions, 2 w hyperplasia, 4 and 12 w papilloma, 20 w TCC 0.05% BBN 4 w and 8 w simple hyperplasia and dysplasia; 12, 16, 20 and 26 w simple hyperplasia, dysplasia, CIS, invasive carcinoma. 0.01%BBN 4 w simple hyperplasia; 8, 12, 16 w simple hyperplasia and dysplasia; 20 and 26w simple hyperplasia, dysplasia, CIS and invasive carcinoma. 0.005% BBN 4 w no lesions; 8 and 12 w simple hyperplasia, 16 and 20 w simple hyperplasia and dysplasia; 26 w simple hyperplasia, dysplasia, CIS and invasive carcinoma.
	50 or 100 mg gastric intubations, 2 x w, 9 w [6] 0.05% water, 20 w [32] 0.05% water (16 w and 32 w) [60]	B6D2F1, mice ♂ Wistar rats ♂ Fisher 344 rats ♂ (6 w)	TCC, SCC, Adenocarcinoma Hyperplasia, TCC, papillary tumors 16 w and 32 w TCC
	Gavage (3 fractionated doses at 24h intervals, BBN dissolved in 1:2 propandiol 300 mg/Kg BW) [54]	Wistar rats ♀	19 months later: Papillomas and invasive TCC
EHBN	0.025% water (4 w, 12 w, 20 w, 28 w, 36 w) [93]. 0.05% water 32 w [44]	B6C3F1 mice ♂ (6 w) B6C3F1 mice ♀ (6 w)	4w-dysplasia; 12w, 20w, 28w and 36w-dysplasia, papiloma, TCC, SCC, adenocarcinoma, CIS; metastasis TCC (invasive), SCC
FANFT	Dietary: 0.1% (45 w) [65] Dietary 0.02% [22]	C3H/He mice ♀ (6 w) Fisher 344 rats ♂ (4 w)	Dysplasia, CIS and TCC Hyperplasia (6 w) Nodular hyperplasia (8 w) Papillary tumours (10, 12, 14 w) Dysplasia, CIS and TCC
MNU	Dietary: 0.1% (38w) [63] Intravesical 5 mg/Kg BW [52] 1.5 mg every 2 weeks (x 4), intravesical [91] 0.15 ml intravesical, 4 doses over 6 weeks [76]	C3H/He mice ♀ (6 w) Wistar rats ♀ Fisher 344 rats ♀ Fisher 344 rats ♀	15 months latter exophytic papiloma and invasive TCC Hyperplasia, papillary lesions, squamous metaplasia 10 weeks after last treatment TCC

Notes: w – weeks; x – times; TCC – transitional cell carcinoma; SCC – squamous cell carcinoma; CIS – carcinoma *in situ*, BW – body weight; ♂ – male; ♀ – female

quickly in rats than in mice. Calculi-induced proliferation appears to be considerably greater in rats than in mice. This difference is particularly due to a primary papillomatous reaction in rats, whereas in mice it is predominantly a nodular response [23]. There is also a difference between sexes; male rodents are more frequently affected than females [24]. The process involved in urothelial carcinogenesis related to calculus formation may be influenced by factors such as pH, volume, osmolality, cationic and anionic concentration and quantitative and qualitative differences in the presence of urinary protein [16].

Calculi can be surgically produced in rats by performing a portacaval shunt, which leads to significant alteration of uric acid metabolism and eventual production of uric acid calculi, ulceration, regenerative proliferation, and carcinoma [26, 45].

Promoting agents. Multistage models of carcinogenesis proposed to explain the patterns of tumor development observed in the urinary bladder involve the initiation of neoplastic change in a few cells by a threshold dose of carcinogen followed by conversion of these latent tumor cells into an autonomous cancer by further doses of the same and/or other carcinogens, and/or promoting agents. In the urinary bladder of mice and rats, neoplastic change can be initiated by a few weeks treatment with low doses of chemical carcinogens above described. Animals exposed subsequently to promoter compounds will develop bladder cancer [33, 73].

Urinary bladder promoters can be classified into at least seven categories as follows: 1) sodium and potassium salts associated with increased concentration of urinary levels of sodium and potassium ions and alkaline urine; 2) urolithiasis inducing agents; 3) antioxidants; 4) anticancer drugs; 5) amino acids;

6) drugs and others [31, 43]. Table 3 shows the classification of bladder cancer promoters.

Irradiation. Treatments with ionizing radiation have been shown to cause bladder cancer in humans. Irradiation with X-rays is capable of inducing preneoplastic urothelial hyperplasia and development of urothelial carcinoma in rodents. The rate of bladder tumors achieved with irradiation depends on the dosage time of exposure and time of follow up. A single dose of irradiation generated from a linear accelerator induced hyperplasia of the urothelium when mice were examined 3–19 months later [50, 92].

Table 3. Classification of urinary bladder cancer promoters

Type	Cancer promotor
Sodium or potassium salts	Sodium saccharin
	Sodium cyclamate
	Sodium L-ascorbate
	Sodium citrate
	Sodium erithorbate
	Sodium phenobarbital
	Sodium barbital
	Sodium chloride
	Potassium carbonate with or without ascorbic acid
	Urolithiasis-inducing chemicals
Antioxidants	Diphenyl
	Butylated hydroxyanisole
	Butylated hydroxytoluene
	Ethoxyquin
Anticancer drugs	<i>t</i> -Butylhydroxyquinone
	2- <i>t</i> -Butyl-4-methylphenol
	Adriamycin
Amino acids	Mitomycin C
	DL-Tryptophan
	L-Leucine
Drugs Others	L-Isoleucine
	Phenacetin
	Components of urine
	Allopurinol
	Dimethylarsinic acid

CELL CULTURE

Cell culture represents an excellent opportunity to study bladder cancer *in vitro*. The growth of normal and neoplastic urothelial cells, obtained from rodents, or man, in tissue culture, offers the possibility of studying “pure” populations of cells without necessarily having significant contamination by other tissues [77]. Different methods for the culture of urothelial cells *in vitro* are described: the propagation of primary cultures and cell lines derived from normal urothelial cells exposed to chemical carcinogens and continuous cell lines derived from bladder tumors [59]. Such cultures provide model systems for studying the factors that control the growth and differentiation of both normal and neoplastic urothelial cells, and for defining the morphological, biochemical and genetic changes associated with the development and progression of bladder cancer [58, 59]. Furthermore, they provide the opportunity to observe the evolution of cancer within a population of cells exposed to a carcinogen and to study the effectiveness of anticancer drugs [58, 77, 82]. Cell cultures also offer the opportunity to study the chromosomal changes associated with early development of bladder cancer.

TUMOR IMPLANTATION

As we described before, bladder tumors can be induced in rodents using a variety of chemicals. However, these tumors required 8–12 months to develop, they grew very slowly and many animals are required [43, 62, 96]. While induction of bladder tumors in animals by chemical means has allowed the study of carcinogenesis and, in some cases, chemotherapeutic agents, the majority of research leading towards clinical applications requires models based upon human cancer cells [41].

Currently there are two more fundamental murine bladder tumor models: the xenograft model (transplantation of human urothelial cell carcinoma into immunodeficient mice) [41, 80], and the syngeneic model (transplantation of carcinogen-induced bladder cancer in syngeneic, i.e. immunocompetent mice) [78, 88]. Syngeneic tumor cells can be implanted subcutaneously (heterotopic) [42] or intravesically (orthotopic) [37].

For reasons of easy access, heterotopic models (subcutaneous, intravenous and intraperitoneal) have been preferred for human tumor implantation. However, it is questionable if the tumor development at these sites parallels the development of the tumor in the organ of its origin [42].

Orthotopic tumor implantation is more difficult. However, the refinement of the various methods of orthotopic implantation now allows this system to be used routinely for a variety of applications. This model of bladder cancer allows the growth and spread of bladder cancer to be better observed in a normal physiologic environment and permits novel approaches to cancer therapy or imaging of tumor growth. The tumor cells used for intravesical instillation

may be obtained from urothelial cell cultures exposed to chemical carcinogens (MBT-2 and AY-27) or human bladder neoplastic cells may be used (MGH-U3) [96]. Cells can be introduced into the bladder cavity after chemical urothelial denudation (leading to multifocal lesions) or after mechanical urothelial lesion (requiring cystotomy) [8, 42]. The first method is less time-consuming, requires no surgery and does not bring up diffuse ulceration, oedema or urinary stones. Its tumor implantation success rate ranges from 28 to 97%. The tumors are multifocal and their localization is unpredictable [8]. Transplantable animal models are not technically complicated, are well tolerated by the animals and result in minimal morbidity associated with occasional haematuria subsequent to tumor cell instillation [42, 96]. More importantly is that these tumors are produced intravesically and arise from the urothelium. Consequently, the tumor can be directly exposed to intravesical anticancer drugs in its natural environment [49]. However, this model is not perfect. Its one disadvantage is that the tumors prove to be invasive carcinomas from the start; this is related to the mechanical urothelial abrasion [8].

TRANSGENIC MODELS FOR BLADDER CANCER

Genetically engineered animals, usually transgenic or gene targeted mice, allow the examination of whether genetic changes, including oncogene over expression/mutation or tumor suppressor gene loss, can increase the risk of neoplastic progression; whether genetic changes can cooperate during bladder carcinogenesis; and how the genetic signature of a neoplasm correlates with particular biological aspects of tumor development [36]. Transgenic mice carrying the human C-Ha-ras proto-oncogene, v-Ha-ras transgenic mice, pim-1 transgenic mice, and several knockout strains of mice deficient in tumor suppressor genes such as *p53* have been shown to exhibit increased carcinogen susceptibility [35]. *p53* knockout mice are much more sensitive to BBN urinary bladder carcinogenesis than the parental mice strain C57Bl/6 [74, 99]. For studies of chemical carcinogenesis rats are generally more frequently used than mice. However, only limited types of transgenic rats have been developed for studying carcinogenesis. The establishment of a rat model with similarities may be the ideal solution. A transgenic rat line carrying three copies of the human *c-Ha-ras* proto-oncogene was developed with its own original promoter region. This rat is highly susceptible to BBN carcinogenesis and may be utilized as a rat model for analysis of bladder tumor development [71]. Tsuda et al. [95] concentrated their attention on the generation of transgenic rats with the same human *c-Ha-ras* proto-oncogene used for the establishment of transgenic mice. The rat line developed by this team called Hras128 is highly susceptible to BBN bladder carcinogenesis [94]. Table 4 shows the utility of each model in function of the subject to be studied [77].

Table 4. Utility of different models to study bladder cancer

Subject for study	Tumors chemically induced	Cell cultures	Tumor implantation	Transgenic models
Conventional histology	yes	no	yes	yes
Ultrastructure	yes	yes	yes	yes
Immunohistochemical	yes	yes	yes	yes
Histochemical	yes	no	yes	yes
Molecular genetics and proteomics	yes	yes	yes	yes
Image cytometry	yes	yes	yes	yes
Image analysis	yes	yes	yes	yes
Treatment	yes?	yes?	yes	yes?
Kinetics	yes	yes?	yes	yes
Carcinogenesis	yes	yes	no	yes

Note: ? – not fully validated.

EVALUATION OF NEOPLASIC DEVELOPMENT IN RODENTS

Urinary cytology has been useful in the diagnosis and intermittent observation of patients with urinary bladder carcinoma. Surprisingly, urinary cytology has received little study in rodents. Confirmation of tumor development and therapeutic effects in rodents is made possible by the detection of a palpable suprapubic mass, gross haematuria, weight loss or when laparotomy or cystectomy is performed [8]. However, these techniques do not have reliable parameters which enable the tumor growth to be assessed. Only large tumors (> 200 mg) are clearly detected by abdominal palpation [38]. To solve this limitation non-invasive diagnosis of superficial bladder tumors in animals can be applied. It is possible to detect and follow the development of induced bladder tumors using ultrasonography, a non-invasive imaging technique that accurately estimates tumors' morphologic characteristics [3, 8]. Magnetic resonance has also been reported for this purpose [96]. However, due to the spatial resolution of magnetic resonance imaging accurate diagnosis of small early lesions could not be obtained. The use of the non invasive ultra thin endoscope, developed by Asanuma et al. [5] allows the detection of bladder tumors with a minimal lesion of 1 mm. Cytoscopic examination is a reliable noninvasive method for detecting and monitoring superficial tumors [5]. All methods described above proved to be suitable means of monitoring intravesical growth, but each imaging procedure required anaesthesia and catheterization. Almost 20% of the animals died prior to the end of the study due to procedural mishaps [3].

CONCLUSION

We have reviewed experimental data related to the induction of bladder cancer in rodents. Our intention is not to propose any specific model for researching bladder cancer but to give enough information to develop a framework in each instance to encourage questioning of the process. Multiple experimental approaches have been employed to study bladder cancer, including the use of rodents. Rodents have the advantage that they can be manipulated to address specific hypotheses concerning the mechanisms underlying this disease. However, it should not be forgotten that the direct study of human bladder cancer continues to be vitally important in understanding the biology of bladder cancer. In the human bladder, it is likely that the urothelium is

chronically exposed to sub-cytotoxic doses of genotoxins and, consequently, acute exposure may not be representative of the process in man. Each rodent model to study bladder carcinogenesis has strong and weak points. The one that is most suitable for the purpose of each study must be selected. The rodent urinary bladder is very similar to the human urinary bladder, which helps to understand bladder carcinogenesis. Like in humans, the spectrum of lesions observed in urinary bladder in mice and rats include preneoplastic lesions, papillary tumors and invasive carcinoma. The selection between mice and rats may be based on the aims of the study. For instance, the small bladder size of mice limits both histologic characterization and the application of intravesical therapeutics.

Chemical induction of bladder cancer in rodents usually requires 8–12 months. However, the administration of chemicals in water or diet although effective present inherent risks to the safety of laboratory personnel. Moreover, it is difficult to quantify the amount of carcinogen ingested by each animal. This disadvantage of carcinogen dosing may be eliminated by the administration of the carcinogen via gavage.

Transgenic rodent models of bladder cancer also have both advantages and disadvantages. Since all feature a specific transgene and the latent period of cancer development is relatively short, they should provide useful tools for analysing the fundamental biology and roles of specific genes in tumor development and progression.

By monitoring the responses to chemical carcinogens using experimental models, it has been possible to identify many of the mechanisms through which tumors developed. Animal tumors also provide an opportunity to study the chromosomal changes associated with the early development of bladder cancer. In addition, such studies may establish whether different carcinogens are associated with specific cytogenetic abnormalities, hence defining the specific gene(s) involved in the development of bladder cancer. Finally, with the progress in the development of the rat and mouse genome programs, without difficult ethical problems and with much shorter generations, as yet undescribed genes related to cancer development in human cases should be successfully detected.

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ЭКСПЕРИМЕНТАЛЬНЫЕ ОПУХОЛИ МОЧЕВОГО ПУЗЫРЯ: МОДЕЛИ НА ГРЫЗУНАХ

Для изучения механизмов развития рака мочевого пузыря было создано несколько экспериментальных моделей на грызунах. Целью обзора была сравнительная оценка различных экспериментальных моделей для изучения канцерогенеза мочевого пузыря, профилактики и терапии. За исключением трансгенных и нокаутных животных, для получения высокого выхода опухолей в любой экспериментальной модели требуется 8–12 мес. Модели спонтанного канцерогенеза мочевого пузыря крайне редки. Выбор экспериментальной модели с определенными параметрами определяет значимость полученных результатов. В статье описаны различные методики, используемые для индукции рака мочевого пузыря *in vivo*, ряд методических подходов, таких как культура клеток, имплантация опухоли и трансгенные модели рака мочевого пузыря и современные методы мониторинга опухолевой прогрессии.

Ключевые слова: рак мочевого пузыря, экспериментальные модели, грызуны.