Upper urinary tract urothelial carcinoma experimental models

Modelos experimentais de carcinoma urotelial do trato urinário superior

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ABSTRACT
Cancer research on animals is an important complement to clinical investigations. Particularly, the use of animal models in researches on urinary tract cancer has a primary role in demonstrating that carcinogenesis is a multiple-stage process. These models are used to induce tumors in order to analyze the development of immunity, chemotherapy, and new techniques. This article discusses the role of animal models using rodents in urothelial carcinoma, the validity of animal models in carcinogen-induced tumors, the primary animal models available of transitional cell carcinoma and carcinoma of the upper urinary tract, and the advantages and disadvantages of the main experimental models in use.

Keywords: Models, animal; Urinary tract/pathology; Carcinoma, transitional cell/pathology

INTRODUCTION
Transitional cell carcinoma (TCC) is the most common neoplasm phenotype affecting the urinary tract, and its location in the renal pelvis is responsible for 5 to 10% of all renal tumors, and for 5% of all urothelial tumors. Its ureteral occurrence is less common, with a rate of approximately one fourth (25%) of what is noted in the renal pelvis(¹-²).

Different from vesicle tumors, recurrence is of greater concern when it appears in the upper tract, to where the access and surveillance are more difficult. The renal pelvis, ureter, and bladder are lined by continuous transitional epithelium, the urothelium, which constitutes an extensive area of homogeneous tissue, even though anatomical and embryonic differences do exist at these different sites(³).

Animal models of bladder TCC are well established and allow a progression in the knowledge of tumor biology, growth patterns, differentiation and dissemination, antigenic expression, metastatic and invasive potential as well as therapeutic response(⁴). Animal models are the center of experimental researches and, at the same time, the connection between experimental and clinical research.

In contrast with the high concentration of studies of the urinary bladder, among the investigations involving TCC, few reports of molecular analysis of the upper urinary tract (UUT) have been published(⁵-⁷), which is also true in clinical studies. Although the histopathological alterations in TCC and UUT are similar to those found in the bladder(⁸), they are less frequently studied(⁷).

In many clinical circumstances, studies of bladder TCC are extrapolated for UUT tumors. Even though they display the same histological strains, there are significant differences with regard to diagnosis, treatment and prognosis of ureter and renal pelvis lesions, as the latter are more pernicious. These

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discrepancies are possibly related to difficulties in early diagnosis, topical treatment, besides peculiar cellular biology and anatomy\(^{(4)}\).

**Role of the animal model using rodents in urothelial carcinoma**

The use of animal models has an important role in cancer research, demonstrating that carcinogenesis is a multiphasic process, namely: initiation – irreversible fixation of the genetic modification (mutation); promotion – reversible expansion of the tumor cell population (proliferation of the initiated cells), and progression – irreversible transition to an invasive malignant tumor (accumulation of lesions and undifferentiation)\(^{(9-10)}\).

Using rodents brings various advantages for medical research. Their physiology and genetics are well known, and their handling is easy and cheap. A mammal model shares various important aspects with humans. Rodents further develop cancer with relative facility in response to low doses of chemical carcinogens, and their tumors develop rapidly over periods of weeks to months\(^{(4)}\).

The structure and function of the urinary tract in rodents is extremely similar to that of humans, and both species share similar profiles of genetic expression in urothelial cancer, suggesting that the urothelial tumor in rodents may prove to be an interesting experimental model\(^{(11-12)}\).

One important aspect of the biology of the urothelium that may influence carcinogenesis is related to its function of self-renewal of the urinary barrier. Although normal urothelium in all species displays an extremely low rate of cellular substitution and a virtual absence of mitoses, it shows a high regenerative capacity with rapid proliferation during development in response to a lesion\(^{(13)}\).

The carcinogens excreted in the urine may promote carcinogenesis not only by direct genotoxic lesion, but also through epithelial damage and consequent cellular proliferation for restoration of the urinary barrier\(^{(13)}\).

New therapeutic agents may be tested in animal models, since they allow an evaluation of the route of administration (including intravesical), pharmacokinetics, efficacy, and potential toxicity.

**Validity of the animal models induced by carcinogens**

A significant number of risk factors, such as exposure to chemical carcinogens, have been related to the development of TCC. Smoking is one of the major risk factors known, and is responsible for up to one third of the cases\(^{(14)}\). It contains approximately 4,700 different chemical compounds, many of them genotoxic, such as n-nitrous and aromatic amine compounds (4-aminobiphenyl and o-toluidine)\(^{(15)}\).

The urine of smokers presents mutagenic characteristics due to the presence of twice the amount of aromatic amines as compared to the urine of non-smokers\(^{(16)}\).

Different experimental approaches have been used in the study of TCC. In various models of bladder carcinogenesis, multiple doses of chemical carcinogens are instilled into the bladder of rodents and canines\(^{(17)}\). Autochthonous induction of bladder cancer by intravesicle instillation of the carcinogen n-methyl-n-nitrosourea (MNU) in female Fisher 344 rats proved to be very useful in studies of urothelial carcinoma\(^{(18)}\). The same authors suggested that the cytotoxic lesion induced in the urothelium would initiate a “reparation response” with rapid proliferation, allowing the accumulation and perpetuation of mutations. Associated treatments using carcinogens and promoters may play synergic or additive roles in the induction of TCC\(^{(19)}\).

The administration of sodium citrate in rats promotes the induction of preneoplastic lesions and neoplasms in bladders previously initiated with chemical carcinogens\(^{(20)}\).

While laboratory animals differ from humans in some aspects, the use of these models is based on evidence of more genetic, genomic, physiologic, biochemical, and metabolic similarities than there are differences among the species of mammals.

Historically, studies involving lab animals, especially rodents, have brought numerable contributions to physiology, biochemistry, pharmacology, toxicology, and biology of cancer.

The use of the models utilizing rodents is based on the premise and experimental evidence that rodents show sufficient similarities in their physiology, biochemistry, metabolism, and genetics, which guarantee their use as human substitutes, as well as their relative availability, low cost, and short life cycle. These studies were and continue to be fundamental in the discovery of new treatments.

Pre-clinical studies including laboratory animals precede clinical and population studies for new drugs and therapies. Animal models contribute to an understanding of the modulating and pathogenesis mechanisms. Presently, the animal model involving rodents is the best tool available for studying cancer, as long as false-positive and false-negative results, metabolic differences between the species, and species-specific responses are identified and taken into consideration\(^{(21)}\).

One cannot consider, therefore, that rodents are perfect substitutes for humans, but to date, they are
Integration of animal experimentation with new genomic methods, computational biology, dynamic systems, and bioinformatics methods may, in the future, incorporate the existing models even further(23).

The challenge will be the selection of instruments for each issue presented.

Principal animal models available of TCC of the UUT

Even though no ideal model is available, attempts to induce cancer in the UUT of rodents using carcinogens have been carried out with success. Stagnation of urine containing the carcinogen and prolonged contact of the target cells with the carcinogen through the urine may be necessary in inducing UUT tumors in experimental models, which happens in conditions such as hydronephrosis(24).

In 1980, 18.6% of all microscopic transitional cell tumors were found in Wistar rats, most of them in the renal pelvis, in 43 animals with deficiencies of essential fatty acids (EFA) as compared to the absence of the tumor in 36 controls. Therefore, rats with EFA deficiencies were considered to be a useful model for studying human urothelial cancer. Calculi were observed in the renal pelvis and bladder in proportions not yet described(25).

The stagnation of urine containing a carcinogen in the renal pelvis may be related to local carcinogenesis. NON/Shi mice (NON for non-obese non-diabetic that had glucose intolerance, but not diabetes) showed spontaneous hydronephrosis in 10 to 30% and when for 12 weeks they received orally 0.05% n-butyl-n-(4-hydroxybutyl)nitrosamine (BBN) dissolved in water, 35% developed renal pelvis carcinomas similar to what happens in humans. Out of these, 38% were squamous cell carcinomas (SCE). Using strains that do not develop spontaneous hydronephrosis (DS/Shi and B6C3F1) submitted to the same carcinogenic treatment, no UUT tumor was observed. The rate of bladder carcinoma varied from 83 to 94% of the animals in the different strains, with no significant difference, showing susceptibility similar to that of the urothelium to BBN. In this manner, the NON/Shi strain was proposed as an experimental model of UUT in humans(26).

Although the time of exposure of the epithelium to the carcinogen and the rate of hydronephrosis may determine greater rates of carcinoma, there is no evidence of high rates of induction to date using this protocol. Inducing 240 animals as per the protocol described above, 75% presented bladder cancer and 7% cancer of the renal pelvis, and only 12 of these were microscopic tumors (12/240, 5%)(7).

In 1995, the first implantation model of human transitional carcinoma cells (TCC) in the UUT of immunosuppressed experimental animals was proposed. Implantation in the collector system occurred in only 45% (18 of 45 animals). Homozygote ("nude") rats, which do not develop the thymus, were inoculated with 1-5 x 10⁶ well-differentiated human RT4 TCC cells through surgical puncture of the inferior kidney pole. Previous unilateral ligation of the ureter to produce urinary stasis increased the rate of mucosal implantation to 67% (4 of the 6 animals studied), as well as mortality (25%). There was no ureteral tumor implantation(27).

Greater sensitivity of the renal pelvis of SD/cShi rats to the carcinogenic action of phenacetin was demonstrated. Eighty-one per cent (43/53) of the rats fed with 1 g/kg of body weight/day of phenacetin developed renal pelvis carcinoma in an average period of 78 weeks, and of these, 96% were TCC and 4% SCE. Ureteral and vesicle TCC occurred in 15 and 24%, respectively. SD/cShi rats were obtained from Sprague-Dawley (SD) rats in 1961 by Aburahi Lab. of Shionogi & Co., Ltd. (Shiga, Japan), of the Charles River Breeding Laboratory in the United States. The cause of the hydronephrosis, inherited by a recessive gene, was described as secondary to the stenosis of the ureteral meatus, although there are no specific studies on this topic. Only 38% of the animals that received phenacetin survived until the end of the protocol, 85 weeks(28).

Chart 1, modified from Reis et al., shows the advantages and disadvantages of the main experimental models of urothelial carcinoma induction(29).

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced by carcinogen</td>
<td>Human carcinogens, Immunocompetent, Multiple stages, causes, factors, process, Physiopathology similar to the human</td>
<td>Formation of calculi, Cystitis</td>
</tr>
<tr>
<td>Transgenic</td>
<td>Immunocompetent, Allow the identification of specific genes regarding carcinogens</td>
<td>Loss of tumor heterogenicity</td>
</tr>
<tr>
<td>Implanted tumor</td>
<td>Identical human cancer, Useful for the study of metastasis mechanisms, Immunosuppressed</td>
<td>Difficulty of orthotopic implantation, Invasive since the beginning</td>
</tr>
</tbody>
</table>

Source: modified from Reis et al.(29)
REFERENCES


