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Urinary Bladder Cancer - Old Models, New Opportunities

Oliveira PA*, Vala H, Gil da Costa RM, Pinto-Leite MR, Arantes-Rodrigues R and Vasconcelos-Nóbrega C

Department of Cytogenetic Laboratory, Hospital Center of Trás-os-Montes and Alto Douro, Portugal

Abstract

Urinary bladder cancer remains as one of most frequent tumours. To elucidate the reasons for the development of tumours, to find out which factors determine the tumour progression and to develop new and better treatments with fewer side effects, intensive research, with the combination of *in vitro* and *in vivo* studies is mandatory. So, in this manuscript we performed a revision concerning the different methodologies to study this disease. In authors' opinion, the best strategy to improve scientific knowledge for UBC should always rely in the association of *in vivo* and *in vitro* results. Currently, there are still many challenges in UBC diagnosis and therapy. These challenges must be faced as new opportunities. The molecular diagnostics and genomic revolution will be fundamental to develop new therapeutic modalities, and also to promote personalized therapies.

Keywords: Rat; Mouse; Carcinogenesis

Introduction

Urinary Bladder Cancer (UBC) remains as one of most frequent tumours, being the second most frequent tumour of the genitourinary tract, only surpassed by the prostate cancer [1-3]. UBC ranges from mild disease, with a low mortality rate (however with high recurrence), to extremely high-grade tumors, associated with metastasis and high mortality. Notably, UBC is one of the most costly cancers to treat, primarily due to the considerable costs associated with life-long clinical management of patients with non-muscle invasive disease, as well as those associated with the cost of caring for patients after surgical removal of the urinary bladder [4-6]. With a clear correlation with environmental exposures, such as tobacco smoke, industrial chemicals, dietary nitrates and arsenic [1,7-12] UBC affects more men than women (2-3x).

Nevertheless, despite its prevalence and adverse impact on human health, UBC has been remarkably understudied relative to other cancers [6].

To elucidate the reasons for the development of tumours, to find out which factors determine the tumour progression and to develop new and better treatments with fewer side effects, intensive research, with the combination of *in vitro* and *in vivo* studies, is mandatory.

In Vitro Studies: Cell Lines

Cancer cell lines are routinely used for various kinds of biomedical research, from drug-sensitivity tests to identify potential therapy targets and pharmacologically useful compounds [13-15]. For the study of UBC, several cell lines have been established. T24, HT1376, 5637, UM-UC-3 are some examples of human bladder cancer cell lines [13]. Some have origin in superficial tumours but the majority are from invasive and metastatic ones [13,14]. Cell lines from experimental UBC are fewer than those of human origin. AY-27 and NBT-II are UBC cell lines derived from rats [13,16-18]. BTT-T739 and MB49 are examples of bladder cancer cell lines with origin in mice [13,19].

In vitro studies with cell lines demand a great amount of care concerning the origin of cell lines. It is crucial to ensure that they are reliable, because cell cross-contamination is a common problem during cell culturing and use. Cross-contamination provides misleading research results leading to unusable therapeutic products. The unwitting use of misidentified cell lines may, ultimately, expose patients to inappropriate, or even harmful, treatments.

In urological research, cell lines, particularly human urothelial cell lines, are well-established tools for preclinical trials. For most cytotoxic agents, if it does not work *in vitro*, it will most certainly not work *in vivo*. If it works *in vitro*, then there is the possibility it may be effective *in vivo*. They are a cost-efficient method of searching for drug activity and can further our understanding of drugs' action on several tumours. Other advantages in using cell lines can also be highlighted: they are easy

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Correspondence:
Oliveira PA, Department of Cytogenetic Laboratory, Hospital Center of Trás-os-Montes and Alto Douro, Portugal, E-mail: Portugal.pamo@utad.pt

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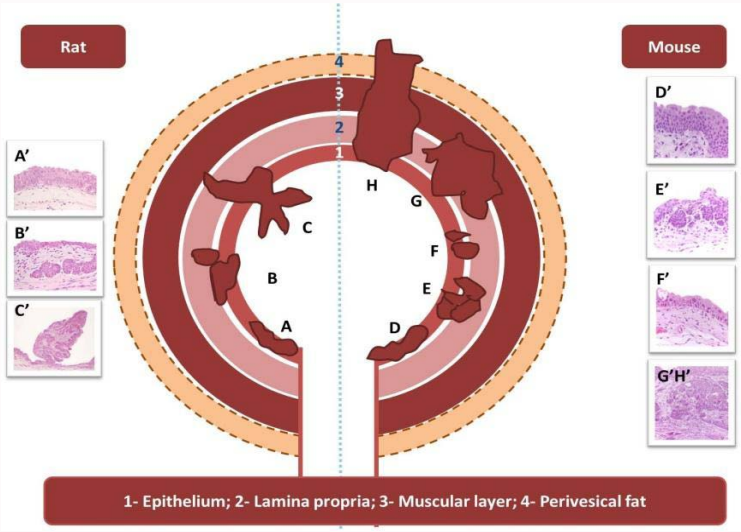

Figure 1: Scheme of the invasion status of bladder tumors in rat and mouse models. **Rat** – Non-invasive tumours. Lesions are located primarily at the epithelium and eventually lamina propria. [A and A': Simple hyperplasia; A': (H&E, 200x); B and B': Nodular hyperplasia; B': (H&E, 200x);C and C': Papilloma; C': (H&E, 100x)]; **Mouse**: Invasive tumors that can penetrate deeper layers of the bladder such as muscular layer, perivesical tissue and extravesical organs. [D and D': Simple hyperplasia; D': (H&E, 600x); E and E': Nodular hyperplasia; E': (H&E, 400x); F and F': Carcinoma *in situ*; F': (H&E, 400x); G, G', Hand H': Invasive carcinoma; G', H': (H&E, 200x)].

to handle and can be replicated almost infinitely. Additionally, they exhibit a relatively high degree of homogeneity. Cell lines, however, have some disadvantages. They are prone to genotypic and phenotypic drift during their continual culture. Subpopulations may arise and cause phenotypic changes over time by the selection of specific, more rapidly growing clones within a population [20].

In Vivo Studies: Animal Models

There is a dire need for relevant animal models for research to improve the treatment and management of humans (and animals) with UBC.

Animal models have greatly contributed to the understanding of carcinogenesis, establishing the bridge between *in vitro* laboratory investigations and studies in humans [21]. Animal models allow the investigation of aspects that cannot be studied under clinical conditions, such as the evaluation of new chemotherapeutic, immunotherapeutic or prophylactic agents, drug regimens, or other treatment methods and can also provide further information on basic mechanisms of tumour growth and spread [22,23].

Although there is the possibility to use several animal species, rodents (rats and mice) are those most often used in animal experimentation [24-38]. UBC can be established subcutaneously (heterotopically) by transplantation of tumour cells, or intravesically (orthotopically) either by transplantation of tumour cells or by chemical induction [19,22,30].

Our team has developed an intense research work with chemically induced bladder cancer models, namely rats and mice [39-49]. They are orthotopic models, developed in immunocompetent animals. These models have the great advantage of simulating the local cancer environment where the influence of the immune system and the anatomical and physiological factors of the tissue of origin, which undoubtedly influence the metastatic process, are not affected [19,30,32,39].

Rodent Models of UBC Chemically Induced

The rodent models of chemically induced UBC can be used to test prophylactic drugs, to test therapeutic drugs and also to define the impact of chemical carcinogens on other organs. There are several chemical carcinogens that can be used to induce bladder cancer in experimental animals. N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), N-[4-(5-nitro-2furyl)-2-thiazolyl] formamide (FANFT) and N-methyl-N-nitrosourea (MNU), are some examples [33-34,50-52], being the first one (BBN) the most used carcinogen in UBC experimental oncology studies.

BBN is an indirect carcinogen: after ingestion it is metabolized mainly in the liver, but also in the urinary bladder, into several metabolites that reach the urinary bladder through urine and come into contact with the urothelium, binding covalently to cellular macromolecules and initiating the carcinogenic process [53,54-57].

BBN-induced urothelial lesions in rodents resemble human urothelial lesions in their morphological characteristics. However, the spectrum of urothelial lesions is different in rats and in mice.

Rat model of UBC resembles non-invasive bladder cancer of humans, with papillary neoplasm, while mouse model resembles flat urothelial lesions evolving to invasive bladder cancer. In rat model of UBC, it is possible to observe simple hyperplasia, dysplasia, nodular hyperplasia, papilloma and papillary neoplasm. In mouse model, urothelial lesions that arise from the BBN administration are different. They can be initiated with simple hyperplasia, progress to displasia, carcinoma *in situ* and later on invasive carcinoma.

Figure 1, summarizes the development of urothelial lesions in rats and mice, also showing the invasion grade of the lesions.

Both rodent models of chemically induced bladder cancer are extremely useful in urologic oncology research, since they represent two variables of the same disease in humans. If the non-invasive pathway has a better prognosis, it is also true that it can relapse. Invasive

pathway, studied in mice models, similarly with the development of UBC in humans, can metastasize. It is important to understand these differences in order to delineate better experiments, according to its main purpose and to achieve improved and translatable results. If the idea of the use of these models is not new, the study possibilities that they offer are never ending.

In authors' opinion, the best strategy to improve scientific knowledge for UBC should always rely in the association of *in vivo* and *in vitro* results. Currently, there are still many challenges in UBC diagnosis and therapy. These challenges must be faced as new opportunities. The molecular diagnostics and genomic revolution will be fundamental to develop new therapeutic modalities, and also to promote personalized therapies.

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