



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

OPEN ACCESS

*Correspondence:

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

Received Date: 06 Jul 2017

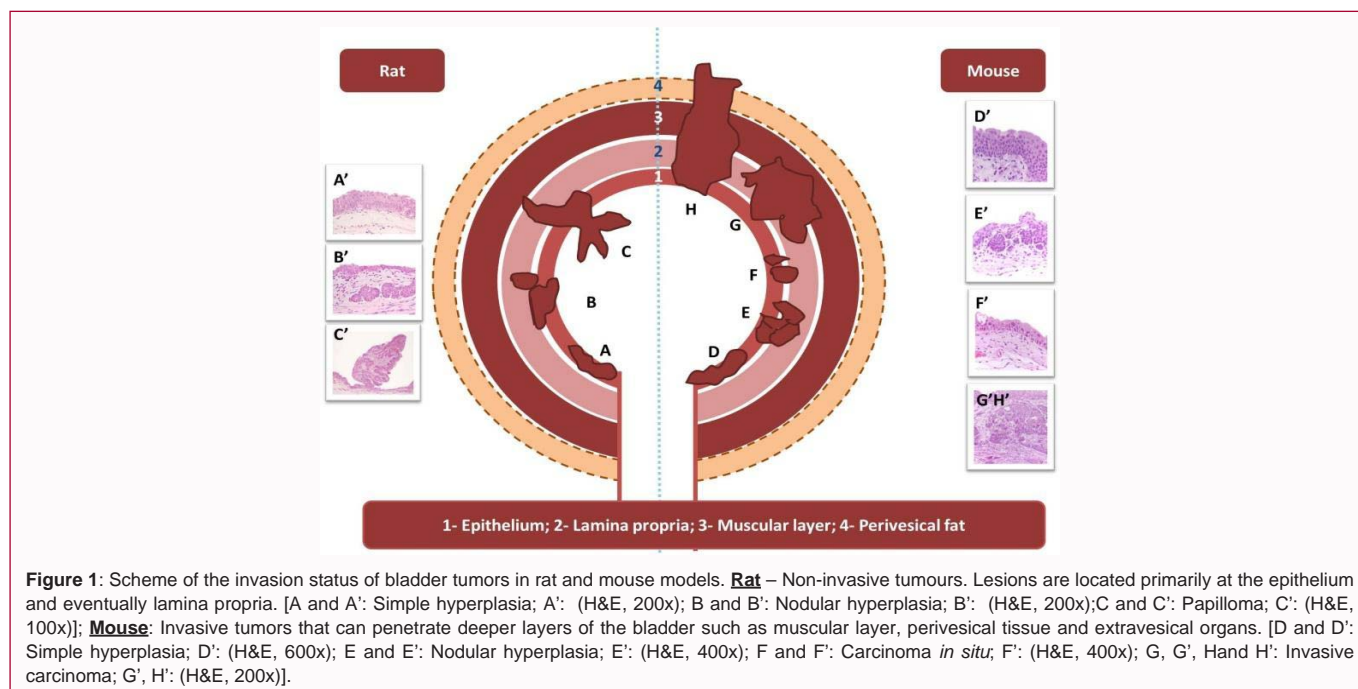
Accepted Date: 20 Aug 2017

Published Date: 14 Sep 2017

Citation:

Oliveira PA, Vala H, Gil da Costa RM, Pinto-Leite MR, Arantes-Rodrigues R, Vasconcelos-Nóbrega C. Urinary Bladder Cancer - Old Models, New Opportunities. *Clin Oncol.* 2017; 2: 1349.

Copyright © 2017 Oliveira PA. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



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 dysplasia, 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.

Acknowledgments

This work is financed by national funds through FCT - Fundação para a Ciência e Tecnologia, I.P., under the project UID/Multi/04016/2016. Furthermore we would like to thank the Instituto Politécnico de Viseu and CI&DETS for their support.

This work is supported by European Investment Funds by FEDER/COMPETE/POCI- Operacional Competitiveness and Internacionalization Programme, under Project POCI-01-0145-FEDER-006958 and National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2013. Furthermore we would like to thank to UTAD and CITAB for their support.

References

- Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017; 67(1): 7-30.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011; 61(2): 69-90.
- Winer E, Gralow J, Diller L, Karlan B, Loehrer P, Pierce L, et al. Clinical Cancer Advances 2008: Major research advances in cancer treatment, prevention, and screening-a report from the American Society of Clinical Oncology. *J Clin Oncol*. 2009; 27(5): 812-826.
- Botteman MF, Pashos CL, Hauser RS, Laskin BL, Redaelli A. Quality of life aspects of bladder cancer: a review of the literature. *Qual Life Res*. 2003; 12(6): 675-688.
- Yeung C, Dinh T, Lee J. The Health Economics of Bladder Cancer: An Updated Review of the Published Literature. *Pharmacoeconomics*. 2014; 32(11): 1093-1104.
- Kobayashi T, Owczarek TB, McKiernan JM, Abate-Shen C. Modelling bladder cancer in mice: opportunities and challenges. *Nat Rev Cancer*. 2015; 15(1): 42-54.
- de Braud F, Maffezzini M, Vitale V, Bruzzi P, Gatta G, Hendry WF, et al. Bladder cancer. *Crit Rev Oncol Hematol*. 2002; 41(1): 89-106.
- Fadl-Elmula I. Chromosomal changes in uroepithelial carcinomas. *Cell Chromosome*. 2005; 4: 1.
- Volanis D, Kadiyskaa T, Galanis A, Delakasa D, Logothetic S, Zoumpourlisc V. Environmental factors and genetic susceptibility promote urinary bladder cancer. *Toxicol Lett*. 2010; 193(2): 131-137.
- Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of some aromatic amines, organic dyes, and related exposures. *Lancet Oncol*. 2008; 9(4): 322-323.
- Clapp RW, Jacobs MM, Loehler EL. Environmental and occupational causes of cancer: new evidence 2005-2007. *Rev Environ Health*. 2008; 23(1): 1-37.
- Takkouche B, Regueira-Méndez C, Montes-Martínez A. Risk of cancer among hairdressers and related workers: a meta-analysis. *Int J Epidemiol*. 2009; 38(6): 1512-1531.
- Gabriel U, Bolenz C, Michel MS. Experimental Models for Therapeutic Studies of Transitional Cell Carcinoma. *Anticancer Res*. 2007; 27(5A): 3163-3171.
- Hatina J, Huckenbeck W, Rieder H, Seifert HH, Schulz WA. [Bladder carcinoma cell lines as models of the pathobiology of bladder cancer. Review of the literature and establishment of a new progression series]. *Urologe A*. 2008; 47(6): 724-34.
- Tsuji K, Kawauchi S, Saito S, Furuya T, Ikemoto K, Nakao N, et al. Breast cancer cell lines carry cell line-specific genomic alterations that are distinct from aberrations in breast cancer tissues: Comparison of the CGH profiles between cancer cell lines and primary cancer tissues. *BMC Cancer*. 2010; 10: 10-15.
- Nishi M. A cell line derived from BBN (N-butyl-N-[4-hydroxybutyl]-nitrosamine)-induced rat bladder cancer: establishment and scanning electron microscopic cell surface characteristics. *Acta Med Okayama*. 1978; 32(3): 181-205.
- Chen JJ, Ye ZQ, Koo MW. Growth inhibition and cell cycle arrest effects of epigallocatechin gallate in the NBT-II bladder tumour cell line. *BJU Int*. 2004; 93(7): 1082-1086.
- Hojo H, Kaneko A, Kayagaki N, Saki M, Hashimoto Y. Subcellular localization and characterization of interleukin-1 alpha produced by rat bladder cancer cells. *Immunol Lett*. 1994; 43(3): 215-220.
- Arentsen HC, Hendricksen K, Oosterwijk E, Witjes JA. Experimental rat bladder urothelial cell carcinoma models. *World J Urol*. 2009; 27(3): 313-317.
- Burdall SE, Hanby AM, Lansdown MR, Speirs V. Breast cancer cell lines: friend or foe? *Breast Cancer Res*. 2003; 5(2): 89-95.
- Reis LO, Pereira TC, Favaro WJ, Cagnon VHA, Lopes-Cendes I, Ferreira U. Experimental animal model and RNA interference: a promising association for bladder cancer research. *World Journal of Urology*. *World J Urol*. 2009; 27(3): 353-361.
- Günther JH, Jurczok A, Wulf T, Brandau S, Deinert I, Jocham D, et al. Optimizing syngeneic orthotopic murine bladder cancer (MB49). *Cancer Res*. 1999; 59(12): 2834-2837.
- Steele VE, Lubet RA. The use of animal models for cancer chemoprevention drug development. *Semin Oncol*. 2010; 37(4): 327-338.
- Okajima E, Hiramatsu T, Hirao K, Ijuin M, Hirao Y, Babaya K, et al. Urinary bladder tumors induced by N-butyl-N-(4-hydroxybutyl) nitrosamine in dogs. *Cancer Res*. 1981; 41(5): 1958-1966.
- Cohen AE, Weisburger EK, Weisburger JH, Ward JM, Putnam CL. Cystoscopy of chemically induced bladder neoplasms in rabbits administered the carcinogen dibutyl nitrosamine. *Invest Urol*. 1975; 12(4): 262-266.
- Cohen SM. Cell proliferation and carcinogenesis. *Drug Metab Rev*. 1998; 30(2): 339-357.
- Hueper WC. Aniline tumors of the bladder. *Arch Pathol. Invest Urol*. 1975; 12(4): 262-266.
- Kunze E, Chowanec J. Pathology of tumours in laboratory animals. Tumours of the rat. Tumours of the urinary bladder. *IARC Sci Publ*. 1990; (99): 345-397.
- Clayson DB, Fishbein L, Cohen SM. Effects of stones and other physical factors on the induction of rodent bladder cancer. *Food Chem Toxicol*. 1995; 33(9): 771-784.
- Chan ES, Patel AR, Smith AK, Klein JB, Thomas AA, Heston WD, et al. Optimizing orthotopic bladder tumor implantation in a syngeneic mouse model. *J Urol*. 2009; 182(6): 2926-2931.

31. Chan E, Patel A, Heston W, Larchian W. Mouse orthotopic models for bladder cancer research. *BJU Int.* 2009; 104(9): 1286-1291.
32. Levett D, Flecknell PA, Rudland PS, Barraclough R, Neal DE, Mellon JK, et al. Transfection of S100A4 produces metastatic variants of an orthotopic model of bladder cancer. *Am J Pathol.* 2002; 160(2): 693-700.
33. Lijinsky W. Chemistry and biology of N-nitroso compounds. In: Cambridge Monographs on Cancer Research. Cambridge University Press, Cambridge UK. 1992; 67-82.
34. druckrey H, Preussmann R, Ivankovic S, Schmidt Ch, Mennel Hd, Stahl Kw. [Selective Induction Of Bladder Cancer In Rats By Dibutyl- And N-Butyl-N-Butanol(4)-Nitrosamine]. *Z Krebsforsch.* 1964; 66: 280-290.
35. Swaminathan S, Bryan GT. Biotransformation of the bladder carcinogen N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide in mice. *Cancer Res.* 1984; 44(6): 2331-2338.
36. Cui L, Shi Y, Daib G, Panb H, Chen J, Song L, et al. Modification of N-methyl-N-nitrosourea initiated bladder carcinogenesis in Wistar rats by terephthalic acid. *Toxicol Appl Pharmacol.* 2006; 210(1-2): 24-31.
37. Reis LO, Fávoro WJ, Ferreira U, Billis A, Fazuoli MG, Cagnon VH. Evolution on experimental animal model for upper urothelium carcinogenesis. *World J Urol.* 2010; 28(4): 499-505.
38. Martín-Sanz P, Mayoral R, Casado M, Boscá L. COX-2 in liver, from regeneration to hepatocarcinogenesis: what we have learned from animal models? *World J Gastroenterol.* 2010; 16(12): 1430-1435.
39. Oliveira PA, Pires MJ, Nóbrega C, Arantes-Rodrigues R, Calado AM, Carrola J, et al. Technical report: Technique of bladder catheterization in female mice and rats for intravesical instillation in models of bladder cancer. *Scand J Lab Anim Sci.* 2010; 37(4): 253-259.
40. Vasconcelos-Nóbrega C, Colaço A, Lopes C, Oliveira PA. Review: BBN as an urothelial carcinogen. *In Vivo.* 2012; 26(4): 727-739.
41. Oliveira PA, Vasconcelos-Nóbrega C, Gil da Costa R, Arantes-Rodrigues R. The N-butyl-N-4-hydroxybutyl Nitrosamine Mouse Urinary Bladder Cancer Model. *Methods in Molecular Biology. Methods Mol Biol.* 2018; 1655: 155-167.
42. Oliveira PA, Gil da Costa RM, Vasconcelos-Nóbrega C. Challenges with in vitro and in vivo experimental models of urinary bladder cancer for novel drug discovery. *Expert Opin Drug Discov.* 2016; 11(6): 599-607.
43. Oliveira PA, Arantes-Rodrigues R, Vasconcelos-Nóbrega C. Animal models of urinary bladder cancer and their application to novel drug discovery. *Expert Opin Drug Discov.* 2014; 9(5): 485-503.
44. Alvarado A, Arantes-Rodrigues R, Vasconcelos-Nóbrega C, Da Costa R, Pinto-Leite MR, Faustino-Rocha AI. Urinary bladder chemical carcinogenesis in laboratory rodents as an experimental model [carcinogénesis química de vejiga urinaria en roedores de laboratorio como modelo experimental]. *Revista Venezolana de Oncología.* 2015; 27: 57-63.
45. Pinto-Leite R, Carreira I, Melo J, Ferreira SI, Ribeiro, Ferreira J, et al. Genomic characterization of three urinary bladder cancer cell lines: Understanding genomic types of urinary bladder cancer. *Tumor Biology. Tumour Biol.* 2014; 35(5): 4599-4617.
46. Vasconcelos-Nóbrega C, Pinto-Leite R, Arantes-Rodrigues R, Ferreira R, Brochado P, Cardoso ML, et al. *In vivo* and *in vitro* effects of RAD001 on bladder cancer. *Urologic Oncology: Seminars and Original Investigations.* 2013; 31: 1212-1221.
47. Arantes-Rodrigues R, Colaço A, Pinto-Leite R, Oliveira PA. *In Vitro* and *In Vivo* experimental models as tools to investigate the efficacy of antineoplastic drugs on urinary bladder cancer. *Anticancer Res.* 2013; 33(4): 1273-96.
48. Palmeira C, Oliveira PA, Lameiras C, Amaro T, Silva VM, Lopes C, et al. Biological similarities between murine chemical-induced and natural human bladder carcinogenesis. *Oncol Lett.* 2010; 1(2): 373-377.
49. Oliveira PA, Colaco A, De la Cruz P LF, Lopes C. Experimental bladder carcinogenesis-rodent models. *Exp Oncol.* 2006; 28(1): 2-11.
50. Swaminathan S, Bryan GT. Biotransformation of the bladder carcinogen N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide in mice. *Cancer Res.* 1984; 44(6): 2331-2338.
51. Cui L, Shi Y, Dai G, Pan H, Chen J, Song L, et al. Modification of N-Methyl-N-Nitrosourea initiated bladder carcinogenesis in Wistar rats by terephthalic acid. *Toxicol Appl Pharmacol.* 2006; 210(1-2): 24-31.
52. Reis LO, Fávoro WJ, Ferreira U, Billis A, Fazuoli MG, Cagnon VH. Evolution on experimental animal model for upper urothelium carcinogenesis. *World J Urol.* 2010; 28(4): 499-505.
53. Cohen SM. Urinary bladder carcinogenesis. *Toxicol Pathol.* 1998; 26(1): 121-127.
54. Iida K, Itoh K, Maher JM, Kumagai Y, Oyasu R, Mori Y, et al. Nrf2 and p53 cooperatively protect against BBN-induced urinary bladder carcinogenesis. *Carcinogenesis.* 2007; 28(11): 2398-2403.
55. Suzuki E, Okada M. Metabolic fate of N-butyl-N-(4-hydroxybutyl) nitrosamine in the rat. *Gan.* 1980; 71(6): 856-862.
56. Mochizuki M, Suzuki E, Okada M. [Structure and metabolic fate of N-nitrosodialkylamines in relation to their organotropic carcinogenicity with special reference to induction of urinary bladder tumors]. *Yakugaku Zasshi.* 1997; 117(10-11): 884-894.
57. Cohen SM, Ohnishi T, Clark NM, He J, Arnold LL. Investigations of rodent urinary bladder carcinogens: collection, processing, and evaluation of urine and bladders. *Toxicol Pathol.* 2007; 35(3): 337-347.