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Review

Appraising Animal Models of Prostate Cancer for Translational Research: Future Directions

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Abstract. *The growing incidence of prostate cancer has prompted a great investment in basic biology and translational studies to develop new therapies. Multiple animal models have been established to study etiological factors, cancer-preventive strategies and the molecular determinants of aggressiveness and metastases. The rat model of prostate cancer induced by chemical carcinogen N-methyl-N-nitrosourea (MNU) and testosterone exposure has become an important tool to study prostatic carcinogenesis and chemopreventive approaches. Over prolonged treatment, this model develops prostatic lesions that closely mimic those observed in human patients. By modifying the experimental conditions, different research groups have been able to induce a vast spectrum of lesions, ranging from early prostatic intraepithelial neoplasia to metastatic cancer. These carefully tuned experimental settings allowed researchers to test lifestyle interventions, and different pharmacological and chemopreventive strategies. However,*

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this model's great flexibility requires careful planning to ensure that the experimental conditions are adequate to obtain the spectrum of lesions intended. The present review addresses such issues, highlighting the value of the rat prostate cancer model and the multiple challenges and opportunities it offers to researchers worldwide.

Prostate cancer (PCa) is among the most prevalent cancers worldwide. In 2020, it was estimated to affect 1.4 million men and to have caused 375,304 deaths worldwide, according to the latest World Health Organization report (1). To understand the complex biopathology of PCa, and therefore be able to rationally develop preventive and therapeutic strategies, it is necessary to use animal models as well as alternative non-animal models (*e.g.*, *in vitro*). There are several in vivo models available for the study of PCa, as previously reviewed by our group and others (2-8). However, these models have to be validated, considering that some of them may be more useful to evaluate specific aspects of the disease while other models may be suited for other purposes.

Among the animal models available for the study of PCa, chemically and/or hormonally-induced rat models are widely used in chemopreventive studies (4, 7, 9). However, before choosing the model, it is important to take into consideration that the rat prostate is composed for four lobes with different histological characteristics and physiological functions. These lobes are named according to their relative position to the urinary bladder: ventral, dorsal, lateral, and anterior (also classified as the coagulating gland) (5, 6, 10). The human prostate is anatomically different from its rat counterpart, consisting of a compact encapsulated gland, pyramid-shaped, with a fibromuscular stroma, located between the urinary bladder and the rectum (11). Therefore, these anatomical differences must be taken into consideration when using rats to study PCa (4), particularly at the time of sample collection, after the animals' sacrifice. Although some authors consider dorsal and lateral prostate lobes homologous to human prostate, it remains a controversial issue (12).

N-nitrosobis-(2-oxopropyl)-amine (BOP), 3,2-dimethyl-4-aminobiphenyl (DMAB), 2-amino-1-methyl-6-phenylimidazol [4,5-b]pyridine (PhiP) and N-Methyl-N-nitrosourea (MNU) are the four chemical compounds described in literature to induce PCa in laboratory rats (4). BOP belongs to the family of nitrosamines and induces tumors not only in the prostate, but also in the nasal cavity, colorectum and urothelium in rats (13-15). Due to this, BOP is not the most suitable to promote PCa, because inducing tumors in so many organs will create confusion in the interpretation of the results, particularly in those from body fluids analysis. Testosterone treatment may be used in combination with BOP and has been reported to induce the development of adenocarcinomas and squamous cell carcinomas in the dorsolateral and ventral prostate in MRC rats (16). The aforementioned problems associated with BOP are not overcome with the use of this carcinogen. The DMAB is classified as a polycyclic aromatic hydrocarbon with multi-organ tropism, inducing tumors in the colon, urinary bladder, pancreas, prostate, mammary glands, preputial glands, seminal vesicles, and Zymbal glands (6, 17). Chronic administration of high doses of testosterone through subcutaneous implants in combination with DMAB may be used to promote tumor development (16). This combination was reported to produce a high incidence of invasive adenocarcinomas in the dorsolateral and anterior prostate lobes, but not in the ventral prostate in F344 rats (18). The tumors developed by the administration of DMBA plus testosterone are histologically and biologically indistinguishable from those induced by MNU in combination with testosterone (6). PhIP is a heterocyclic amine and may be metabolized to biologically active metabolites (N-hydroxy-PhIP and N-acetoxy-PhIP) that form DNA adducts (19). Shirai and colleagues exposed F344 rats to PhIP, at a dose of 400 ppm mixed in the diet, for 52 weeks (20) and reported the development of adenocarcinomas in the ventral prostatic lobe, histopathologically identical to those induced by DMAB. MNU does not require metabolic activation, being a direct-acting alkylating agent that methylates guanines. This carcinogenic agent may induce tumors in various organs, such as the breast, colon, urinary bladder, retina, and prostate (4, 21), but organ specificity depends on the animals' age and sex, and the dose and route of administration. Pollard and colleagues developed a method to induced prostate cancer in Lobund-Wistar rats through the administration of MNU associated with hormonal treatment (22, 23). This protocol induced adenocarcinomas and atypical hyperplastic lesions in the ventral, dorsolateral, and anterior prostate. Later, Marteen C. Bosland developed a chemical carcinogen plus testosterone rat model of prostate carcinogenesis that resembles human PCa in several aspects and became the most widely used animal model (7, 24). First, rats are treated daily with an antiandrogen, such as flutamide or cyproterone acetate, to inhibit prostate epithelial cell proliferation, followed by the administration of testosterone propionate to induce a synchronous cell proliferation peak. Following this step, a single intraperitoneal MNU injection is given, targeting the proliferating cell population. Finally, the rats receive low-dose testosterone via slow-release silastic implants until the end of experiment to sustain tumor development. This long multistep protocol was reported to induce a high incidence of adenocarcinomas in the rat dorsolateral prostate, 12-13 months after MNU administration (24-26). Considering the wide use of the Bosland rat model for PCa chemoprevention studies, the present work aimed to critically evaluate this animal model of cancer, appraising tumor incidence, location, and histological characteristics.

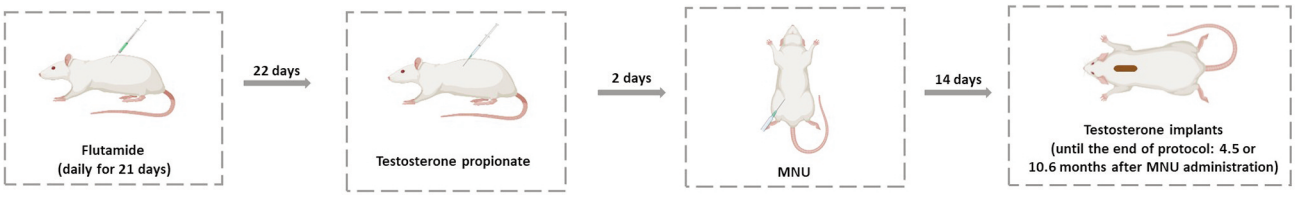

Figure 1. *Prostate cancer induction protocol.*

The Follow up of the Boslands' Prostate Cancer Model

As mentioned above, careful modulation of the experimental conditions allows researchers to study a different spectrum of prostatic lesions using variations of the Boslands' rat model. Our group performed an experimental animal protocol with two different timepoints of animals' sacrifice: 4.5 or 10.6 months after MNU administration to understand the spectrum of dorsolateral prostate lesions induced. To achieve this goal, we used male Wistar Unilever rats (Rattus norvegicus) at 12 weeks of age, based on the original Bosland protocol (7, 25) (Figure 1).

Shortly, the anti-androgenic drug flutamide (50 mg/kg; TCI Chemicals, Portland, OR, USA) was administered subcutaneously for 21 consecutive days. Twenty-four hours after the last flutamide administration, testosterone propionate (TCI Chemicals) was dissolved in corn oil and administered subcutaneously (100 mg/kg). Two days later, the rats were intraperitoneally injected with MNU (30 mg/kg; Isopac®, Sigma Chemical Co., Madrid, Spain). Two weeks later, testosterone implants were subcutaneously implanted in the interscapular region of animals and maintained until the end of the experimental protocol. For testosterone implantation, the animals were anesthetized with ketamine (75 mg/kg, Imalgene® 1000, Merial S.A.S., Lyon, France) and xylazine (10 mg/kg, Rompun® 2%, Bayer Healthcare S.A., Kiel, Germany). The testosterone implants were made from silastic tubing, sealed with G.E. RTV-108 adhesive sealant, filled with 3 cm tightly packed crystalline testosterone (Sigma Chemical, Madrid, Spain) with the aid of a small spatula and sealed with a clip previously sterilized in an autoclave. The implants were weighed to ensure that all tubes had the same amount of testosterone. The tubes were always kept upright, and the sealant was placed on the tube ends after they were completely filled. These silastic tubes remained in the induced animals until the end of the experimental protocol. The biggest difficulty associated with this induction protocol was the preparation of the flutamide, because it precipitates after preparation, even in the needle, thus it is necessary to be in constant agitation, not only during the preparation but also in the syringe.

Using this experimental protocol, no macroscopic prostate lesions nor metastases were observed in any experimental group in either the first or the second timepoint. Prostatic lesions were observed in the first sacrifice, including: low-grade dysplastic lesions (40% of animals), prostatic intraepithelial neoplasia (PIN) (20% of animals), and microinvasive carcinomas (10% of animals) in the dorsolateral prostate. As expected, a significantly higher number of dorsolateral prostate lesions were observed at 61 weeks of age, with dysplasia occurring in 85.7% of animals, and PIN and microinvasive carcinomas in 64.3% of animals. The animals of the second sacrifice were exposed to the implants for 44 weeks whereas the animals sacrificed first were only exposed for 18 weeks. This is in agreement with other studies and demonstrated that the longer the exposure to testosterone by slow-release implants, the greater the number of lesions (24, 26). It is worth noting that control rats sacrificed at 61 weeks-old also developed lesions, although at a much lower frequency than those observed in treated rats. These spontaneous lesions observed in the control group may be explained by the animals' advanced age and seemed to mimic what happens in older men (27, 28), who are more susceptible to alterations and prostate lesions development. Therefore, age matters in both men and rats.

However, contrary to what is described in other studies (24, 29-31), our animals did not develop macroscopic lesions in the dorsolateral prostate lobes, nor metastases. In a detailed review about this chemical and hormonally rat model of PCa, Bosland *et al*. reported that neoplastic development requires at least eight to nine months (and more commonly, 12-13 months) after the MNU injection (24). In our first sacrifice, animals were sacrificed 4.5 months after the MNU injection, what was probably an insufficient period for the development of large malignant lesions and metastases. In the second experiment, animals were euthanized 10.6 months after MNU injection, logically increasing the incidence of microinvasive lesions.

The dorsolateral prostate also showed acute inflammation of the acini, focal necrosis, and reactive hyperplasia, with small focal areas of chronic stromal inflammation. Focal chronic inflammation with stromal fibrosis and mononucleated cell infiltration was identified in all groups. Inflammation was also frequently reported by other studies (32, 33) and reported

as more common and severe in the dorsolateral prostate as observed by our research team. All groups also developed acute serous or purulent inflammation in the dorsolateral prostate acini. The most extensive and severe inflammation foci were observed at the second timepoint. These findings highlight the usefulness of this model to study the role of chronic inflammation in prostatic carcinogenesis. Curiously, no changes in the levels of circulating inflammatory markers (*e.g.*, C-reactive protein, albumin, interleukin-6) were observed in this model, which may be justified by the anti-inflammatory role of testosterone. Indeed, the serum levels of testosterone and 17beta-estradiol were approximately 30 times higher in PCa rats compared to control ones.

Liver histological analysis did not reveal significant alterations promoted by the administration of flutamide, testosterone and MNU, and these data were corroborated by serum biochemistry results, with no significant changes in the levels of hepatic functions parameters, such as alanine aminotransferase. However, as this model was dependent on testosterone, the serum levels of this hormone were high in PCa animals, compared to the control animals (as mentioned above).

Appraising Animal Welfare in the Rat Model of Prostate Cancer

Animal models of cancer are prone to develop severe pain, weight loss and other distressing conditions. These conditions must be adequately monitored and controlled by the research team, potentially imposing a premature animals' sacrifice to avoid further suffering, or in some cases, to avoid biasing results (34). It is therefore important to appraise rats' health status, especially due to the long time course of the experiments.

During our protocols, we observed that rats displayed a normal mental status, normal eyes aspect, ears and whiskers position, response to handling, breathing and hydration status. Despite this, we noted that rats with PCa were less active when compared with matched controls, especially towards the end of the experimental protocol. No animals died during the experiment and there was no need to sacrifice any rat before the end of the protocol. Thus, contrary to what is observed in other cancer models, this model does not induce animals' suffering.

Discussion

Presently, there is a growing amount of animal models to study PCa. However, most of these are mouse models, including xenografts carrying PCa cell lines (35) or tissues transplanted from PCa patients (36), syngeneic cell-based models (37), or genetically-modified mouse strains [*e.g.*, employing the probasin gene promoter to target oncogene expression to the prostate (38)]. The rat model of prostate cancer presents considerable advantages to study the multi-step development of prostate cancer induced by factors known to be involved in human prostatic carcinogenesis, such as androgen stimulation, chemical carcinogens exposition, and to evaluate the effects of chemopreventive agents and lifestyle interventions. Furthermore, this model may be very promising to study the role of chronic prostatic inflammation in tumorigenesis and tumor progression. Inflammation is one of the hallmarks of cancer, and several studies have implicated chronic inflammation in the occurrence and progression of PCa (39, 40). However, downsides of this model include the long time required to induce cancer and the limited molecular data available, compared with other PCa rodents' models (36).

The rat model of PCa has been mainly used to identify substances that could prevent PCa development and test their chemopreventive properties (29, 41-50). In most published studies concerning the model of PCa induced by MNU and testosterone, animal body weight variation and the incidence of histological prostatic lesions are the most analyzed variables (29, 41-50). However, other variables must be collected and analyzed to draw more information from induced rats, namely, water and food consumption, relative organs' weight, blood serum concentration (hematological and biochemical), and histopathological analysis. Our group also performed the first follow-up study of the rat's prostatic dimensions using ultrasonography (51). This monitorization allowed a detailed study of the rat prostate and the monitoring of prostate size during PCa induction or normal animal growth (51). The follow-up of aspects related to animal welfare are of paramount importance for in vivo experiments. While this has been attempted by our team, a more systematic characterization, especially at more prolonged timepoints, remains missing. Taken together, these approaches will contribute to the addition of layers of complexity to this valuable research tool and direct future investigations.

Conclusion

The Boslands' rat model of PCa is robust, reproducible and allows researchers to study multi-step prostate lesions over time. This has been particularly useful for testing potential chemopreventive approaches. We suggest that further refinement will require a more detailed knowledge of the timeline of prostate lesions development at earlier timepoints and establishing the genomic, transcriptomic, and DNA methylation profile of each kind of lesion. This approach may potentially help elucidating the molecular determinants that underpin highly aggressive metastatic, castration-resistant PCa and the development of prostate small-cell carcinomas. This rat model is also useful for elucidating the role of prostate inflammation at the various

stages of prostatic carcinogenesis. Finally, this model holds promise for the development of liquid biopsies from blood and urine to try to validate markers of prostatic lesions at an early stage.

Conflicts of Interest

All Authors declare no actual, potential, or perceived conflicts of interest that would prejudice the impartiality of the study.

Authors' Contributions

E.N-G. and R.M.G.C. drafted the manuscript. All Authors revised and edited the manuscript and approved the final submitted version.

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References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *71(3)*: 209-249, 2021. PMID: 33538338. DOI: 10.3322/caac.21660

2 Nascimento-Gonçalves E, Ferreira R, Oliveira PA and Colaço BJA: An overview of current alternative models for use in the context of prostate cancer research. Altern Lab Anim *48(2)*: 58-69, 2020. PMID: 32614643. DOI: 10.1177/0261192920929701

3 Nascimento-Gonçalves E, Seixas F, Ferreira R, Colaço B, Parada B and Oliveira PA: An overview of the latest in state-of-the-art murine models for prostate cancer. Expert Opin Drug Discov *16(11)*: 1349-1364, 2021. PMID: 34224283. DOI: 10.1080/17460441.2021.1943354

4 Nascimento-Gonçalves E, Faustino-Rocha AI, Seixas F, Ginja M, Colaço B, Ferreira R, Fardilha M and Oliveira PA: Modelling human prostate cancer: Rat models. Life Sci *203*: 210-224, 2018. PMID: 29684445. DOI: 10.1016/j.lfs.2018.04.014

5 Roy-Burman P, Wu H, Powell WC, Hagenkord J and Cohen MB: Genetically defined mouse models that mimic natural aspects of human prostate cancer development. Endocr Relat Cancer *11(2)*: 225-254, 2004. PMID: 15163300. DOI: 10.1677/erc.0.0110225

6 Shirai T, Takahashi S, Cui L, Futakuchi M, Kato K, Tamano S and Imaida K: Experimental prostate carcinogenesis - rodent models. Mutat Res *462(2-3)*: 219-226, 2000. PMID: 10767633. DOI: 10.1016/s1383-5742(00)00039-9

7 Bosland MC: Animal models for the study of prostate carcinogenesis. J Cell Biochem Suppl *16H*: 89-98, 1992. PMID: 1289679. DOI: 10.1002/jcb.240501221

8 Valkenburg KC and Williams BO: Mouse models of prostate cancer. Prostate Cancer *2011*: 895238, 2011. PMID: 22111002. DOI: 10.1155/2011/895238

9 Bosland MC: Is there a future for chemoprevention of prostate cancer? Cancer Prev Res (Phila) *9(8)*: 642-647, 2016. PMID: 27099271. DOI: 10.1158/1940-6207.CAPR-16-0088

10 Oliveira DS, Dzinic S, Bonfil AI, Saliganan AD, Sheng S and Bonfil RD: The mouse prostate: a basic anatomical and histological guideline. Bosn J Basic Med Sci *16(1)*: 8-13, 2016. PMID: 26773172. DOI: 10.17305/bjbms.2016.917

11 Lee C and Holland JM: Anatomy, histology, and ultrastructure (correlation with function), prostate, rat. In: Genital System. Jones TC, Mohr U and Hunt RD (eds.). Berlin, Heidelberg, Germany, Springer, pp. 239-251, 1987.

12 Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R, Ward JM and Cardiff RD: Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. Cancer Res *64(6)*: 2270-2305, 2004. PMID: 15026373. DOI: 10.1158/0008-5472.can-03-0946

13 Lawson TA, Gingell R, Nagel D, Hines LA and Ross A: Methylation of hamster DNA by the carcinogen N-nitroso-bis (2-oxopropyl)amine. Cancer Lett *11(3)*: 251-255, 1981. PMID: 7248930. DOI: 10.1016/0304-3835(81)90116-6

14 Rao MS, Subbarao V and Scarpelli DG: Effects of N-nitrosobis(2-oxopropyl)amine in newborn and suckling hamsters. Br J Cancer *41(6)*: 996-999, 1980. PMID: 6252924. DOI: 10.1038/bjc.1980.181

15 Rao MS and Pour P: Development of biliary and hepatic neoplasms in guinea pigs treated with N-nitrosobis(2-oxopropyl)amine. Cancer Lett *5(1)*: 31-34, 1978. PMID: 210926. DOI: 10.1016/s0304-3835(78)80007-x

16 Shirai T, Yamamoto A, Iwasaki S, Tamano S and Masui T: Induction of invasive carcinomas of the seminal vesicles and coagulating glands of F344 rats by administration of N-methylnitrosourea or N-nitrosobis(2-oxopropyl)amine and followed by testosterone propionate with or without high-fat diet. Carcinogenesis *12(11)*: 2169-2173, 1991. PMID: 1934306. DOI: 10.1093/carcin/12.11.2169

17 Shirai T, Tada M, Kojima M, Hasegawa R, Masui T and Ito N: DNA adducts in target and nontarget tissues of 3,2'-dimethyl-4-aminobiphenyl in rats. Environ Health Perspect *102(Suppl 6)*: 167-172, 1994. PMID: 7889841. DOI: 10.1289/ehp.94102s6167

18 Shirai T, Tamano S, Sano M, Imaida K, Hagiwara A, Futakuchi M, Takahashi S and Hirose M: Site-specific effects of testosterone propionate on the prostate of rat pretreated with 3,2'-dimethyl-4-aminobiphenyl: dose-dependent induction of

invasive carcinomas. Jpn J Cancer Res *86(7)*: 645-648, 1995. PMID: 7559081. DOI: 10.1111/j.1349-7006.1995.tb02447.x

19 Takayama K, Yamashita K, Wakabayashi K, Sugimura T and Nagao M: DNA modification by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in rats. Jpn J Cancer Res *80(12)*: 1145-1148, 1989. PMID: 2516840. DOI: 10.1111/j.1349-7006.1989.tb01644.x

20 Shirai T, Sano M, Tamano S, Takahashi S, Hirose M, Futakuchi M, Hasegawa R, Imaida K, Matsumoto K, Wakabayashi K, Sugimura T and Ito N: The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) derived from cooked foods. Cancer Res *57(2)*: 195-198, 1997. PMID: 9000552.

21 Faustino-Rocha AI, Silva A, Gabriel J, Gil da Costa RM, Moutinho M, Oliveira PA, Gama A, Ferreira R and Ginja M: Long-term exercise training as a modulator of mammary cancer vascularization. Biomed Pharmacother *81*: 273-280, 2016. PMID: 27261604. DOI: 10.1016/j.biopha.2016.04.030

22 Pollard M and Luckert PH: Promotional effects of testosterone and high fat diet on the development of autochthonous prostate cancer in rats. Cancer Lett *32(2)*: 223-227, 1986. PMID: 3756849. DOI: 10.1016/0304-3835(86)90123-0

23 Pollard M and Luckert PH: Autochthonous prostate adenocarcinomas in Lobund-Wistar rats: a model system. Prostate *11(3)*: 219-227, 1987. PMID: 3684782. DOI: 10.1002/pros.2990110303

24 Bosland MC, Schlicht MJ, Horton L and McCormick DL: The MNU plus testosterone rat model of prostate carcinogenesis. Toxicol Pathol *50(4)*: 478-496, 2022. PMID: 35588266. DOI: 10.1177/01926233221096345

25 Bosland MC: Chemical and hormonal induction of prostate cancer in animal models. Urol Oncol *2(4)*: 103-110, 1996. PMID: 21224148. DOI: 10.1016/s1078-1439(97)82840-2

26 Bosland MC: Testosterone treatment is a potent tumor promoter for the rat prostate. Endocrinology *155(12)*: 4629-4633, 2014. PMID: 25247471. DOI: 10.1210/en.2014-1688

27 Campolina-Silva GH, Werneck-Gomes H, Maria BT, Barata MC, Torres MJ, Contreras HR, Mahecha GAB and Oliveira CA: Targeting Wistar rat as a model for studying benign, premalignant and malignant lesions of the prostate. Life Sci *242*: 117149, 2020. PMID: 31830481. DOI: 10.1016/j.lfs.2019.117149

28 Sengupta P: The laboratory rat: Relating its age with human's. Int J Prev Med *4(6)*: 624-630, 2013. PMID: 23930179.

29 McCormick DL, Rao KV, Dooley L, Steele VE, Lubet RA, Kelloff GJ and Bosland MC: Influence of N-methyl-N-nitrosourea, testosterone, and N-(4-hydroxyphenyl)-all-trans-retinamide on prostate cancer induction in Wistar-Unilever rats. Cancer Res *58(15)*: 3282-3288, 1998. PMID: 9699656.

30 Bosland MC and Prinsen MK: Induction of dorsolateral prostate adenocarcinomas and other accessory sex gland lesions in male Wistar rats by a single administration of N-methyl-N-nitrosourea, 7,12-dimethylbenz(a)anthracene, and 3,2'-dimethyl-4-aminobiphenyl after sequential treatment with cyproterone acetate and testosterone propionate. Cancer Res *50(3)*: 691-699, 1990. PMID: 2137026.

31 Bosland MC, Prinsen MK and Kroes R: Adenocarcinomas of the prostate induced by N-nitroso-N-methylurea in rats pretreated with cyproterone acetate and testosterone. Cancer Lett *18(1)*: 69-78, 1983. PMID: 6218869. DOI: 10.1016/0304-3835(83)90119-2

32 Suwa T, Nyska A, Peckham JC, Hailey JR, Mahler JF, Haseman JK and Maronpot RR: A retrospective analysis of background lesions and tissue accountability for male accessory sex organs in Fischer-344 rats. Toxicol Pathol *29(4)*: 467-478, 2001. PMID: 11560252. DOI: 10.1080/01926230152500086

33 Bosland MC, Prinsen MK, Dirksen TJ and Spit BJ: Characterization of adenocarcinomas of the dorsolateral prostate induced in Wistar rats by N-methyl-N-nitrosourea, 7,12-dimethylbenz(a)anthracene, and 3,2'-dimethyl-4-aminobiphenyl, following sequential treatment with cyproterone acetate and testosterone propionate. Cancer Res *50(3)*: 700-709, 1990. PMID: 2105161.

34 Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA and Committee of the National Cancer Research Institute: Guidelines for the welfare and use of animals in cancer research. Br J Cancer *102(11)*: 1555-1577, 2010. PMID: 20502460. DOI: 10.1038/sj.bjc.6605642

35 Wang X, An Z, Geller J and Hoffman RM: High-malignancy orthotopic nude mouse model of human prostate cancer LNCaP. Prostate *39(3)*: 182-186, 1999. PMID: 10334107. DOI: 10.1002/(sici)1097-0045(19990515)39:3<182::aid-pros6>3.0.co;2-b

36 Labrecque MP, Coleman IM, Brown LG, True LD, Kollath L, Lakely B, Nguyen HM, Yang YC, da Costa RMG, Kaipainen A, Coleman R, Higano CS, Yu EY, Cheng HH, Mostaghel EA, Montgomery B, Schweizer MT, Hsieh AC, Lin DW, Corey E, Nelson PS and Morrissey C: Molecular profiling stratifies diverse phenotypes of treatment-refractory metastatic castration-resistant prostate cancer. J Clin Invest *129(10)*: 4492-4505, 2019. PMID: 31361600. DOI: 10.1172/JCI128212

37 Bianchi-Frias D, Damodarasamy M, Hernandez SA, Gil da Costa RM, Vakar-Lopez F, Coleman IM, Reed MJ and Nelson PS: The aged microenvironment influences the tumorigenic potential of malignant prostate epithelial cells. Mol Cancer Res *17(1)*: 321-331, 2019. PMID: 30224545. DOI: 10.1158/1541-7786.MCR-18-0522

38 Masumori N, Thomas TZ, Chaurand P, Case T, Paul M, Kasper S, Caprioli RM, Tsukamoto T, Shappell SB and Matusik RJ: A probasin-large T antigen transgenic mouse line develops prostate adenocarcinoma and neuroendocrine carcinoma with metastatic potential. Cancer Res *61(5)*: 2239-2249, 2001. PMID: 11280793.

39 de Bono JS, Guo C, Gurel B, De Marzo AM, Sfanos KS, Mani RS, Gil J, Drake CG and Alimonti A: Prostate carcinogenesis: inflammatory storms. Nat Rev Cancer *20(8)*: 455-469, 2020. PMID: 32546840. DOI: 10.1038/s41568-020-0267-9

40 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144(5)*: 646-674, 2011. PMID: 21376230. DOI: 10.1016/j.cell.2011.02.013

41 Arunkumar A, Vijayababu MR, Venkataraman P, Senthilkumar K and Arunakaran J: Chemoprevention of rat prostate carcinogenesis by diallyl disulfide, an organosulfur compound of garlic. Biol Pharm Bull *29(2)*: 375-379, 2006. PMID: 16462049. DOI: 10.1248/bpb.29.375

42 Boileau TW, Liao Z, Kim S, Lemeshow S, Erdman JW Jr and Clinton SK: Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. J Natl Cancer Inst *95(21)*: 1578-1586, 2003. PMID: 14600090. DOI: 10.1093/jnci/djg081

43 Rao KV, Johnson WD, Bosland MC, Lubet RA, Steele VE, Kelloff GJ and McCormick DL: Chemoprevention of rat prostate

carcinogenesis by early and delayed administration of dehydroepiandrosterone. Cancer Res *59(13)*: 3084-3089, 1999. PMID: 10397249.

44 McCormick DL, Rao KV, Steele VE, Lubet RA, Kelloff GJ and Bosland MC: Chemoprevention of rat prostate carcinogenesis by 9-cis-retinoic acid. Cancer Res *59(3)*: 521-524, 1999. PMID: 9973192.

45 McCormick DL, Johnson WD, Kozub NM, Rao KV, Lubet RA, Steele VE and Bosland MC: Chemoprevention of rat prostate carcinogenesis by dietary 16alpha-fluoro-5-androsten-17-one (fluasterone), a minimally androgenic analog of dehydroepiandrosterone. Carcinogenesis *28(2)*: 398-403, 2007. PMID: 16952912. DOI: 10.1093/carcin/bgl141

46 McCormick DL, Rao KV, Johnson WD, Bosland MC, Lubet RA and Steele VE: Null activity of selenium and vitamin e as cancer chemopreventive agents in the rat prostate. Cancer Prev Res (Phila) *3(3)*: 381-392, 2010. PMID: 20145190. DOI: 10.1158/1940-6207.CAPR-09-0176

47 Wang J, Eltoum IE and Lamartiniere CA: Dietary genistein suppresses chemically induced prostate cancer in Lobund-Wistar rats. Cancer Lett *186(1)*: 11-18, 2002. PMID: 12183070. DOI: 10.1016/s0304-3835(01)00811-4

48 Wang J, Eltoum IE, Carpenter M and Lamartiniere CA: Genistein mechanisms and timing of prostate cancer chemoprevention in lobund-wistar rats. Asian Pac J Cancer Prev *10(1)*: 143-150, 2009. PMID: 19469643.

49 McCormick DL, Johnson WD, Haryu TM, Bosland MC, Lubet RA and Steele VE: Null effect of dietary restriction on prostate carcinogenesis in the Wistar-Unilever rat. Nutr Cancer *57(2)*: 194-200, 2007. PMID: 17571953. DOI: 10.1080/01635580701277494

50 McCormick DL, Johnson WD, Bosland MC, Lubet RA and Steele VE: Chemoprevention of rat prostate carcinogenesis by soy isoflavones and by Bowman-Birk inhibitor. Nutr Cancer *57(2)*: 184-193, 2007. PMID: 17571952. DOI: 10.1080/01635580701277478

51 Faustino-Rocha AI, Oliveira PA, Duarte JA, Ferreira R and Ginja M: Ultrasonographic evaluation of gastrocnemius muscle in a rat model of N-methyl-N-nitrosourea-induced mammary tumor. In Vivo *27(6)*: 803-807, 2013. PMID: 24292585.

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