

## Modelling human prostate cancer: rat models

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## **Abstract**

Prostate cancer is the second most common cancer in men, affecting approximately 1.1 million men worldwide. In this way, the study of prostate cancer biopathology and the study of new potential therapies is of paramount importance. Several rat models were developed over the years to study prostate cancer, namely spontaneous models, chemically-induced models, implantation of cancer cell lines and genetically-engineered models. This manuscript aimed to provide the readers with an overview of the rat models of prostate cancer, highlighting their advantages and disadvantages, as well as, their applications.

**Keywords:** animal models, image monitoring, prostate carcinogenesis, rats

## **1. Introduction**

Prostate cancer is the second most common cancer in men, affecting approximately 1.1 million men worldwide. In the year 2012, prostate cancer was responsible for 307,000 deaths, being considered the fifth leading cause of death from cancer in men [1].

Although the causes of prostate cancer are not fully understood, many risk factors have been considered for the development of this type of cancer, such as age, race, family history, diet, hormone exposure, particularly to androgens and estrogens, and inflammation [2–4]. Since the prostate gland is an androgen-dependent tissue and consequently the prostate cancer is also androgen-dependent [2,3], the important role of androgenic hormones for prostate cancer development is well recognized [4].

Animal models have been used to study several diseases, like cancer, cerebral palsy, diabetes, Alzheimer, obesity and cardiac disease. The animal models may contribute invaluable information to better understand many aspects involved in disease development, and for the discovery and development of new pharmacological and non-pharmacological therapies (lifestyle) and preventive strategies, which may then be tested in clinical trials.

The animal models may be spontaneous, chemically-induced, transgenic/mutant animals with modifications in targeted genes, or implanted models (syngeneic or xenograft) [5–8]. An ideal animal model of human disease should be simple, not expensive and mimics the Human disease as much as possible. The rodents are commonly used in experimental research as cancer models, because they are relatively easy and cheap to maintain, their physiology and genetics are well known, they are mammals like Humans and the tumor's development is fast (all steps of carcinogenesis - initiation, promotion, progression and metastasis - may be observed) [6,8].

This review focused on rat prostate cancer models that have been established over the years for prostate cancer study, highlighting their advantages and disadvantages, as well as, their applications. We also describe the works performed in these prostate cancer models for the evaluation of several drugs and natural compounds.

## **2. Rat prostate: anatomy and histology**

Prostate is an accessory gland of the reproductive system of male mammals, found below the bladder in front of the rectum. Despite analogies found in prostate morphogenesis in different species, the variability of its anatomy among mammals is remarkable. While the prostate is a compact solitary structure in men and dogs, the prostate of rats and mice consists of distinct lobes. The rat prostate consists of four distinct lobes: the ventral, lateral, dorsal and anterior lobes, according to their relative position to urinary bladder [2,9] (Fig. 1). Each lobe has different morphological characteristics. The **ventral lobe**, unlike the others, does not have a human homologue, and consists of two discrete lobes ventro-lateral to the urinary bladder and attached to the urethra by connective tissue; the **dorso** and **lateral lobes**, commonly referred as dorsolateral prostate, are located along the urethra, forming a macroscopic mass. The **anterior lobes** are thin tubular structures attached to the seminal vesicles [10]. The knowledge of the different rat prostate lobes is important, because the ability to develop carcinomas vary among them [2]. The man prostate is a walnut-sized gland located beneath the urinary bladder and it is just one tubule-alveolar gland [11]. Despite these anatomical differences, recent studies found similarities in the molecular mechanisms underlying prostate cancer development in rats and men, making the rat as a valid animal model to study human prostate cancer.

### 3. Rat as a model of prostate carcinogenesis

Despite many research projects in the field of prostate cancer are carried out using cells lines (*in vitro* studies), which allow the understanding of biological aspects related to the development of this disease, they fail to mimic the complex cellular interaction that occurs in tumor microenvironment. To overcome this limitation, researchers employed their efforts for several years on the development of animal models to study this disease.

In 1937, Moore and Melchionna were the first ones to induce prostate carcinoma in the anterior lobe of the White rat prostate through the direct injection of 1:2 benzpyrene into prostate [12]. Metastasis development was not reported in this model, being considered a model limitation. Some years later, in 1945, Dunning and colleagues, induced the development of metastasizing prostate carcinomas in albino Fisher 344 rats and black agouti Irish AxC 9935 rats [13], through the implantation of methylcholanthrene crystal into prostate. Since then, several experimental researches were conducted to discover chemical carcinogenesis with tropism for prostate tissue [14]. Highlight the N-nitrosobis (2-oxopropyl) amine (BOP) discovered in 1981 by Pour [15], the *N-Methyl-N-nitrosourea* (MNU) discovered in 1986 by Pollard [9], the 3,2'-dimethyl-4-aminobiphenyl (DMAB) discovered in 1986 by Katayana [16] and the 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhiP) discovered in 1997 by Shirai [17].

An adequate rat model of prostate carcinogenesis should develop androgen-sensitive adenocarcinomas in a short period of time, mimic the physiology and characteristics of man tumors, and the tumors must metastasize, preferably to bones. The tumor

development should be from dorsal, lateral or anterior prostate lobes that are human homologue [14].

Concerning chemical inducers, several chemical and natural compounds may be used to assess their effect on prostate carcinogenesis. Among the agents used are: lycopene [18], dehydroepiandrosterone (DHEA) [19], fluasterone [20], quercetin [21], zinc [22], vitamin E [23], selenium [24], celecoxib [25], flutamide [26], cadmium [27], pioglitazone [28], pomegranate [29,30] and cholesterol [31].

#### **4. Rat models of prostate carcinogenesis**

Several animal models are available for the study of prostate cancer: spontaneous tumors, chemically or hormonally-induced, implantation of cancer cells and genetically engineered animals [2,32].

##### **4.1 Spontaneous tumors**

The first report of prostate spontaneous tumor was in 1963 by Dr. W.F. Dunning, who detected a single adenocarcinoma without metastasis in a 22 months-old Copenhagen rat [33–35]. Later, in 1973, Pollard reported spontaneous prostatic carcinomas in dorso-lateral and anterior prostate lobes, in a germfree Wistar rats with 26 months of age [34]. In 1957, spontaneous tumors of the ventral prostate were reported in 34-37 months-old AxC rats [33]. Aging male, on average 24 months, ACI/Seg rats demonstrated high susceptibility to develop spontaneous ventral prostate adenocarcinomas [36]. Since a long latency period is needed for tumor development (around 2-3 years) and tumor incidence is low, these spontaneous tumors are not advantageous as animal models [32].

## **4.2 Induced tumors**

The prostate tumors development may be easily induced through the administration of chemical carcinogens alone or by the combination of chemical carcinogens and hormones [2].

### **4.2.1 Chemically-induced prostate cancer**

Up to now, four chemical compounds are recognized for the induction of prostate carcinomas development in rats: BOP, MNU, DMAB and PhiP [2].

#### **4.2.1.1 N-nitrosobis (2-oxopropyl) amine (BOP)**

The BOP belongs to the family of nitrosamines and it acts as a potent carcinogen in different species and organs, like hamsters (pancreatic tumors) [37,38] and guinea pigs (biliary and hepatic neoplasms) [39]. When ingested by the animals, this compound is converted into carbon dioxide becoming a DNA methylating agent. BOP is also converted in two nitrosamine metabolites, N-nitrosobis (2-hydroxypropyl)amine (BHP) and N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) [38,40]. In 1981, Pour [15] reported for the first time that application of BOP induced tumors in the prostate dorsal lobe in MRC rats. Some years later, the same author conducted a study to evaluate the effects of dietary fat on the development of prostatic cancer in Wistar-derived MRC rats: testosterone propionate (100 mg/kg of body weight, subcutaneous injection) was daily administered for five days, and BOP (20 mg/kg body weight subcutaneous injection) was daily given to rats for three days, beginning with the third testosterone injection. This study lasted 72 weeks and showed that dietary fat did not influence the patterns of prostatic cancer [41]. Despite this, the BOP only induced

squamous cell carcinomas development in the ventral prostate lobe, the only lobe that does not have a human homologue, making it an animal model not widely used [14].

#### **4.2.1.2 *N*-methyl-*N*-nitrosourea (MNU)**

The MNU is a nitro-compound that acts as direct carcinogen agent by methylation of the guanine nucleosides without previous metabolic activation [42,43]. Pollard developed a chemically and hormonally-induced prostate cancer model in Lobound-Wistar rats through the administration of MNU associated with hormonal treatment [2,9]. This model consists of an intravenous injection of MNU (30-40 mg/kg), followed by a long-term administration of testosterone (10-40 mg) via silastic implants [9]. The tumors developed within a year in the dorsolateral prostate, showing invasive growth and metastases to the lymph nodes and lungs [9]. Over the years, this model was improved and now the sequential treatment with an antiandrogen (cyproterone acetate or flutamide), followed by a single intravenous or intraperitoneal injection of MNU and chronic treatment with silastic implants filled with testosterone is frequently used for prostate cancer development [9,44,45]. This approach leads to a high incidence of carcinomas in various rat strains [14]. Maarten Bosland described this method in detail: cyproterone acetate (50 mg/kg/day) may be administered by gavage as suspension in water, or by subcutaneous injection in oil, during 2-3 weeks, followed by the administration of testosterone propionate (10-100 mg/kg) by subcutaneous injection during three consecutive days. Forty-eight hours later, MNU must be administered by intravenous or intraperitoneal injection in doses between 30 to 50 mg/kg, and finally, testosterone propionate in silastic implants are placed subcutaneously in interscapular region by surgical approach under general anesthesia



[46]. This method, although complex in execution, allows a higher incidence of rat prostate cancer. To achieve the maximal tumor incidence, the experimental work should be conducted until 50-60 weeks after MNU injection [9]. Over the years many authors used this method to study prostate carcinogenesis [47].

The animal models of chemically-induced prostate cancer makes possible the evaluation of the chemopreventive effect of different compounds. Chemoprevention may be defined as the use of natural, synthetic or biological agents to reverse, suppress or prevent initial phases of carcinogenesis or progression of malignant cells to invasive disease [32,48]. The studies may be consulted in Table 1.

#### **4.2.1.33,2'-dimethyl-4-aminobiphenyl (DMAB)**

The DMAB is a classical polycyclic aromatic hydrocarbon with carcinogenic properties for multiple organs, such as colon, urinary bladder, mammary glands and Zymbal glands [2]. This compound needs to be previously activated in the liver. Then, the metabolites may be also metabolized and their oxidation products interact with DNA causing transversions in nucleotides, inducing irreversible changes [49]. The ability of DMAB to induce the development of carcinomas in ventral prostate lobe was reported by Katayama in 1986 [50] and confirmed by other authors over the years. The carcinogenic potential of DMAB is dose-depend. It was demonstrated that a low dosage of DMAB given over a long period (around 48 weeks) was more effective to induce prostate cancer development when compared with a high dosage over a short period of time (10-25 weeks) [51]. High doses of testosterone propionate may be used to promote induction of invasive carcinomas in the dorso-lateral and anterior prostate lobes [2,52]. The rat strains have different susceptibilities to DMAB, F344 and ACI rat

strain are more susceptible, while Wistar and Sprague-Dawley rats are resistant strains [53]. The DMAB method consists of a subcutaneous administration, normally at dose of 50 mg/kg/body weight, 10 times at 2-week intervals [52]. Silastic tubes filled with testosterone propionate may be used to promote tumor development. The implants should be placed subcutaneously and replaced at 6-week intervals [54]. In a general way, chemopreventive studies with this compound last 60 weeks, on average [55]. More details may be consulted in Table 2.

#### **4.2.1.4 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhiP)**

The PhiP is a heterocyclic amine isolated from cooked fish and meat. This compound may be metabolized to biologically active metabolites (N-hydroxy-PhiP and N-acetoxy-PhiP) that form DNA adducts [56–58] and induce cancer development in mammary gland, intestine and prostate [59,60]. Prostate cancer histopathological features are similar to those induced by DMAB. The rats exposed to PhiP develop prostate carcinomas in the ventral lobe, but not in the dorsolateral or anterior lobes. The experimental protocols using PhiP as prostate carcinogen consists of the administration of this compound mixed into the diet [32,59] or administrated intragastrically by gavage (70-200 mg/kg) [56,61,62]. This model can be used for chemoprevention studies. Detailed information concerning to these studies may be consulted in Table 3.

#### **4.2.2 Hormonally-induced prostate cancer**

Since the rat prostate is an androgen-sensitive tissue, the administration of testosterone may induce the development of invasive adenocarcinomas in dorso-

lateral and in anterior prostate, with low incidence in various rat strains [9,14,32]. The association of testosterone with estrogen, like 17 $\beta$ -estradiol, induces a higher carcinoma incidence in the dorsal, lateral and anterior prostate in Noble and Sprague-Dawley rats [14]. The methodology to induce prostate tumors with this compound consist of the use of silastic tubes filled with testosterone (crystalline testosterone or testosterone propionate) alone or in combination with estradiol (in two silastic tubes separated) implanted under the skin [14]. The wall thickness determines the speed of hormone release, as well as, the length of the tubing over which the hormone can diffuse out. The amount of the hormone in silastic tubes determine the duration of release. This model may be used in chemopreventive studies [63–65], but it is not very advantageous because of the early death of the rats due to the concomitant development of estrogen-induced pituitary tumors, which prevent the prostate carcinomas from growing and metastasis [14].

#### **4.3 Implantation of cancer cell lines**

Prostate cancer cells lines may be implanted in rats for tumor development. The cell lines may be either obtained from human prostate tumors and implanted into rats (xenograft model) [66], or obtained from chemically-induced or spontaneous rat prostate cancer (syngeneic model) [67–71]. Furthermore, these models may be orthotopic, if the prostate cancer cells are implanted in the tumor site of origin, in this case the prostate [72–74]; or heterotopic, if the cancer cells are implanted in a different place, for example subcutaneously [68] or in subcutis of the dorsal surface of the rat tail [71].

PAIII cancer cell line is an example of syngeneic model. This androgen insensitive cancer cell line is derived from a spontaneous prostate carcinoma from a Lobund-Wistar rat and it has the ability to form large primary tumors when injected subcutaneously [68,75]. Harvey Pollard and colleagues [68] used this model to test the influence of ascorbic acid on tumorigenesis and concluded that pharmacological doses of this compound may suppress tumor growth and metastasis. PLS 10 is another cancer cell line, it was established from DMAB plus testosterone-induced carcinomas in the dorsal prostate of male F344 rats, which may be implanted into rats [67]. The PSL10 cell line was used as a syngeneic heterotopic model into the flank of F344 rats in a study aiming to understand hyperthermic effects of magnetic particles on rat prostate cancer. The authors concluded that hyperthermia is an effective therapy for prostate cancer [67].

The xenograft prostate cancer models in rats are not widely used in experimental research due to the few immunodeficient rat strains available for use. Highlight the work of Tumati [66] that developed an advanced orthotopic prostate cancer model using a modified human prostate cancer cell line (PC3) implanted into the prostate of nude or Copenhagen rats to assess the effects of high-dose image-guided radiation therapy combined with biological agents [66]. In another work, Andressen [76] used immunodeficient male homozygous Sprague-Dawley rats injected with CRW22 human prostate cancer cell line to investigate the ability to induce bone metastasis.

#### **4.3.1 Dunning model**

Most of the *in vivo* rat tumor lines for testing new drugs for the treatment of hormone-dependent prostate cancers originated from the Dunning R3327 adenocarcinoma of

the Copenhagen rat. This cell line was developed from a spontaneous dorsal prostatic adenocarcinoma in a Copenhagen male rat in 1963. The tumor was identified in a necropsy and grafts of the tumor were transplanted into syngeneic rats and F<sub>1</sub> hybrids of a Copenhagen x Fischer cross. The transplanted tumor was histologically, biochemically and biologically similar to the rat dorso-lateral prostate [35]. Since then, several sublines were developed from Dunning R3327, such as AT-1, MAT-Ly-Lu, AT-4-R3327-5 and PAP [35]. Each subline has different characteristics and exhibits a range of phenotypes that mimic aspects of the man prostate cancer, like the slow-growing androgen-responsive tumor [7]. These cell lines may be passed *in vivo* without lose tumorigenicity and metastatic ability [35]. Since the tumor associated with this model is slow-growing, non-metastatic, androgen-responsive, and maintained the histologic appearance and biochemical properties of the rat dorsal prostate gland, it is considered an appropriate model for cancer research, namely for chemoprevention studies [35] (Table 4).

The Dunning model has the main advantage of not requiring exposure to chemical compounds for its execution, thus reducing the health risk for the investigator, the environmental effects and costs.

#### **4.4 Genetically-engineered models**

Both chemically or hormonally-induced prostate cancer models are labor intensive and need a long period of latency for tumor development: at least 60 weeks for the DMAB and PhIP models, 50 weeks for the MNU models, 70 weeks for BOP models and 40 weeks for hormonally-induced models on average. As an attempt to overcome this disadvantage, genetically-engineered models were developed [77,78]. In 2001, Shirai

and colleagues created a transgenic rat prostate cancer model, named “transgenic rat with adenocarcinomas of the prostate (TRAP)”, using the probasin gene promoter and the Simian Virus-40 T (SV40-T) antigen in the genetic background of Sprague-Dawley rats. The rat probasin gene encodes prostate-specific proteins, while androgen and zinc regulates specific proteins to the dorsolateral epithelium of the prostate. The prostate tumors developed by this animal model are androgen-receptor positive and androgen-dependent [78], and develop mostly in the ventral, lateral and dorsal prostate lobes at high incidence [32,78]. The initial lesions in prostate epithelial cells may be observed at four weeks and the carcinomas become extensive at 15 weeks [32], providing a useful animal model to investigate the mechanisms underlying prostate cancer development, as well as, the effects of modifying factors. Another transgenic rat prostate cancer model was developed in the Lewis rat, crossing the Sprague-Dawley SV40-T rats with Lewis strain to study prostate cancer immunotherapy (Table 5). The prostate tumors developed with 100% penetrance and were androgen sensitive [79].

## **5. Follow-up of animal models - prostate imaging**

Although the prostate cancer remains the fifth cause of death by cancer among men, the mortality associated with this type cancer has decreased in the past decades, mainly due to the widespread use of screening strategies. Despite the only definitive way to confirm prostate cancer is through a prostate biopsy (frequently ultrasound guided biopsy) [80], screening for this kind of cancer includes digital rectal examination (DRE) focused on prostate size and consistency, or more typically, a change on serum prostate-specific antigen (PSA) [81]. Furthermore, the prostate changes may be non-

invasively evaluated through imaging modalities, such as ultrasonography (transrectal ultrasound) [82]. Nowadays, ultrasonography provides not only anatomic information (prostate dimensions and macroscopic structure of parenchyma) through the use of B-mode (grayscale ultrasonography) [66,83], but also functional information on tumor microenvironment (evaluation of prostate vascularization) through the use of Power Doppler, Color Doppler, Pulsed Doppler, B Flow, and ultimately contrast agents. Contrast-enhanced ultrasound (CEUS) is based on the intravenous administration of a contrast agent [80] and allows not only to quantify and evaluate the pattern of distribution of the vessels inside the parenchymal prostate (tortuous vessels, centripetal/centrifugal enhancement of contrast agent), but also to perform dynamic studies, evaluating the arrival time of the contrast agent, peak intensity, time to peak, ascending-slope, descending-slope and area under the curve [84].

When compared with other imaging modalities, the ultrasonography has some advantages, namely the ultrasound apparatus are portable, they are less expensive, they are recommended for claustrophobic patients and, the most important one, does not impose ionizing radiation. Although the ultrasonography is the oldest and the most widely used imaging modality for prostate imaging [80], it is limited on the detection and location of prostate cancer, and on the tissue contrast between benign and cancerous tissues due to its lower sensitivity and specificity [82]. To overcome these limitations, other imaging modalities, such as Magnetic Resonance Imaging and Positron Emission Tomography have been employed on prostate screening.

Magnetic Resonance Imaging allows the visualization of the prostate contours and internal anatomy, providing functional and structural information of tumor vasculature and physiology [84] with great sensitivity, but with low specificity [85]. This imaging

technique is usually combined with other techniques, like Diffusion Weighted Imaging that improves the detection and localization of prostate cancer; Magnetic Resonance Spectroscopy that assesses the relative concentration of different chemical compounds in tissue, and Dynamic Contrast-Enhanced Magnetic Resonance imaging whose principles are based on tumor angiogenesis [85,86]. Positron Emission Tomography is a non-invasive imaging modality that uses radiolabel tracers and gamma cameras to measure sensitively and quantitatively the concentration of these radioactive molecules in the prostate [84]. This imaging modality is often combined with Magnetic Resonance Imaging for anatomical localization of the spots for radiotracers [80]. Positron Emission Tomography is more used in later stage cancer than in diagnosis, being useful on the detection of biochemical relapse, cancer recurrence [80] and metastasis. This imaging modality has the disadvantages of not detect small lesions, not distinguish benign and malignant processes, the higher costs, and requirement of advanced training and skills [87]. It is worth to note that these imaging modalities may also be used in rat models for prostate cancer diagnosis and to monitor the effects of new potential therapies for cancer treatment [88], however the published works are scarce.

Our research team has some ultrasonographic, Magnetic Resonance Imaging and Computed Tomography studies on the rat model of prostate cancer. The effects of long-term exercise training on prostate carcinogenesis were studied using a chemically and hormonally-induced rat model. For this, a multistep experimental protocol was employed using Wistar rats. At 12 weeks of age, the experimental protocol was initiated with the subcutaneous administration of the anti-androgenic drug flutamide (50 mg/kg of body weight) for consecutive 21 days. Twenty-four hours after the last



flutamide administration, they received a subcutaneous injection of testosterone propionate at a dose of 100 mg/kg of body weight, followed by a single intraperitoneal administration of the carcinogen agent MNU (30 mg/kg of body weight) 48 h after the testosterone administration. Finally, two weeks after the MNU administration, testosterone filled tubes were subcutaneously implanted on the cervical region under anesthesia, and remained until the end of the experiment. The prostate dimensions and macroscopic appearance was non-invasively monitored throughout the experimental protocol by ultrasonography using a 12 MHz linear probe. The preliminary results showed a reduction of prostate volume after the flutamide administration (more evident on ventral lobes) (Fig. 2A and B). An inverse effect was observed six weeks after the MNU administration, with the carcinogen promoting the increase of prostate volume (with higher incidence on ventral lobes) and compressing the neck of urinary bladder (Fig. 2C and D). The prostate volume continued increasing overtime, until the end of the experimental work, with a marked volume enhancement of dorsal prostate lobes and the vesicular glands placed near the prostate lobes (Fig. 2E and F). We also performed a study of prostate vascularization using Power Doppler, B Flow and contrast-enhanced ultrasound. An increase on prostate vascularization was observed between the 35 weeks and 61 weeks after MNU administration. Some Magnetic Resonance Imaging and Computed Tomography studies were also performed in normal and cancerous prostates (Fig. 3).

## **6. Sample collection and histological evaluation**

A standardized protocol to collect prostate tumors was not yet established. Some researchers remove accessory sex glands together with urinary bladder and separate

the different prostate lobes from urinary bladder after fixation in formalin [19,63,89]. Other researchers remove the accessory sex glands, fix them in formalin and cut them into slices (where include urethra and seminal vesicles) to paraffin inclusion [27,31,62,90,91]. Bosland suggests that the accessory sex glands are best removed and fixed together with urinary bladder and then they should be separated [9]. According to our experience, this seems to be the most appropriate method to identify the different prostate lobes.

## **7. Spectrum of prostate lesions**

As mentioned elsewhere, cancer is a multifactorial disease and factors that are responsible for prostate tumorigenesis remain largely unknown. As prostate gland is an endocrine-responsive tissue, many studies focused on the effect of androgens, estrogens and their metabolites on prostate tissues.

Over the years, several rat prostate models using chemical carcinogens have been established. In 1977, Fingerhut and Veenema [92] reported carcinomas induced by DMBA in gonadectomized animals. Later in 1983, Pour described hyperplastic and metaplastic lesions, and squamous cell carcinomas, on MCR rat ventral prostate after repeated BOP administration [93]. These lesions in the ventral prostate, which has no homologue in humans [36,94,95], occur spontaneously in some rat strains, especially in old animals, while chemical carcinogens induce neoplastic changes predominately in the dorsolateral lobe [96,97] .

Carcinomas *in situ* of the dorsolateral prostate (an embryological homologous to the human prostate), were reported in other chemical induction models using DMABP [16] or a combination of DMABP and ethinyl estradiol [98]. Carcinomas of the dorsolateral

prostate were also described by Pollard and Luckert (1986) on the Lobund-Wistar rat, after a single MNU administration [99].

Bosland and Prinsen (1990) [95] using MNU or DMBA, following chemical castration and testosterone propionate pretreatment, induced invasive adenocarcinomas and carcinomas *in situ* of the dorsolateral prostate on Wistar rats, 63 weeks after carcinogen injection, mainly in the MNU treated animals. They also reported reactive hyperplasia of the dorsolateral prostate associated with acute and chronic inflammatory processes as a common finding in all experimental group. Apparently, reactive hyperplasia is a consequence of inflammation [100,101].

Later in 1998, McCormick and colleagues [102] reported a high incidence of accessory sex glands cancer, mostly in dorsolateral and anterior prostate lobes, in rats treated sequentially with MNU and testosterone, but lower cancer incidence in rats receiving only MNU or treated with testosterone only; no lesions were observed in untreated animals, suggesting that carcinogenesis depends on both chemical and hormonal stimulation.

In 1999, Rao *et al.* [19] also described dorsolateral and anterior prostate carcinomas in Wistar-Unilever rats using cyproterone acetate and testosterone propionate, followed by a single dose of MNU in animals with chronic androgen stimulation. They also reported that some animals developed seminal vesicle tumors. Previously, Lucia *et al.* (1995) [91] reported a high incidence of seminal vesicle tumors, rather than prostate tumors, induced by MNU and testosterone propionate in Lobund-Wistar rats.

More recently, in 2014, Bosland [46] reported a high frequency of prostate carcinomas after MNU injection in rats with subcutaneous testosterone implants. The frequency was lower in rats exposed to testosterone alone (not exposed to MNU) and tumors did

not occur in rats given MNU or not treated. Based on these findings, Bosland [46] suggested that testosterone is a carcinogen and a tumor-promoting agent.

Our group studied the influence of chemical carcinogen and hormonal stimulation on the induction of prostate cancer on Wistar rats using a multistep experimental protocol where the animals were submitted to a chemical castration through the administration of flutamide, followed by the administration of testosterone propionate, a single injection of the carcinogen MNU and a chronic exposure to testosterone using subcutaneous implants. Twenty-three weeks after the beginning of the experiment, we observed mainly prostate hyperplastic lesions, and occasionally low grade dysplastic lesions, mostly in dorsolateral prostate, which also showed acute inflammation of the acini, focal necrosis and reactive hyperplasia, with small focal areas of chronic stromal inflammation. In fact, all groups of the experiment showed acute and/or chronic inflammation. Inflammation was also frequently reported by other studies [96,101] and reported as more common and severe in the dorsolateral prostate [101], as we observed. Some animals also developed atypical hyperplasia of anterior prostate and seminal vesicle (Fig. 4).

Rats sacrificed 49 weeks after the beginning of the study showed more commonly dysplasia, *in situ* carcinoma or invasive carcinomas. Our data suggest that chronic exposure to testosterone acts as a tumor promoter in cells initiated by MNU, fostering the transition from hyperplasia to dysplasia and finally the progression from *in situ* to invasive carcinoma, being this promoter effect apparently time-dependent.

## **8. Conclusions**

Experimental data concerning to the rat models of prostate carcinogenesis was reviewed in this work. Although several animal models are available to study prostate cancer and a perfect model does not exist, they provide an important tool to study human and animal prostate carcinogenesis, and to evaluate the effects of potential preventive and therapeutic strategies. The model should be chosen by the researchers, taking into account the aims of their studies, the costs, and the advantages and disadvantages of each one.

Although complex, time-consuming and labor-intensive, the model of prostate cancer hormone and chemically-induced in male rats is the most frequently used due to its advantages when compared with the remaining models. When applying the sequential treatment with an anti-androgen, the carcinogenic agent MNU and the chronic treatment with silastic implants filled with testosterone, a high incidence of prostate carcinomas is observed in several rat strains, with the maximal incidence reached 50-60 weeks after the carcinogen administration. Furthermore, the carcinogenesis process may be non-invasively monitored using different imaging modalities, and the tumors developed by the animals are hormone-dependent and histologically similar to those developed by men, allowing the translation of the results from animals to humans.

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**Figure legends:**

**Figure 1.** Schematic representation of rat prostate. It consists of four distinct lobes according to their relative position to urinary bladder: ventral (V), lateral (L), dorsal (D) and anterior (A) lobes.

**Figure 2.** Ultrasonographic monitoring of rat prostate during an assay of hormone and chemically-induced prostate carcinogenesis performed by our research team. Prostate lobes are visible around the urinary bladder (asterisks). Rat prostate immediately before the beginning of the experimental protocol (A). A reduction of prostate volume was observed after flutamide administration for 21 consecutive days, this decrease was more evident on the ventral lobes (B). Six weeks after the *N*-methyl-*N*-nitrosourea (MNU) administration, an increase of the prostate dimensions was observed (C), with consequent compression of the neck of the urinary bladder (arrow) (D). The prostate volume continued increasing until the end of the experimental work, with a marked volume enhancement of the dorsal prostate lobes and the vesicular glands placed near the prostate lobes (E and F). Arrowheads in figures D and E indicate the prostate lobes. All images were obtained with the animal in supine position. Images A, B, C and E were obtained in transverse plane; images D and F were obtained in longitudinal plane.

**Figure 3.** Monitoring of rat prostate by Computed Tomography (A and B) and Magnetic Resonance Imaging (C and D). It was evaluated the prostate of animals exposed to the protocol of prostate cancer induction (A and C) and the prostate of control animals (B and D).

**Figure 4.** Influence of chemical carcinogen (MNU, *N*-methyl-*N*-nitrosourea) and hormonal stimulation (testosterone) on the induction of prostate cancer using a multistep protocol where the animals were submitted to a chemical castration (flutamide administration). Prostate cells' atrophy was observed after flutamide administration for 21 consecutive days. Cell hyperplasia was observed after a single injection of MNU and chronic exposure to testosterone for 18 weeks (23 weeks after the beginning of the protocol). The exposition of testosterone for 44 weeks (49 weeks after the beginning of the study) promoted the transition of cell hyperplasia to dysplasia, and finally the progression of carcinoma *in situ* to invasive carcinoma.

Table 1

Table 1. *In vivo* studies using MNU model of prostate cancer to assess the efficacy of several therapeutic strategies.

MNU Model			
Animal Strain	Drugs or compounds evaluated (classification)	Dose/Treatment	Therapeutic effects (Ref)
Wistar-Unilever	Acticoa powder (polyphenol)	Gavage (24 and 48 mg/kg; 7 days/week) 2 wks before MNU	Positive effects of acticoa powder at dose of 24 mg/kg [103]
	Celcoxib (non-steroidal anti-inflammatory drugs)	p.o. (500 mg/kg) 21 days after MNU for 52 wks	Positive effects against prostate cancer [25]
	Celecoxib and exisulind (non-steroidal anti-inflammatory drugs)	p.o. (celecoxib 250 or 500 ppm or exisulind 500 or 1000 ppm) 21 days after MNU for 52 wks	Positive effects of celcoxib plus exisulind at low doses [104]
	Chronic dietary restriction (caloric intake reduction)	Dietary restriction ( 0% [ad libitum control], 15%, or 30%) 2 weeks after MNU for 12 months	No effect on the induction of prostate cancer by reducing caloric intake [105]
	Dehydroepiandrosterone (DHA) (adrenal steroid)	p.o. (2000 mg/kg diet) 1, 20 or 40 wks after MNU and 13 months	Inhibited prostate carcinogenesis in a dose-related [19]
		p.o. (1000 or 2000 mg/kg diet) 1 wk before MNU for 13 months	
	N-(4-Hydroxyphenyl)-all-trans-retinamide (4-HPR) (synthetic retinoid)	p.o. (1 mmol/kg diet for 450 days) on the day after MNU	No chemopreventive effect [102]
	9-cis-retinoic acid (9-cis-RA) (natural metabolite of retinoic acid)	Diet supplemented (100 mg/kg or 50 mg/kg diet) 1 week before MNU for 13 months	Chemopreventive against prostate cancer [89]
	Selenium and vitamin E (vitamins)	p.o. (L-selenomethionine 3 or 1.5 mg/kg diet, vitamin E, 4 or 2 mg/kg diet and L-selenomethionine + vitamin E 3+2 mg/kg or 3+500 mg/diet ) 1 wk after MNU and for 13 months	No effects of the compounds alone or together [24]
	Soy isoflavone mixture PTI G-2535 and a soy-derived protease inhibitor (BBIC) (soy isoflavones/protease inhibitor)	p.o. (0, 200 or 2000 mg/kg diet of PTI G-2535 or BBIC) 1 wk after MNU for 13 months	The combination of PTI G-2535+ BBIC was more effective in prostate cancer prevention than the compounds alone [106]
γ-tocopherol (vitamin)	p.o. (20 mg/kg) for 16 wks	Protected against the development of cancer [107]	

	Tomato Powder and Lycopene (spray-dried product made from heat-processed tomato paste/water-dispersible lycopene beadlets)	Diet supplemented (Lycopene 2.5 mg/kg diet and tomato powder 100 g/kg diet)	Tomato powder but not lycopene inhibited prostate carcinogenesis [108]
Sprague-Dawley	Diallyl disulfide (component of garlic)	p.o. (150 mg/kg b.w.; 2 times/wk for 16 wks)	Inhibited prostate carcinogenesis [109]
	Fluasterone (dehydroepiandrosterone analog)	PO (2000 and 1000 mg/kg diet) 1 wk before MNU, for 13 months	Suppressed prostate cancer induction [110]
	Zinc chloride (trace element)	d.w. (100 ppm 3 times/wk for 20 wks) 1 wk before administration of initial dose of testosterone propionate; administration for 20 wks	Reduced tumor incidence [21]
<b>Albino wistar</b>	Calcitriol (vitamin D metabolite)	i.p. (0.5 µg/kg b.w. 3 times/wk) 1 wk before administration of cyproterone acetate and for 16 wks	Chemo preventive activity [22]
<b>Wistar</b>	Monascus cursory extraction (MCE) (red mold rice metabolite)	p.o. (0.2% or 0.5%) 21 days after MNU for 52 wks	Reduced incidence and size of tumors [111]
<b>Lobund-Wistar</b>	Genistein (phytoestrogen component of soy)	p.o. (250 mg/kg/diet) from birth until 35 days or start at 90 days of age, 20 days after cancer initiation, until 11 months or from birth throughout life	Inhibited prostate cancer was more effective by life time exposure [112]
		p.o. (25 and 250 mg/kg/diet) start at conception and continued until euthanasia	Protective effects of lifetime dietary [113]
	Glutaraldehyde-fixed tumor (GFT) cell vaccine	Vaccination when tumors were first palpated and weekly until the rats became clinically debilitated	Tumor regression and reduction of metastases [114]
	Linomide (quinoline-3-carboxamide)	d.w. (0-25 mg/kg daily) after testosterone implantation and for 12 months	Inhibited 60% of incidence of prostate cancer at maximum dose [115]
		i.p. (100 mg/kg b.w. daily) or in d.w. (1 mg/1 ml water) when small palpable tumors (0.5-cm diameter) were detected until the tumors reached 3-4 cm in diameter	Minimal therapeutic benefit on tumor development [116]
	N-(4-Hydroxyphenyl)-all-trans-retinamide (4-HPR) (synthetic retinoid)	p.o. (1mmol/kg diet) after 7 months MNU	Prevented prostate cancer [117]

	<p>Tamoxifen, N-(4-Hydroxyphenyl)retinamide (4-HPR) and <math>\alpha,25</math>-dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol (Ro24-5531) (selective estrogen receptor modulators synthetic retinoid; Vitamin D analogue)</p>	<p>p.o. (2.5 or 1.25 nmol/kg diet of Ro245331, 2 or 1 nmom/kg diet of 4-HPR and 5 or 0.5 mg/kg diet of tamoxifen)</p>	<p>Reduced tumors development [91]</p>

p.o.: *per os*; wks: weeks; d.w.: drinking water; i.p.: intraperitoneal injection; b.w. body weight.



**Table 1.** *In vivo* studies with DMAB model to study different therapeutic approaches for prostate cancer.

DMAB Model			
Animal Strain	Drugs or compounds evaluated (classification)	Dose/Treatment	Therapeutic effects (Ref)
Fischer 344	Bicalutamide (antiandrogen)	p.o. (30 mg/kg/diet 3 times/wk) 40 wks after DMAB for 14 wks	Decrease prostate cancer if treatment started in later period of disease [118]
	Cadmium (heavy metal)	i.m. ( 30 $\mu$ mol/kg b.w. and 10 $\mu$ mol/kg b.w. on the 1 <sup>st</sup> day; or 30 $\mu$ mol/kg b.w. and 10 $\mu$ mol/kg b.w. after DMAB; or 10, 20 and $\mu$ mol/kg b.w. at wk 40; or 10 $\mu$ mol/kg b.w. at wk 20 and then 5 $\mu$ mol/kg b.w. at wk 30, 40 and 50.)	Promoted prostate cancer [27]
	Cholesterol, saponin, clofibrate (sterol; glycosides; fibrate)	p.o. (1% and 2% of cholesterol or 0.3% clofibrate) and SC (1mg saponin) after DMAB for 40 wks	No effects of cholesteremia were observed [31]
	Fat diets (corn oil, beef tallow and perilla oil) (polyunsaturated fatty acids)	p.o. (20% of corn oil, beef tallow or perilla oil/diet) from the beginning of experiment during 60 wks	Promotion of prostate carcinogenesis by beef tallow, but not corn oil or perilla oil [119]
	Finasteride and casodex (5-alpha reductase inhibitor; antiandrogen)	p.o. (5 or 15 mg/kg/diet of finasteride 2 times/wk and 15, 30 or 50 mg/kg/diet casodex 3 times/wk), 20 wks after DMAB for 40 wks	Inhibited prostate cancer in a dose-dependent manner [120]
	Genistin and daidzin (isoflavones)	p.o. (0,1% genistin and 0,1% daidzin/diet) after DMAB for 40 wks	Reduced the numbers of ventral prostate carcinomas and decrease in incidence with both compounds [121]
	Indomethacin (nonsteroidal anti-inflammatory drug)	d.w. (20 ppm) after DMAB for 37 wks	No inhibited tumor development [122]
	p-nonylphenol (NP) (alkylphenols)	p.o. (0, 25, 250 or 2000 ppm/diet) from 3 wks of rat age for 3 wks	No modulating effects on prostate carcinogenesis late neonatal treatment with NP [90]
	4-n-octylphenol (estrogenic compound)	p.o. (0, 10 or 100ppm) 1 wk after DMAB for 20 wks	No effects were observed [55]
4-tert-octylphenol (tOP) and benzyl	p.o. (100/10 ppm of tOP or 100/10 ppm of BBP/diet)	No effects were observed [123]	

butyl phthalate (BBP) (estrogenic compounds)		
Silymarin (polyphenolic flavonoid antioxidant)	p.o. (100 or 500 ppm/diet) 1wk after the last dosing of DMAB for 40 wks	Chemo preventive ability [124]
Soybean isoflavone mixture (74% genistein and 21% daidzein)	p.o. (100 and 400 ppm/diet) for 50 wks	Suppressed the development of adenocarcinoma [20]
Testosterone propionate (TP) and diethylstilbestrol (DES) (anabolic steroid; synthetic, nonsteroidal estrogen)	Silastic tubes (40 mg of TP and 10 mg of DES) 20 wks after DMAB for 30 days then removed for 10 days repeated 7 times for 40 weeks	Inhibited prostate cancer development by combination of TP and DES [125]

p.o.: *per os*; wks: weeks; i.m.: intramuscular injection; b.w.: body weight; s.c.: subcutaneous injection

**Table 1.** *In vivo* studies using PhiP model to assess the efficacy of several compounds on prostate cancer prevention.

PhiP model			
Animal Strain	Drugs or compounds evaluated (classification)	Dose/Treatment	Therapeutic effects (Ref)
Fischer 344	Fujiflavone (commercial isoflavone supplement)	p.o. (0.25%) 10 wks after PhiP for 50 wks	Inhibited prostate cancer [61]
	Lycopene and curcumin (carotenoid pigment; component of rhizomatous herbaceous perennial plant)	p.o. (45 ppm/diet of lycopene, 500 ppm/diet of curcumin or lycopene + curcumin) after PhiP for 50 wks	No effects on prostate tumor development with the compounds alone or together [62]
	Nobiletin (5,6,7,8,3',4'-hexamethoxy flavones) (polymethoxy-flavonoid extract from citrus fruits)	p.o. (0.05%) 16 wks of rat age to 66 wks of rat age	Inhibited prostate cancer [56]
	Whole tomato powder + broccoli powder (fruit; vegetable)	p.o. (10% of freeze dried powders) for 20 wks	Reduced and prevented prostate cancer [59]

p.o.: *per os*; wks: weeks

Table 1. *In vivo* prostate cancer chemopreventive studies using the Dunning model sublines.

Dunning model			
Cell line: Dunning R3327-AT-1 model			
Animal Strain	Drugs or compounds evaluated (classification)	Dose/Treatment	Therapeutic effects (Ref)
Copenhagen	Physical activity	TR (60-min 5 day/wk for 7 wks) 7–10 days after tumor inoculation	Exercise training increased tumor microvascular Po <sub>2</sub> and reduced tumor hypoxia [72]
	Pomegranate juice and exercise training	p.o. (15µl pomegranate juice 5 days/wk) and TR (40 min 5 days/wk for 2 wks) 15 days after cell line inoculation or p.o. (750 µl pomegranate juice) + TR (60 min 5 days/wk 2 wks)	Decreased prostate tumor proliferation by treadmill running, but the combination of the two approaches was not efficient when compared with the treatments conducted separately [126]
	Soy protein isolate and conjugated linoleic acid (isoflavone-rich protein isolate from genistein; natural positional isomer of linoleic acid)	p.o. (5, 10 and 20%/diet of soy protein isolate and 0.5 and 1%/diet of conjugated linoleic acid) for 35 wks	No inhibitory effects of the compounds alone or together were observed on tumor growth and development [127]
Cell line: Dunning R3327H model			
Copenhagen	Lycopene, selenium (in the form of methylselenocysteine) and vitamin E (γ-tocopherol) (vitamins)	Diet supplemented (250 mg/kg of lycopene beadlets, 1mg/kg of selenium and 200 mg/kg vitamin E) 4-6 weeks before tumor inoculation for 22-24 weeks	Reduced growth of prostate tumor by selenium, but not lycopene or vitamin E [128]
	Hyperthermia	Hyperthermia treatments (46.5°C twice) for 2 hours when the tumor volume reached 0.5 to 2.0 cm <sup>3</sup>	Death of tumor cells [129]
	PNU 157706 ((N-(1,1,1,3,3,3-hexafluorophenylpropyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide)) and flutamide (5α-reductase inhibitor ;non-steroidal antiandrogen)	Oral (10 mg/kg/day of PNU 157706 and 1 or 5 ml/kg/day of flutamide 6 days/week) for 9 weeks	Inhibited prostate cancer of both compounds [130]

<b>F1 hybrid</b>	Cyclophosphamide, ketoconazole, aclacinomycin-A, methotrexate and methotrexate--5-Fluorouracil (alkylating agente antifungal drug; antracycline; chemotherapy agent; antimetabolite)	Exp1: i.p. (100 mg/cyclophosphamide once every 3 to 4 wks and 7.5 mg/kg of methotrexate followed in 90 min by 50 mg/kg of 5-fluorouracil once each wk for 8 wks; Exp2: i.p. (100 mg/kg of methotrexate once each wk for 6 wks, 8 mg/kg of aclacinomycin-A once wk for 4 wks and p.o. (60 mg/kg of ketoconazole 5 times/wk for 6 wks)	Significant suppression of tumor growth by Aclacinomycin-A and ketoconazole [131]
<b>Copenhagen x Fischer F1</b>	Estramustine phosphate+radiation treatment (alkylating antineoplastic agente)	s.c. (360µg/day/rat) 3 months after tumor inoculation and 1 day after radiation for 2 wks	Decreased prostate cancer volume and estramustine phosphate potentiated irradiation [132]
<b>Copenhagen x Fischer F2</b>	6-Methylene-4-pregnene-3,20-dione (6MP) + castration (inhibitor of 5α-reductase)	s.c. (10, 20 and 40 mg/kg b.w. of 6MP) or castration+40 mg/kg b.w. of 6MP + 0.1 mg of testosterone propionate 3 months after tumor inoculation for 4 wks	Reduced tumor growth in castrated and testosterone supplemented animals. 6MP may represent an alternative for castration [133]
<b>Cell line: Dunning R3327-AT-2 model</b>			
<b>Copenhagen</b>	Laser interstitial thermotherapy (LITT)	LITT (980nm diode laser for 75s) after 3 wks of tumor inoculation	Induced tumor necrosis [134]
<b>Copenhagen x Fisher</b>	High-intensity focused ultrasound (HIFU) combined with chemotherapy	HIFU alone or with 2 chemotherapy agentes (4 mg/kg paclitaxel s.c. and 15 mg/kg estramustine phosphate i.p.) after 15 days tumor inoculation for 5 days	Inhibited tumor development by combined treatment [135]
<b>Cell line:Dunning R3327-MAT-Ly-Lu model</b>			
<b>Male Copenhagen rat</b>	Brahma rasayana (herbal tonic)	p.o. (1, 250 and 1.5 mg/kg b.w. daily) 2 days after tumor inoculation for 5 wks	Reduced tumor incidence [136]
	Calcitriol and EB1089 (analogue of 1,25(OH)2D) (steroid hormone)	i.p. (0.5 µg/kg calcitriol or 0.5 µg/kg EB1089 3 times/wk) 7 days before tumor implantation until the tumors grew to 10 ml	Both compoundslihibited prostate cancer metastasis [137]
	Doxycycline (DC) and non-antimicrobial chemically modified tetracycline (CMT-3) (tetracycline)	p.o. (40 mg/kg DC and CMT-3 daily) 5 days after tumor implantation until tumors reached a volume > 10ml	Reduced tumor incidence and metastasis [138]
	1,25-dihydroxycholecalciferol	s.c. (0,1 ml 3 times/wk) for 3 wks	Inhibitedtumor volume and reduced the

(1,25-d3) (vitamin D <sub>3</sub> )		number and size of lung metastases [139]
1,25-dihydroxyvitamin D3 (calcitriol) and two analogues (EB1089 and CB1093) (steroid hormone)	i.p. (1µg/kg calcitriol or 2.5 µg/kg analogues 5 days/wk) 1 day after tumor inoculation	All compounds inhibited tumor growth [140]
Physical activity	TR (5 minutes/day) after 21 days tumor inoculation for 5 days	Increased tumor oxygenation [73]
Gabapentin ( 1-(aminomethyl) cyclohexane acetic acid) (analgesic)	p.o. ( 4.6, 9.1 and 16.8 µg/kg/diet daily) 48h after initial inoculation for 21 days	No effect on prostate tumor development [141]
Estramustine and 9-aminocamptothecin (9-AC) (alkylating antineoplastic agent; water-insoluble camptothecin derivative)	i.p. (10mg/kg/day estramustine on days 4 to 13) and s.c. (9-AC on days 4 to 13) for 14 days	Inhibited prostate cancer growth [142]
Estramustine and colchicines (alkylating antineoplastic agent; alkaloid)	i.p. ( 10.1 mg/kg estramustine and 0.1 mg/kg colchicine) on days 4-13	No interactions of compounds inhibited prostate cancer cell growth [143]
Magnetic Fluid Hyperthermia (MFH)	Magnetic fluids (injections of 400µl) and MFH (frequency 100 Hz and field strength 0-18 kA/m) during 45 min 20 days after tumor inoculation	Inhibited growth tumor [74]
Mix of estramustine, etoposide and taxol (alkylating antineoplastic agent; semisynthetic derivative of podophyllotoxin; antineoplastic drug)	i.p. (10 mg/kg/day estramustine on days 4 to 13 and 50mg/m <sup>2</sup> /day etoposide on days 4 to 13) and i.v. (135 mg/m <sup>2</sup> taxol on days 4 to 10)	Inhibited prostate cancer cell growth [144]
Nucleoside analogue BCH-4556 [β-L-(-)-dioxolane-cytidine]	i.p. (75 mg/kg 2 times/day) soon after tumor inoculation or 3 days after for 6 days or IP (25 mg/day or 75 mg/day 2 times/day) 8 days after tumor inoculation for 6 days	The maximum dose prevented tumor growth [145]
Paclitaxel (antineoplastic drug)	Intratumorally injection (polymer loaded with paclitaxel in total of 250 µl of the suspension) 1 time or i.p. 4 mo of paclitaxel) every 3 days for 35 days	Polymeric formulation releasing paclitaxel reduced the tumor mass and metastases [146]

	Tamoxifen and vinblastine (selective estrogen receptor modulator; natural alkaloid)	d.w. (100nM Tamoxifen daily) and IV (1% vinblastine on days 3 and 10) for 14 days	Combined compounds inhibited the prostate tumor growth [147]
	Thermotherapy using magnetic nanoparticles combined with external radiation	Magnetic fluid (200-400µl) and MFH (frequency 100 Hz and field strength 0-18 kA/m); External radiation (2x10 Gy, 2x20 Gy or 2x30 Gy) 20 days after tumor inoculation	Combined treatment with the radiation dose of 20 Gy inhibited tumor growth [148]

TR: treadmill running; p.o.: *per os*; wks: weeks; i.p.: intraperitoneal injection; d.w.: drinking water; s.c.: subcutaneous injection; i.v.: intravenous injection

**Table 1.** *In vivo* studies using transgenic rats to evaluate the effect of various compounds in prostate cancer studies.

Transgenic rats model			
Animal Strain	Drugs or compounds evaluated	Dose/Treatment	Therapeutic effects (Ref)
Trangenic rats with a Sprague-Dawley genetic background	Apocynin (inhibitor of NADPH oxidase)	d.w. (100 or 500 mg/mL) at 6 wks of age for 8 wks	Suppressed the progression of prostate carcinogenesis [149]
	Arctiin (phytoestrogen lignan)	p.o. (0.1, 0.002 or 0.004%) for 18 wks	No effects were observed [150]
	Atrazine (herbicide of the triazine class)	p.o. (500 or 1000 ppm/diet) for 13 weeks	Slight inhibition of prostate carcinogenesis by high dose [151]
	Fermented brown rice and rice bran with <i>Aspergillus oryzae</i>	p.o (5 or 10%/diet) at 6 wks of age for 15 wks	Suppressed the progression of prostate carcinogenesis (54)
	Finasteride and flutamide (5-alpha reductase; non steroidal antiandrogen)	Gavage ( Finasteride 10 mg/kg and flutamide 5 or 20 mg/kg) 5 times/ week for 2, 5 and 7 weeks	Suppressed prostate cancer development [26]
	Genistein and resveratrol (phytoestrogens)	p.o. (250 mg/kg/diet genistein and 250 mg/kg/diet resveratrol or 83 mg/kg/diet genistein + 83 mg/kg/diet resveratrol) at birth for 12 or 30 wks	Compounds alone or in combination suppressed prostate cancer development [152]
	Leuprorelin acetate (luteinizing hormone-releasing hormone agonist)	s.c. (0.28 and 2.8 mg/kg once a wk) for 4 wks	Inhibited prostate carcinogenesis [153]
	Nobiletin and auraptene (pymethoxy-flavonoid extracted from citrus fruits; prenyloxy coumarin antioxidant agent isolated from citrus fruits)	p.o. (500 ppm) at 5 wks of age for 15 wks	Nobiletin may be valuable for prostate cancer prevention [154]
	Pioglitazone (peroxisome proliferator-activated receptor $\gamma$ agonist)	p.o. (0.1 and 5 mg/kg) 5 times/wk for 8 wks	Suppressed prostate carcinogenesis [28]
	Pomegranate fruit juice and ellagic acid (polyphenol)	p.o. (0.1% or 1% ellagic acid ) or DW (5% pomegranate juice) for 10 wks	Suppressed the progression of prostate carcinogenesis ([29]
Raloxifene and nimesulide (Selective estrogen receptor	s.c. (5 mg/kg raloxifene at 5 wks of for 90 days) or p.o. ( 400 ppm/diet nimesulide) + s.c. (5 or 10 mg/kg raloxifene) for 90	Raloxifene inhibited prostate tumor development, but not nimesulide [155]	



	modulators; selective cyclooxygenase-2 inhibitor)	days	
	Resveratrol (phytoestrogen)	d.w. (50, 100 or 200 µg/ml) at 3 wks of age for 10 wks	Inhibited prostate carcinogenesis [156]
	γ-tocopherol (vitamin)	p.o. (50 or 100 mg/kg/diet for 10 wks or 50, 100, or 200 mg/kg/diet for 7 wks)	Inhibited prostate tumor progression [23]

p.o.: *per os*; wks: weeks; d.w.: drinking water; s.c.: subcutaneous injection

Figure 1  
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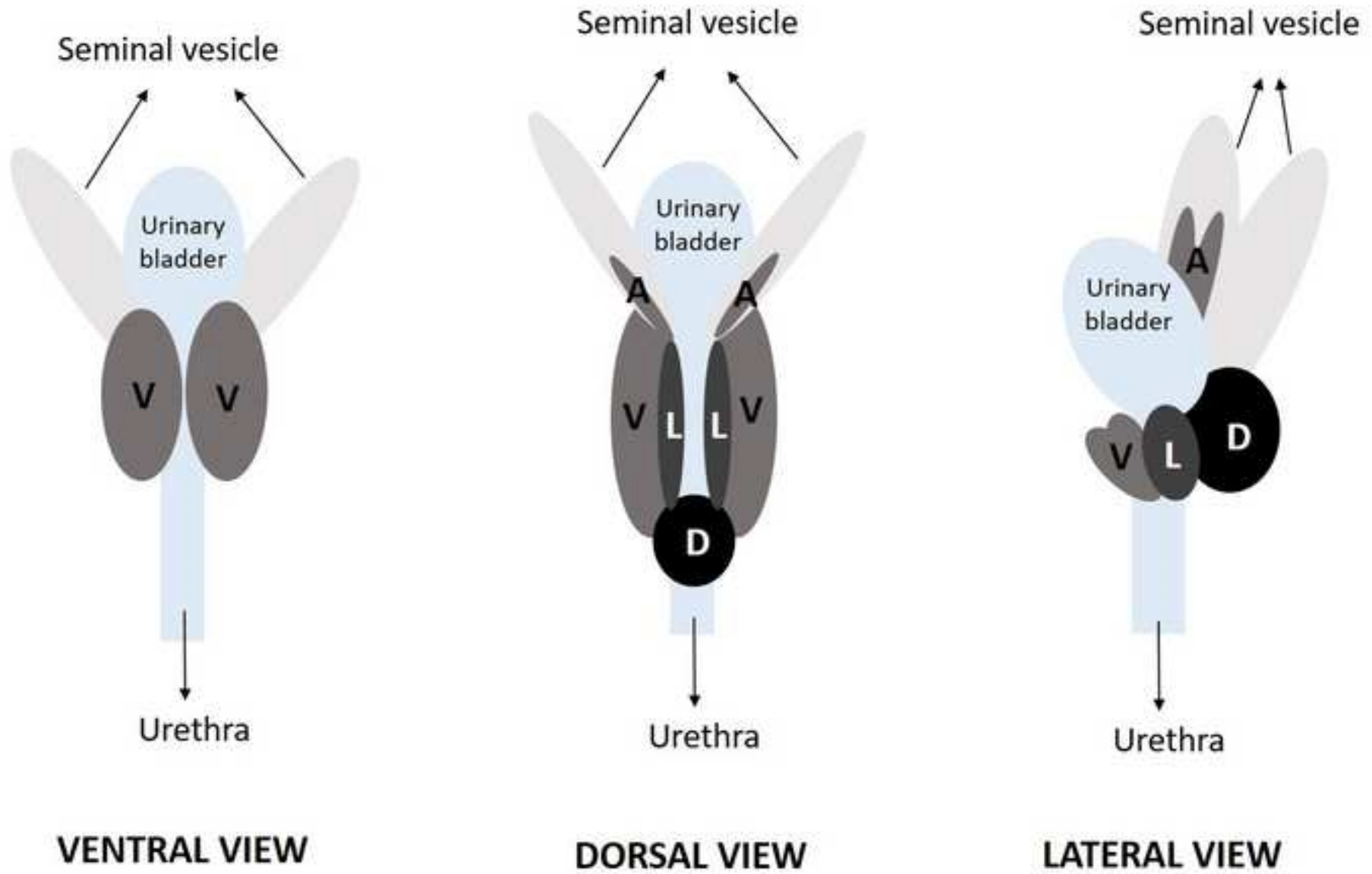


Figure 2  
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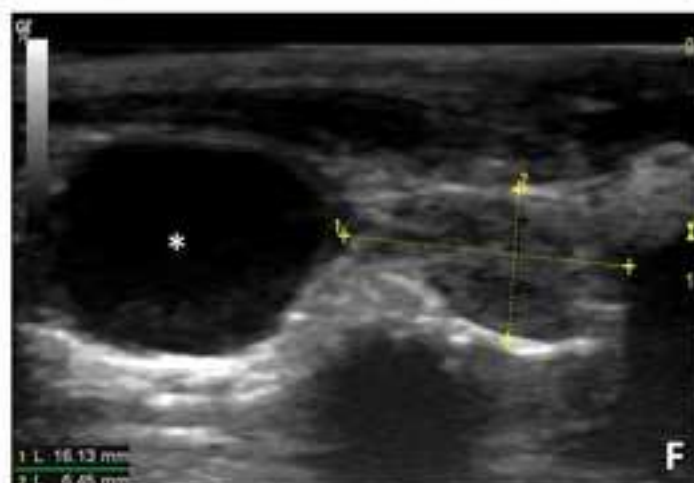
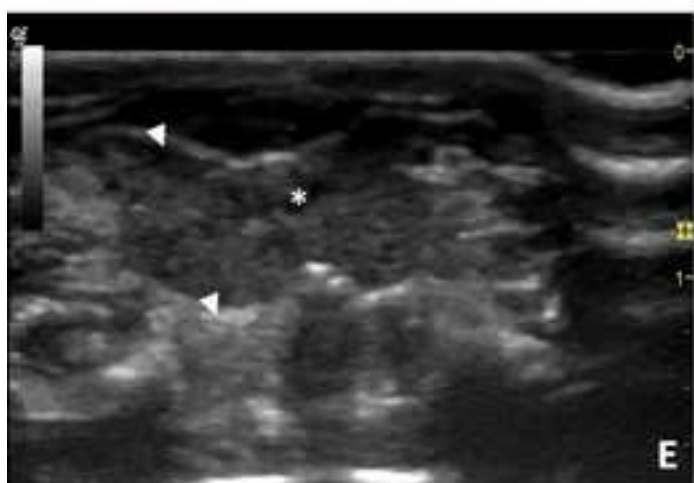
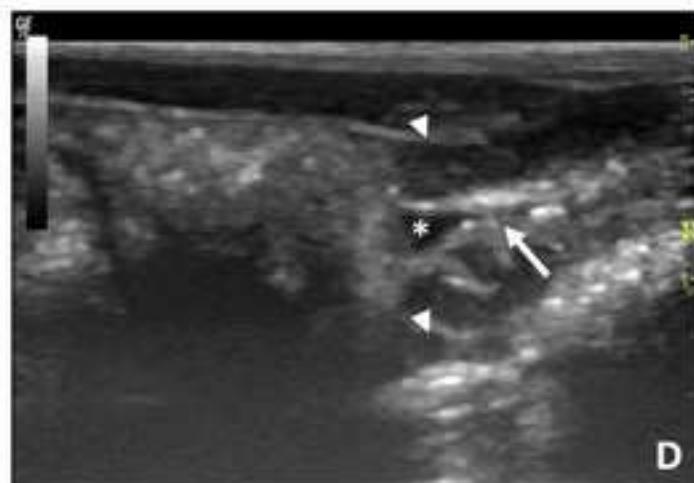
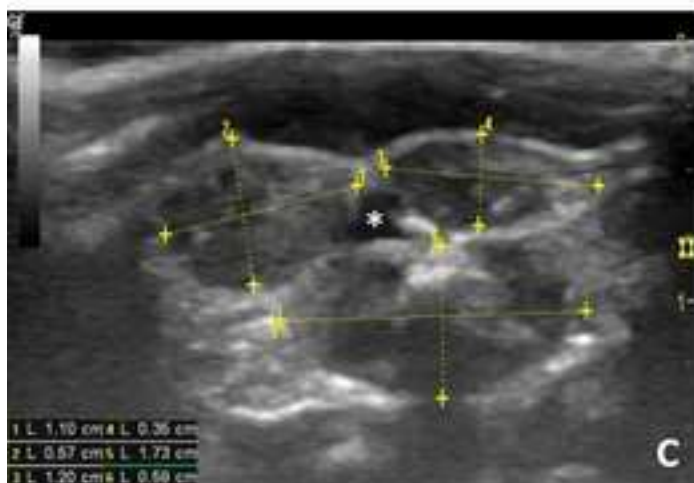
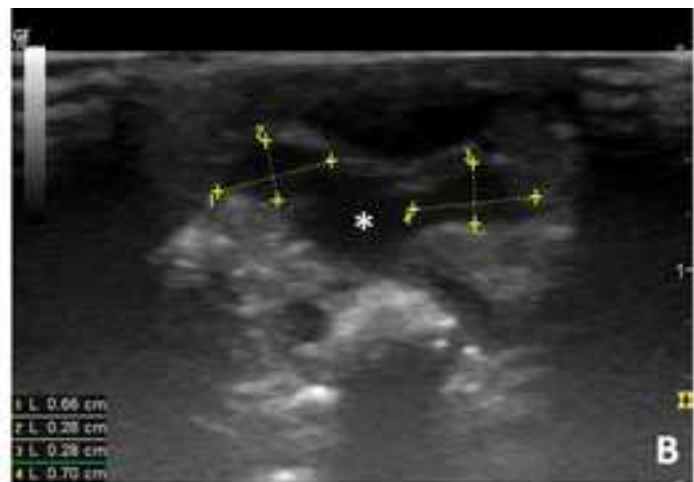
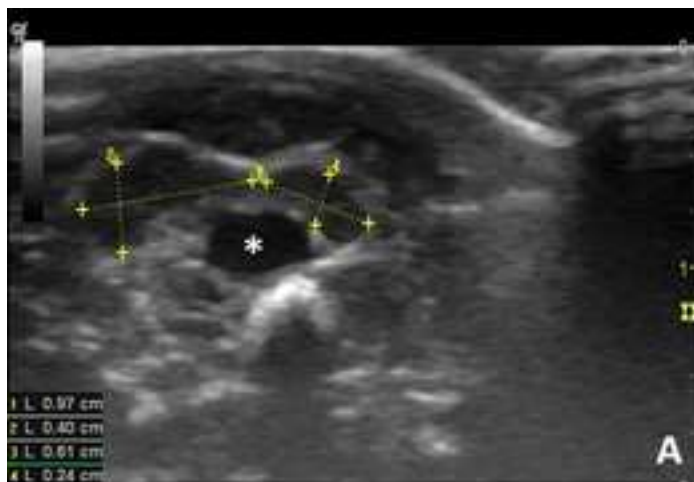


Figure 3  
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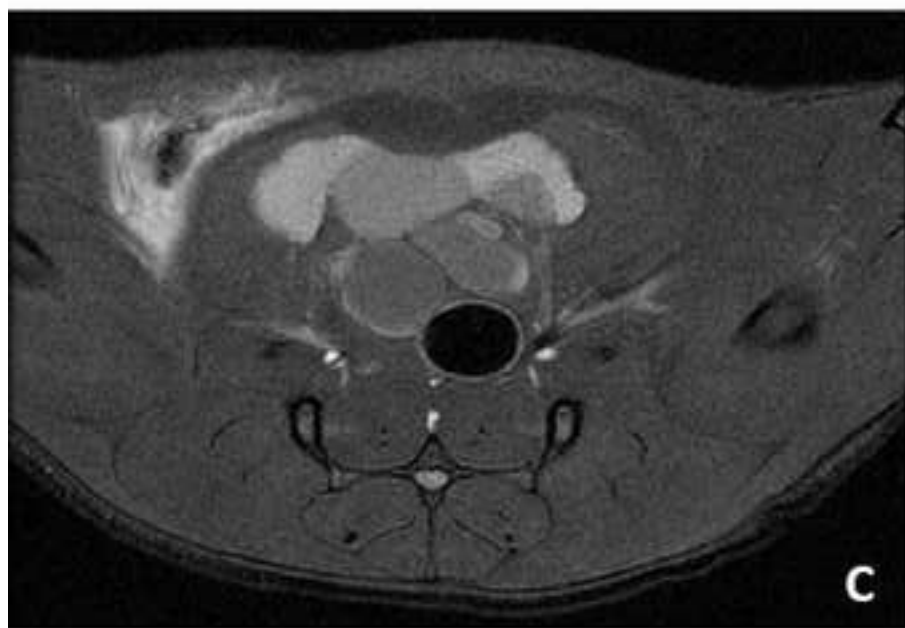
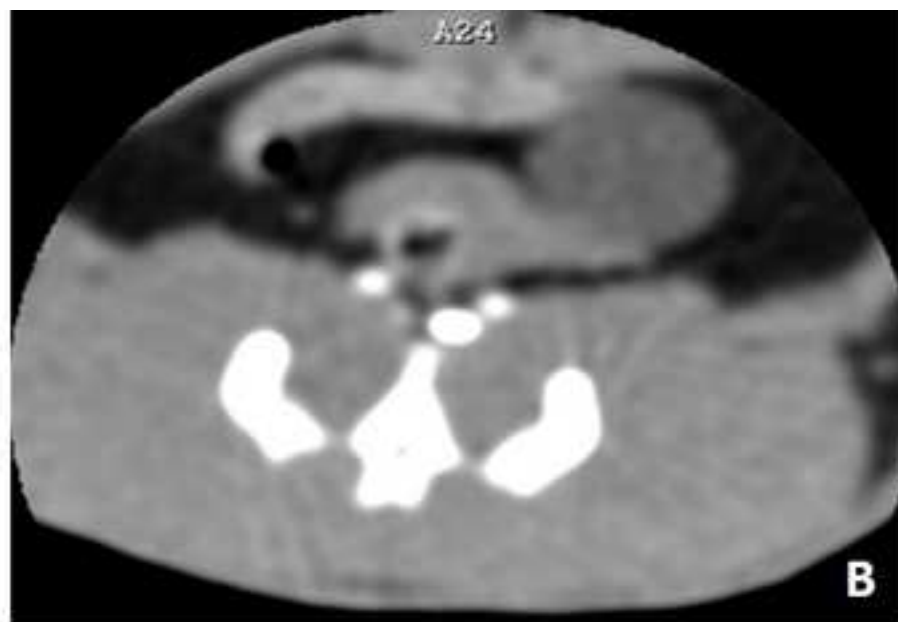
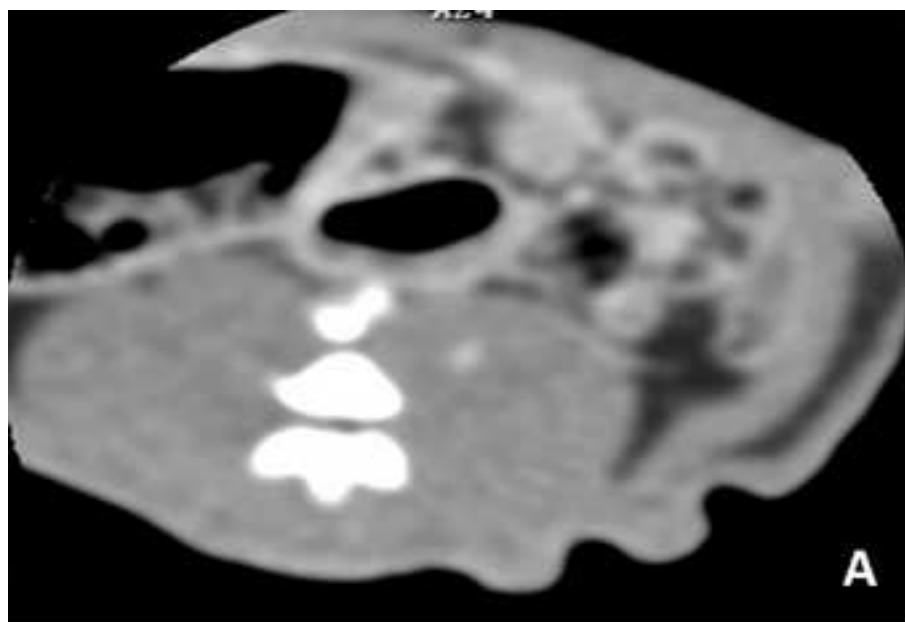


Figure 4  
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