Life Sciences 217 (2019) 141–147

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model: A good alternative to study PCa progression and chemoprevention approaches

Larissa Akemi Kido[a,b,*], Celina de Almeida Lamas[b], Mário Roberto Maróstica Jr[a], Valéria Helena Alves Cagnon[b]

[a] Department of Food and Nutrition, University of Campinas, 80 Monteiro Lobato St, Campinas, São Paulo 13083-852, Brazil
[b] Department of Structural and Functional Biology, University of Campinas, Bertrand Russel Av, Campinas, São Paulo 13083-865, Brazil

ARTICLE INFO

Keywords:
TRAMP
Prostate
Inflammation
Angiogenesis
Reactive stroma

ABSTRACT

The use of genetically modified animals has been studied in scientific research over time as a way to discover new treatments or even a cure for various diseases. Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) is a model for prostate cancer (PCa) that develops lesions that range from preneoplastic to metastasis. Its similarity to human PCa brings essential knowledge about disease development as well as making possible to investigate different degrees of the tumor profile. We reviewed the literature regarding five important areas relating to PCa progression in the TRAMP model. We also present some useful PCa models comparing them to TRAMP. Furthermore, we investigated the effect of some therapies related to these areas highlighting the best approaches that can delay PCa progression. The revised studies showed that TRAMP cancer stages are well established from 8 to 30 weeks of age, which makes possible to interfere in specific times of PCa development. Moreover, inflammatory and angiogenic blockage before the appearance of malignant lesions retarded PCa progression and showed better results than therapeutical approaches in other phases in TRAMP mice. Reactive stroma is less studied than other areas, although it has been showing a particular relevance in PCa as a milestone in malignant transformation through the modulation of TGF-β, vimentin, and αSMA. We concluded that even years after its creation, the TRAMP model is still one of the most essential tools for PCa study, as well as for the development of new strategies to prevent the disease.

1. Introduction

For almost 25 years, the term TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) has been present in a wide variety of studies to designate a specific genetically engineered mouse. This model resembles human prostate cancer (PCa) features, showing different lesion grades and progression in a short period. Thus, researchers have explored several investigative methodologies in this model to understand and uncover the complex process involved in PCa. TRAMP mice were engineered using the minimal rat probasin promoter (−426/+28) to target the large T SV40 virus expression and small t oncoproteins, only in the prostatic epithelium, inactivating p53 and Rb tumor suppressor activity in the prostate [20,21,24]. As a result, this model is able to develop not only progressive PCa, but also androgen independence and metastatic spread [20].

The advantage of monitoring prostatic lesion progression in a short time has brought relevant results for TRAMP studies, including morphological characterization of prostatic tissue and other organs; cancer metabolism; diagnosis; prevention; treatment and novel drug discovery [33,56,57]. According to Balmain [5], the use of mouse models shows advantages since it allows the control of aspects such as environmental exposure that leads to cancer onset, which is as yet not possible in human beings. Furthermore, the genetically engineered mice (GEM) have contributed to elucidate different biological processes involved in aggressive profile PCa not only in basic but also translational research [22]. However, it is important to make clear that until now no single mouse model comprises all features of human PCa progression, therefore many models have been created to reproduce the main characteristics of this disease [22,34,42].

GEM PCa models can be divided mainly into (i) models with viral oncogenes, in which the TRAMP model belongs; (ii) knockout mouse models based on tumor suppressor genes; (iii) transgenic mice with multiple genetic mutations [78]. Different authors brought up in-depth discussions and reviews in the literature about these models, comparing

* Corresponding author at: Department of Food and Nutrition, School of Food Engineering, University of Campinas (UNICAMP), P.O. Box 6109, 13083-852 Campinas, SP, Brazil.
E-mail address: larissa.kido@gmail.com (L.A. Kido).

https://doi.org/10.1016/j.lfs.2018.12.002
Received 12 September 2018; Received in revised form 26 November 2018; Accepted 2 December 2018
Available online 04 December 2018
0024-3205/

them to each other and showing advantages and limitations of their use, which will not be discussed as a whole in this manuscript [22,47,78]. However, we selected some PCa models to compare to TRAMP and to bring forth a new perspective on the different issues here exploited.

In this review, we discussed five extremely relevant areas relating to PCa progression in the TRAMP model: morphological characterization, hormonal response, angiogenesis, inflammation, and reactive stroma. Moreover, we presented studies involving new therapies, strategies to prevent tumorigenesis and new biomarker identification in all these issues above mentioned. Also, we highlight the importance of studying the different prostatic lobes, in which the proliferative lesions could be less frequent and/or more aggressive in the TRAMP model. Therefore, this review aims to highlight the TRAMP mice model as an excellent way to find new opportunities for chemoprevention and therapeutic approaches, establishing new perspectives in PCa studies.

2. Morphology

2.1. Lesions staging in TRAMP mice as a differential factor in the prostate cancer study

Gingrich and Greenberg [21] already stated that the TRAMP model has some advantages concerning other experimental models in PCa. Among them, the fact that the specific transgene is mainly restricted to the prostatic epithelial cells and the tumor histological similarities to human being PCa, such as metastasis and androgen-independence [18,21]. Nevertheless, Chiaverotti et al. [11] consider that PCa development in TRAMP mice is not similar to the human form of the disease, taking into consideration that TRAMP mouse prostatic stroma and epithelium are simultaneously affected by the lesions in a diffuse form, despite showing nuclear atypia.

TRAMP mice develop progressive forms of the prostatic disease, which range from prostatic intraepithelial neoplasia (PIN) to invasive adenocarcinoma, besides a metastatic process occurring in the lymph nodes, lungs, and bones [20,21,24]. The presence of proliferative processes is verified in 6–12 week-old TRAMP mice with different grades of hyperplasia, including PIN with cribriform and papillary patterns, which are an indication of disease severity [21]. Severe hyperplasia and adenocarcinoma are verified in 18 week-old mice, and according to Greenberg [23], 100% of TRAMP mice present primary tumors at 24–30 weeks of age.

TRAMP mice have different backgrounds, which demonstrates differences related to the life expectancy and PCa development. TRAMP mice C57BL/6 TRAMP X FVB rarely live more than 33 weeks of age, whereas mice C57BL/6 TRAMP could live up to 36–40 weeks of age [19]. These differences in life expectancy proved that TRAMP mice with an FVB background presented more aggressive diseases than those with a C57BL/6 background [19]. According to Chiaverotti et al. [11], TRAMP mice from FVB background usually live less than TRAMP mice from a C57BL/6 background due to the fast development and progression of neuroendocrine adenocarcinoma.

Regarding to the presence of proliferative processes in the different prostatic lobes, it was verified that the transgene expression in TRAMP mice is mainly related to the dorsolateral and ventral lobes [21]. Despite this, different studies showed the occurrence of adenocarcinoma in other organs from the male reproductive tract, such as the anterior prostate lobe and seminal vesicle [14,57]. The differential responses among the prostatic lobes were shown in an experiment of dietary restriction from 7 to 11 or 20-week-old TRAMP mice [65]. These authors observed a lobe-specific neoplastic development and a decrease lesion severity in anterior, dorsolateral and ventral lobes [65]. The prostatic lesions could be seen initially as focal proliferation, showing hyperbasophilic epithelium in the anterior and dorsal lobes, whereas diffused lesions in all the epithelium were observed in the lateral and ventral prostatic lobes [66]. Kaplan-Lefko et al. [30] verified that dorsal, ventral and lateral lobes presented PIN as the primary lesion in 8-week-old mice, whereas the anterior lobe remained predominantly healthy. Furthermore, at 8 weeks of age, the dorsal and ventral lobes presented less than 2% of the glandular area which could be classified as cancer [30]. By and large, Kaplan-Lefko et al. [30] highlighted a substantial change in prostatic morphology of TRAMP mice that ranged from healthy and/or PIN, at early PCa stages, to an entirely disease dominated microenvironment.

Research from our group contributed to elucidate the morphological development of PCa in TRAMP mice, as well as create new criteria for lesion staging. Kido et al. [33] verified the presence of low-grade prostatic intraepithelial neoplasia (LGPIN) and high-grade prostatic intraepithelial neoplasia (HGPIN) in the ventral prostate of 8 and 12-week old TRAMP mice, where HGPIN incidence was significantly higher in the older animals. The presence of undifferentiated adenocarcinoma was only detected in the ventral prostate of 22-week-old TRAMP mice. This study also demonstrated that Goniothalamin (GTN), a synthetic compound similar to those found in *Goniothalamus* genus plants with anti-inflammatory properties, led to a PCa progression delay in TRAMP mice, highlighting the inflammation as an important factor for cancer progression. In agreement with previous literature data, Silva et al. [56] also demonstrated the PCa progression according to aging in the prostate ventral lobe from TRAMP mice. Interestingly, these authors described that the angiogenesis blockage with Nintedanib, before PCa onset, was able to delay the disease progression. Nonetheless, therapy with the same antiangiogenic drug also increased healthy tissue areas in the prostate dorsolateral and anterior lobes in 12 and 16-week old TRAMP mice, as well as decreased pre-neoplastic lesion [3,45]. In other words, these studies confirmed that therapeutic approaches at the beginning or before PCa onset, were fundamental to keep the prostatic microenvironment balance during more time, making the cancer progression difficult.

Different authors have shown extraprostatic proliferative lesions in TRAMP mice such as neuroendocrine urethra tumors [66], seminal vesicle tumors [14,68], and renal tubular acinar carcinomas [68]. Berman-Booty et al. [7] identified two types of tumor in TRAMP mice, the first an anaplastic tumor in the midbrain and the second, a poorly-differentiated adenocarcinoma in the submandibular salivary gland. A study involving androgen ablation therapy, showed that more than 40% of castrated TRAMP mice developed a recurrent tumor, which was more poorly differentiated than those found in the non-castrated mice, besides showing an increased incidence of metastasis [30]. These authors detected that castration led to an increased metastatic rate in the liver, salivary gland, and calvaria, even in animals that did not present local disease recurrence, suggesting the metastatic process as an early event able to acquire hormonal independence [30].

It is known that androgens are responsible for the development, growth, and homeostasis of the prostate, and in TRAMP mice it is not different, due to the transgenic expression being directly regulated by androgens [21]. Niu et al. [44] pointed out that the androgen receptor (AR), located in the prostatic stroma has an important role in prostatic proliferation activity in primary PCa in the TRAMP model. In this context, androgen-ablation therapy in the TRAMP model led to hormone-independent PCa at 24 weeks of age associated with a higher neuroendocrine cancer phenotype occurrence, which possibly contributed to a more aggressive PCa after castration [30]. Interestingly, these authors verified that synaptophysin, a neuroendocrine marker, was expressed in most primary tumors of castrated mice presenting metastatic disease. Chiaverotti et al. [11] also point out the possible existence of neuroendocrine precursor lesion in 4-week-old-TRAMP mice, which was associated with a lower or negative AR immunoexpression in neuroendocrine foci. These data show a complex response of TRAMP mice after androgenic ablation, which is relevant considering that the disease recurrence is a natural condition of human PCa. It is believed that androgens act on the prostate of the TRAMP mice primarily through AR, which throughout cancer development can suffer mutations and respond to other stimuli. In this way, TRAMP mice appear to be a suitable animal model to be used when evaluating agents that target this hormone-refractory disease [30].

Nevertheless, the androgen and estrogen imbalance in this gland is directly related to PCa pathogenesis, highlighting the importance of the synergic action of these hormones for prostate regulation [13,77]. Different authors showed that AR and estrogen receptor α (ERα) levels tend to increase in the ventral and anterior prostate during PCa progression in TRAMP mice [56,57]. According to Slusarz et al. [61], TRAMP mice ERα knocked (KO) presented a decrease in poorly differentiated adenocarcinoma in comparison with wild mice. Also, a reduced incidence of poorly differentiated adenocarcinoma was seen in ERβ KO TRAMP mice, indicating that therapies targeting estrogens receptors can prevent PCa [61]. The same authors suggested that in the TRAMP model ERβ stimulation demonstrated a protective role, whereas ERα expression could develop a tumorigenic influence. It was recently verified that TRAMP mice presented an AR increase tendency in both ventral and anterior lobes in 8–22 week old TRAMP mice, as well as a tendency to increase ERα in both epithelial cell cytoplasm and nucleus [56,57]. Silva et al. [57] demonstrated that Goniothalamin treatment decreased both hormone receptors. On the other hand, it was verified that the AR immunoexpression decreased after fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) receptor were blocked with Nintedanib, whereas the ERα signaling did not change. The TRAMP model also exhibits important features regarding hormonal response that can contribute towards studies involving the new therapies.

3. Angiogenesis

3.1. Interfering in neovascularization at the early disease, an efficient tool in the management of tumor microenvironment

The angiogenesis process has been studied in the TRAMP model since it develops PCa spontaneously and presents well-defined progression steps, showing the same phases of angiogenesis observed in clinical PCa [28,56]. Moreover, TRAMP mice allow the study of the initial phase of angiogenesis, which is crucial to tumor onset and is rarely accessed in clinical practice [28,49].

The first stage of angiogenesis, known as the "initiation switch," occurs early in PCa development, in which an increase of hypoxia 1α factor (HIF-1α) in response to low oxygen tension in TRAMP mice is observed. It stimulates the synthesis of pro-angiogenic molecules such as VEGF, FGF2 and its receptor 3 (FGFR3), fact that intensify the vascular permeability and created a favorable microenvironment for tumor survival and metastasis onset [38,56]. In this stage, VEGF acts through its receptor VEGFR1 (Flt-1), which is associated with PIN and well-differentiated adenocarcinoma presence in the TRAMP model [56].

As PCa progresses in the TRAMP model, a second "angiogenic switch" occurs, which is also called the "progression switch". In this stage, there is an HIF-1α and VEGFR1 reduction, associated with an intensification of VEGF and VEGFR2, besides the increase of intraepithelial microvessels, undifferentiated adenocarcinoma and tumor androgen independence [3,28,38]. The presence of two receptors for VEGF, also verified in the TRAMP model, ensures its differentiated effect on tumor development. The VEGFR1 expression is related to cell migration, whereas the VEGFR2 expression is associated with increased cell proliferation and the induction of the anti-apoptotic process in endothelial cells [8,28]. A recent proteomic study showed the overexpression of platelet-derived growth factor B (PDGF-B), a pericyte recruiter, in tumor tissue originated from TRAMP [82]. These authors validated the PDGF-B network in PCa carcinogenesis, confirming the upregulation of angiogenic pathways in the TRAMP model.

Interestingly, there are also anti-angiogenic molecules that exert a protective role in the development of PCa, such as endostatin [38,70]. In TRAMP mice, high levels of this molecule were observed mainly at the PIN stage, which is probably a consequence of the associated increase of metalloproteinases (MMP), which have a proteolytic role in endostatin synthesis [38,39]. However, PCa progression in this model led to reduced levels of this molecule associated with an increase in pro-angiogenic factors such as VEGF and FGF2 [38].

The interference in angiogenesis signaling leads to reduced neovascularization and associated factors such as VEGF, VEGFR1, and FGFR3, and is considered an important intervention strategy in TRAMP PCa [56]. Moreover, the blockage of angiogenesis pathways in TRAMP mice alters mitogenic factors related to cell proliferation; besides contributing to the reduction of pro-inflammatory factors, such as interleukin (IL) 17 and cyclooxygenase 2 (COX-2) [3,45,56]. In addition to this, anti-angiogenic approaches can also modulate structural molecules, such as α and β dystroglycan and laminin, which are important for tumor environment remodeling and cell migration in TRAMP mice [45].

Not only specific anti-angiogenic drugs can interfere on angiogenesis, but also other compounds with different properties have also been demonstrating the ability to modulate the prostate angiogenic status. Polyphenols, for example, showed a capacity to down-modulate angiogenesis key molecules such as VEGF, VEGFR1 and 2, HIFα and MMPs, probably as a result of its interference in insulin-like growth factor 1 (IGF-1) signaling. The inhibition of mitogenic pathways promoted downstream regulation of angiogenesis, preventing this process and PCa progression in TRAMP mice [1,53,55,60]. Anti-inflammatory therapies, including Celecoxib treatment, revealed that COX-2 downregulation reduces Prostaglandin E_2 expression, which is directly related to angiogenic signaling. Thus, the interference in inflammatory pathways could also reduce VEGF expression in TRAMP PCa, collaborating to reduced tissue irrigation and consequently low tumor growth [43].

Thus, the importance of studying mechanisms related to angiogenesis in the TRAMP model is evident, since it is complex and essential for the development, survival, invasiveness, and differentiation of the prostate tumor. Therefore, the interference in the angiogenic process is effective in delaying TRAMP PCa progression, affecting signaling pathways and interfering in extracellular aspects.

4. Inflammation

4.1. The inflammatory pathways are important upstream malignancy signaling

The inflammatory process is triggered by the attempt of the immune system to repair some benign prostatic alterations, such as prostatic inflammatory atrophy and PIN [69]. Studies of TRAMP PCa show that the most critical inflammatory hallmarks in clinical practice are also altered in this mice model. This clinical implication reinforces its importance for the study of inflammation [2,27,51,69].

IL-6, IL-8, and IL-17 are important PCa promoters. Generally, these cytokines are increased according to tumor progression in human PCa [10]. In the TRAMP mice, a progressive increase in the IL-6 serum levels occurs in 20, 24 and 28-week-old [27,80]. Besides, a high IL-17 immunoexpression in the anterior prostate of 12 and 16-week-old TRAMP mice was verified, highlighting the possible influence of inflammatory mediators on tumor development even in a less aggressive PCa [3]. These interleukins presented a multifunctional action that regulate the expression and activity of other inflammatory mediators, such as the signal transducers and activators of transcription 3 (STAT-3), nuclear factor kB (NFkB) and COX-2. These molecules also stimulate mitogenic factors and the continuous secretion of pro-inflammatory cytokines, improving tumor invasiveness, progression and metastatic onset [35,69,73,79].

The STAT-3 and NFkB are transcription factors that are involved in tumor survival stimulus and are generally elevated in PCa [31,54]. It was observed high pSTAT-3 and NFkB levels in the ventral prostate of 22 weeks-old TRAMP mice, confirming the involvement of these molecules in PCa progression. Moreover, the constitutive activation of these transcription factors stimulates positive feedback, amplifying the inflammatory response and deregulating the prostatic microenvironment even more [69].

COX-2 is also considered a key molecule in malignant development since it is related to several processes involved in PCa progression such as angiogenesis, apoptosis, and invasiveness [51,62]. Its expression is upstream stimulated by androgens, inflammatory cytokines and transcription factors such as IL-6, tumor necrosis factor α (TNFα) and NFkB [16]. The COX-2 is expressed in TRAMP mice at the beginning of malignant development and increases with tumor progression [3,33,57]. Recent studies demonstrated that COX-2 inhibition, especially at the beginning of TRAMP PCa (from 8 to 12 weeks of age), reduced cellular proliferation and increased the apoptotic index in the ventral prostate in short-term, as well as reduced the proliferative index and well-differentiated adenocarcinoma frequency in long-term [33,57].

The use of new drugs in PCa treatment with a broader action in different targets of inflammatory pathways have promoted a better morphological recovery. Kido et al. [33] verified that the GTN, administered from 8 to 12-week-old TRAMP mice, presented a broad spectrum of anti-inflammatory action decreasing COX-2, NFkB, STAT-3, TNFα, and IL-1β. These authors also confirmed that the GTN anti-inflammatory potential was one of the main reasons implicated in PCa delay. Silva et al. [57] evaluated the GTN effect in the anterior prostate, revealing that besides reducing the COX-2 level, GTN could also interfere in the AR and ERα levels, confirming the relationship between inflammation and hormonal imbalance in TRAMP PCa.

Also, it is already known that COX-2 can interfere in angiogenesis due to the upregulation of VEGF and FGFs, besides the downregulation of BCL-2, inhibiting endothelial cell apoptosis [17,51]. Recent research verified that the blockage of VEGF, PDGF and FGF receptors was able to reduce COX-2 and IL-17 immunoexpression in TRAMP mice, pointing to the participation of inflammatory mediators in PCa complex pathways [3]. Thus, it is possible to observe that the use of the TRAMP model to study inflammation in PCa development allows not just the verification of chronic and acute inflammation, but it also permits the evaluation of integrated pathways that are essential to carcinogenesis.

5. Reactive stroma

5.1. Targeting the prostatic microenvironment in TRAMP mice

The fundamental role of the stromal environment in tissue homeostasis maintenance and its strong influence in epithelial signaling, wound healing processes, structural plasticity, as well as the development of some diseases is well established in the literature [6,12,71]. Also, the high capacity to respond promptly to tissue homeostasis disturbance can make the stroma a conducive environment for cancer onset, and in this case, it is referred to as the "reactive stroma." According to Tuxhorn et al. [71], this term describes a new microenvironment, which was generated as a consequence of the neoplastic process.

The main features that define the reactive stroma are the presence of stromal cells with a myofibroblastic phenotype adjacent to proliferative epithelial lesions, an increase of ECM remodeling, angiogenesis, and inflammation, which are similar to a wound healing response [6,40,71]. In an attempt towards tissue repair, these events together modify the tissue structure and enhance tumor permeability for growth factors in the host stroma, giving support to cancer development. In the prostatic tissue, reactive stroma is correlated to PIN presence and advances according to cancer progression [72]. Moreover, the reactive stroma phenotype appears to be associated with the elevated expression of some proteins, which are not only linked to stromal transformation but also define the key characteristics of myofibroblasts and cancer-associated fibroblasts, the main cells of the reactive stroma. These cells have a central role in PCa progression through their repair-centric and pro-survival biology, secreting a variety of growth factors and chemokines that contribute to proliferation and angiogenesis [4,6].

Different authors have shown the reactive stroma in the TRAMP model. Montico et al. [40] identified increased immunoexpression of α-actin (αSMA), vimentin (VIM) and the transforming growth factor beta (TGF-β) in the PIN stage of 8-week-old TRAMP mice, which was similar to that found in the prostate of senile non-transgenic animals. In addition, the same authors associated the dual positive immunolabelling of CD34/αSMA and CD34/VIM to lesion degree increase in TRAMP mice, suggesting the existence of a perivascular origin of reactive stromal cells. Both αSMA and VIM are reactive stroma markers, and their co-expression is related to a switch in stromal cell phenotype, which is attributed to myofibroblasts [72]. Likewise, TGF-β is considered a regulator of prostate cancer reactive stroma *in vivo* due to its ability to stimulate phenotypic stromal cell switching and regulate processes such as angiogenesis and ECM remodeling [72]. The crossing between TRAMP and transgenic mice harboring the dominant-negative mutant TGF-β type II showed that TGF-β signaling imbalance quickened neoplastic transformation in the prostate, increasing proliferative index in 12, 16, 20 and 24 week-old mice [52]. Also, this study suggested that TGFβRII inactivation was able to enhance AR expression and macrophage infiltration, inducing endothelial-to-mesenchymal transition and neovascularization, as well as worsening lesion severity in the early stages of tumor development. Together, these studies pointed out that TGF-β, as a reactive stroma key molecule, is significant to PCa progression, and that TRAMP mice can be useful to test possible regulatory agents which interfere in this pathway.

Another aspect about reactive stroma in TRAMP mice was detailed by Yang et al. [81]. These authors characterized the stromal cell phenotype in three PCa mice models (PB-MYC, ERG/PTEN, and TRAMP), and found different proportions of cells with smooth muscle or fibroblast-like features in each model. TRAMP mice between 20 and 33 weeks of age showed PIN predominance and increased αSMA positive cells with an invasive phenotype. On the other hand, in advanced tumor proliferating stromal cells masses invading epithelial compartment were identified and classified as an intraductal adenoma with stroma (IAS). In these areas, most of the stromal cells showed intense αSMA, VIM and or collagen-1 immunolabelling, which implies in reactive stromal features. Notwithstanding, the authors indicated smooth muscle cells as the main cell-of-origin of the stromal cells in TRAMP mice tumors. The same study still reported the hedgehog (HH) signaling role, which regulates the development and regeneration of the adult prostate. The results indicated increased expression of HH ligands in TRAMP mice, especially in PIN lesions, but not in healthy epithelial cells. According to Yang et al. [81], HH signaling can be predictive of a stromal presence in a tumor, which occurs in a paracrine mode.

Recently, Silva et al. [57] indicated that increased αSMA immunoexpression followed the PCa progression in the anterior prostate of TRAMP mice. Also, a long-term decrease of αSMA immunolabelling was observed after GTN treatment, indicating that reactive stroma markers can also be influenced by inflammation blockage. Although this study does not explicitly bring insights into the reactive stroma, other authors have already reported that an inflammatory microenvironment can change genotypic and phenotypic features in the stromal cells, leading to stromal reactivity in the prostate [6]. Considering the human PCa variability and its different rate of tumor progression, it is plausible to suggest the use of the anterior prostate of TRAMP mice as a good candidate in futures studies on reactive stroma, as well as the other prostate lobes.

6. Alternative animal models for PCa and perspectives in preclinical studies

Chemoprevention studies, drug innovation, and strategies to delay PCa progression, as well as other types of cancer, take place with the mandatory use of animal models, at least until now. Although there is no single model which mimics the human being disease, it is fundamental that animal models resemble human neoplastic lesions for PCa study [34]. The two significant pitfalls concerning mouse models for PCa are (i) when they develop bone metastasis, the incidence and progression are low in relation to human beings; (ii) the high incidence of neuroendocrine adenocarcinoma phenotype, which represents just 2% of cases in human being PCa [22,25,34]. Despite that, for chemoprevention studies and PCa progression evaluation, the main concern is restricted to reproducibility and standardization. In this case, TRAMP does not fail, and preclinical approaches should be considered using this model.

Over the last years, a large number of animal models were generated to cover the PCa heterogenicity, including models with the same prostate-specific probasin promoter (PB) utilized in TRAMP. In the LADY model, the promoter is the large PB, which controls SV40 Large T-antigen. Although the LADY shows similarity to pathological features in relation to TRAMP mice, they are different due to the lack of small antigen t in the LADY model. Eleven lines were constructed from original LADY, and all of them developed PIN by 10 weeks of age and undifferentiated adenocarcinoma by 20 weeks [32]. However, the LADY model presents a lower frequency than in the TRAMP [36]. Despite being a good model for PCa, intact mice did not develop metastasis as TRAMP mice do [22]. The literature data are scarce relating the LADY models and the topics addressed in this study; however studies using LADY 12T-10 line have observed that a diet supplemented with micronutrients such as vitamin E, selenium, and lycopene was able to decrease PCa progression as demonstrated by significant PCNA reduction and altered immunoexpression of neuroendocrine markers [46,75]. Cervi et al. [9] also observed that the same micronutrient supplementation for 42 weeks in the LADY model led to PF-4 expression increase, a megakaryocyte-specific protein that is an endogenous antiangiogenic. These authors believe that somehow the supplementation could hinder the angiogenic process in the tumor after mutations promoted by the angiogenic switch, controlling the early steps of tumorigenesis process as well as dissemination and metastasis [9].

Lo-myc and Hi-myc are PCa models also generated by prostate-specific PB (ARR_2PB), and high expression of myc has been observed in more than 30% of prostate tumors and increased according to tumor grade progress [78]. The myc is relevant in PCa context, not only because of its role in several mechanisms and disease progression, but also due to its overexpression as an early event of PCa. Hi-myc presents PIN at 2–4 weeks of age, and invasive adenocarcinoma can be detected from 12 to 24 weeks of age. In contrast, in Lo-myc mice, these same lesions appear by 10–12 months of age, showing a higher latency than Hi-myc [78]. Nevertheless, none of these animals developed metastasis neither neuroendocrine carcinoma, as found in TRAMP [15].

As revised by Grabowska et al. [22], the studies with the Hi-myc model have combined it with other genes relevant to human PCa, somehow limiting the direct comparison to the TRAMP model. Notwithstanding, some studies englobe the issues here described. Melis et al. [37] showed that invasive adenocarcinoma onset is retarded in Hi-Myc mice combined with B and T cell deficiency (RAG1 −/− mice) when compared to Hi-myc intact, as consequence of leucocytes decrease in the prostate. As observed in TRAMP mice, the lymphocytes absence also delays PCa progression [26]. Hi-myc mice with NFκB overexpression became non-sensitive to castration, suggesting that NFκB participation in progression to androgen independent PCa phenotype [29]. Taken together, myc-models also appear to be a practical tool to PCa study, mostly regarding the myc involvement in the onset and first stages of PCa development.

Unlike the models mentioned above, many GEM models have been created by incorporating single or multiples genetic losses and gains based on human PCa genetic lesions, altering tumor suppressor genes [22,74,78]. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a tumor suppressor gene, and its loss or mutation is linked to human being cancers and is a critical early regulator of PCa [34,74]. Pten-deficient models, in general, recapitulates human PCa progression without neuroendocrine formation, being entirely dependent to extent Pten inactivation and intensified by crossing with other knockout models. The Pten-KO (Pten-*loxp/loxp*: PB-Cre4$^+$), a conditional model for PCa, was used in a chemoprevention study to investigate the Withaferin A (WA) phytonutrient effects on disease progression [41]. The authors observed that WA treatment delayed PCa progression by downregulation of AKT pathway and endothelial-mesenchymal transition decrease [41]. Nevertheless, the point that catches our attention is that by using the same protocol, another study verified similar results to TRAMP mice (C57BL/6-Tg [TRAMP] 8247Ng/J) [64]. According to Moselhy et al. [41] the Pten-KO model showed the correlation between Par-4 (proapoptotic tumor suppressor protein) down-regulation and the inhibitory effects of WA on AKT phosphorylation, as seen in TRAMP mice. Despite being different models engineered by different genetic tools, in some methodological approaches we can look at some similarities among their biological responses.

Questions about reactive stroma were also assessed with Pten models. Pten null prostate cancer is able to become androgen insensitive, and after 10 weeks post-castration reactive stroma can be detected [76]. Another study reported that reactive stroma was increased in Pten PCa model under JAG1 stimulation, a Notch ligand [63]. They observed vimentin immunoexpression increase in periglandular stromal cells associated with a disruption of αSMA in stromal cell layers and a robust expression of Tenascin-C, predicting a myofibroblast phenotype [63]. However, JAG1 overexpression did not affect disease progression in a prostate-specific Pten null mouse model for PCa [63]. It is known that Notch is a preserved signaling pathway in mammalians that regulates many processes such as cellular differentiation, proliferation, angiogenesis and cell-fate in both homeostasis and carcinogenesis situations [48,63]. Unlike Su et al. [63], it was founded that JAG-1 upregulation in TRAMP endothelial-specific JAG-1 mutants led to an angiogenesis increase, as well as, PCa development and progression [48].

Therefore, the use of GEM is extremely convenient to PCa studies, mostly to plan and apply chemopreventive strategies. Here, we exposed the TRAMP mice as a joker model available to access different PCa aspects, being reported similar results to those seen in TRAMP and other PCa models.

In 2003 Kaplan-Lefko et al. [30] already postulated that the use of transgenic mice for preclinical tests is advantageous mainly due to cancer development is autochthonous and liable to an appropriate microenvironment and an immune system. Despite neuroendocrine cancer development in advanced phases, the TRAMP model is still used in chemopreventive studies and preclinical trials. Nowadays, there is a trend to test edibles sources compounds that can delay PCa progression. Recently, a preclinical study with diets containing tomato and lycopene showed a significant decrease in cancer incidence in all prostate lobes from TRAMP mice, in other words, the prostatic tissue responded similarly to the treatment. However, in the control groups, the cancer incidence was lobe-specific, even in a less aggressive line like C57BL/6-Tg(TRAMP) 8247Ng/J x FVB/NJ [67]. The probability of developing cancer was 7.37, 5.87, and 2.55 times higher in the dorsal, lateral and ventral prostates, respectively, when compared to the anterior prostate [67]. Another preclinical study found that sulforaphane, a phytochemical present in edible cruciferous vegetables, inhibited fatty acid synthesis in TRAMP mice, which contributed to PCa chemoprevention [59]. This same research group conducted studies in TRAMP mice and human beings to evaluate the effects of another bioactive compound also found in edible cruciferous vegetables with chemopreventive properties, the phenethyl isothiocyanate (PEITC). [50,58]. They observed a decrease in poorly differentiated PCa incidence and lactate serum levels in the TRAMP model, whereas the intervention by one week with PEITC in smoker subjects predisposed to or with PCa did not affect lactate levels [58].

We believe that all topics before mentioned can be explored in the

TRAMP model by methods involving treatments and protocols with natural compounds from edible sources as a chemopreventive approach. Once the treatment efficacy is proven, the administration of natural products is an excellent strategy to advance preclinical and translational studies. In PCa, the challenge for chemopreventive studies is reaching desirable effects that delay disease progression without side effects.

7. Conclusion

In this review, we brought up the importance of morphology; hormones; angiogenesis; inflammation; and reactive stroma as key features of the molecular pathogenesis in the TRAMP. Interestingly, these aspects show several similarities between TRAMP and human being PCa, not only regarding disease stages but also about molecular markers response to the cancer development. The inhibition of these pathways in different stages showed a particular significance in PCa delay, which was especially efficient in the initial phases of tumor development. We also pointed out that cancer progression rate is different in each prostate lobe of TRAMP. Thus, the development of research that associates these lobes is vital to demonstrate the disease variability found in human cancer. Therefore, it can be concluded that even after 25 years of TRAMP being used as a PCa model, they continue to be an efficient tool in PCa study, providing safe results for translational and chemoprevention research.

Conflict of interest

There is no conflict of interest.

Funding source

This study was not funded.

CRediT authorship contribution statement

Larissa Akemi Kido: Conceptualization, Data curation, Investigation, Writing - original draft, Writing - review & editing. **Celina de Almeida Lamas:** Data curation, Investigation, Writing - original draft, Writing - review & editing. **Mário Roberto Maróstica:** Writing - review & editing. **Valéria Helena Alves Cagnon:** Conceptualization, Data curation, Supervision, Investigation, Writing - original draft, Writing - review & editing.

References

[1] V.M. Adhami, I.A. Siddiqui, N. Ahmad, S. Gupta, H. Mukhtar, Oral consumption of green tea polyphenols inhibits insulin-like growth factor-I-induced signaling in an autochthonous mouse model of prostate cancer, Cancer Res. 64 (2004) 8715–8722.

[2] N.I. Affara, L.M. Coussens, IKKalpha at the crossroads of inflammation and metastasis, Cell 129 (2007) 25–26.

[3] L.F. Alves, R.F. da Silva, V.H.A. Cagnon, Nintedanib effects on delaying cancer progression and decreasing COX-2 and IL-17 in the prostate anterior lobe in TRAMP mice, Tissue Cell 50 (2018) 96–103.

[4] G. Ayala, J.A. Tuxhorn, T.M. Wheeler, A. Frolov, P.T. Scardino, M. Ohori, et al., Reactive stroma as a predictor of biochemical-free recurrence in prostate cancer, Clin. Cancer Res. 9 (2003) 4792–4801.

[5] A. Balmain, Cancer as a complex genetic trait: tumor susceptibility in humans and mouse models, Cell 108 (2002) 145–152.

[6] D.A. Barron, D.R. Rowley, The reactive stroma microenvironment and prostate cancer progression, Endocr. Relat. Cancer 19 (2012) R187–R204.

[7] L.D. Berman-Booty, J.M. Thomas-Ahner, B. Bolon, M.J. Oglesbee, S.K. Clinton, S.K. Kulp, et al., Extra-prostatic transgene-associated neoplastic lesions in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, Toxicol. Pathol. 43 (2015) 186–197.

[8] R. Bianco, R. Rosa, V. Damiano, G. Daniele, T. Gelardi, S. Garofalo, et al., Vascular endothelial growth factor receptor-1 contributes to resistance to anti-epidermal growth factor receptor drugs in human cancer cells, Clin. Cancer Res. 14 (2008) 5069–5080.

[9] D. Cervi, B. Pak, N.A. Venier, L.M. Sugar, R.K. Nam, N.E. Fleshner, et al., Micronutrients attenuate progression of prostate cancer by elevating the endogenous inhibitor of angiogenesis, platelet factor-4, BMC Cancer 10 (2010) 258.

[10] N. Chi, Z. Tan, K. Ma, L. Bao, Z. Yun, Increased circulating myeloid-derived suppressor cells correlate with cancer stages, interleukin-8 and -6 in prostate cancer, Int. J. Clin. Exp. Med. 7 (2014) 3181–3192.

[11] T. Chiaverotti, S.S. Couto, A. Donjacour, J.H. Mao, H. Nagase, R.D. Cardiff, et al., Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer, Am. J. Pathol. 172 (2008) 236–246.

[12] M.S. Condon, The role of the stromal microenvironment in prostate cancer, Semin. Cancer Biol. 15 (2005) 132–137.

[13] G.R. Cunha, Y.Z. Wang, S.W. Hayward, G.P. Risbridger, Estrogenic effects on prostatic differentiation and carcinogenesis, Reprod. Fertil. Dev. 13 (2001) 285–296.

[14] C.F. Dal Pozzo, L.A. Kido, F. Montico, M.P. Goncalves, V.H. Cagnon, Morphology and MMP-9, AR and IGFR-1 responses of the seminal vesicle in TRAMP mice model, Tissue Cell 48 (2016) 217–223.

[15] K. Ellwood-Yen, T.G. Graeber, J. Wongvipat, M.L. Iruela-Arispe, J. Zhang, R. Matusik, et al., Myc-driven murine prostate cancer shares molecular features with human prostate tumors, Cancer Cell 4 (2003) 223–238.

[16] E. Fosslien, Molecular pathology of cyclooxygenase-2 in neoplasia, Ann. Clin. Lab. Sci. 30 (2000) 3–21.

[17] E. Fosslien, Review: molecular pathology of cyclooxygenase-2 in cancer-induced angiogenesis, Ann. Clin. Lab. Sci. 31 (2001) 325–348.

[18] B.A. Foster, J.R. Gingrich, E.D. Kwon, C. Madias, N.M. Greenberg, Characterization of prostatic epithelial cell lines derived from transgenic adenocarcinoma of the mouse prostate (TRAMP) model, Cancer Res. 57 (1997) 3325–3330.

[19] J.R. Gingrich, R.J. Barrios, B.A. Foster, N.M. Greenberg, Pathologic progression of autochthonous prostate cancer in the TRAMP model, Prostate Cancer Prostatic Dis. 2 (1999) 70–75.

[20] J.R. Gingrich, R.J. Barrios, R.A. Morton, B.F. Boyce, F.J. DeMayo, M.J. Finegold, et al., Metastatic prostate cancer in a transgenic mouse, Cancer Res. 56 (1996) 4096–4102.

[21] J.R. Gingrich, N.M. Greenberg, A transgenic mouse prostate cancer model, Toxicol. Pathol. 24 (1996) 502–504.

[22] M.M. Grabowska, D.J. DeGraff, X. Yu, R.J. Jin, Z. Chen, A.D. Borowsky, et al., Mouse models of prostate cancer: picking the best model for the question, Cancer Metastasis Rev. 33 (2014) 377–397.

[23] N.M. Greenberg, Androgens and growth factors in prostate cancer: a transgenic perspective, Prostate Cancer Prostatic Dis. 3 (2000) 224–228.

[24] N.M. Greenberg, F. DeMayo, M.J. Finegold, D. Medina, W.D. Tilley, J.O. Aspinall, et al., Prostate cancer in a transgenic mouse, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 3439–3443.

[25] D.J. Grignon, Unusual subtypes of prostate cancer, Mod. Pathol. 17 (2004) 316–327.

[26] Y. He, J. Zha, Y. Wang, W. Liu, X. Yang, P. Yu, Tissue damage-associated "danger signals" influence T-cell responses that promote the progression of preneoplasia to cancer, Cancer Res. 73 (2013) 629–639.

[27] J. Hu, T. Yang, H. Xu, M. Hu, H. Wen, H. Jiang, A novel anticancer agent icaritin inhibited proinflammatory cytokines in TRAMP mice, Int. Urol. Nephrol. 48 (2016) 1649–1655.

[28] W.J. Huss, C.F. Hanrahan, R.J. Barrios, J.W. Simons, N.M. Greenberg, Angiogenesis and prostate cancer: identification of a molecular progression switch, Cancer Res. 61 (2001) 2736–2743.

[29] R.J. Jin, Y. Lho, L. Connelly, Y. Wang, X. Yu, L. Saint Jean, et al., The nuclear factor-kappaB pathway controls the progression of prostate cancer to androgen-independent growth, Cancer Res. 68 (2008) 6762–6769.

[30] P.J. Kaplan-Lefko, T.M. Chen, M.M. Ittmann, R.J. Barrios, G.E. Ayala, W.J. Huss, et al., Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model, Prostate 55 (2003) 219–237.

[31] M. Karin, Y. Cao, F.R. Greten, Z.W. Li, NF-kappaB in cancer: from innocent bystander to major culprit, Nat. Rev. Cancer 2 (2002) 301–310.

[32] S. Kasper, P.C. Sheppard, Y. Yan, N. Pettigrew, A.D. Borowsky, G.S. Prins, et al., Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer, Lab. Investig. 78 (i-xv) (1998).

[33] L.A. Kido, F. Montico, R. Sauce, A.B. Macedo, E. Minatel, D.B. Costa, et al., Anti-inflammatory therapies in TRAMP mice: delay in PCa progression, Endocr. Relat. Cancer 23 (2016) 235–250.

[34] R.D. Klein, The use of genetically engineered mouse models of prostate cancer for nutrition and cancer chemoprevention research, Mutat. Res. 576 (2005) 111–119.

[35] E. Maniati, R. Soper, T. Hagemann, Up for mischief? IL-17/Th17 in the tumor microenvironment, Oncogene 29 (2010) 5653–5662.

[36] N. Masumori, T.Z. Thomas, P. Chaurand, T. Case, M. Paul, S. Kasper, et al., A probasin-large T antigen transgenic mouse line develops prostate adenocarcinoma and neuroendocrine carcinoma with metastatic potential, Cancer Res. 61 (2001) 2239–2249.

[37] M.H.M. Melis, E. Nevedomskaya, J. van Burgsteden, B. Cioni, H.J.T. van Zeeburg, J.Y. Song, et al., The adaptive immune system promotes initiation of prostate carcinogenesis in a human c-Myc transgenic mouse model, Oncotarget 8 (2017) 93867–93877.

[38] F. Montico, L.A. Kido, A.C. Hetzl, V.H. Cagnon, Prostatic angiogenic responses in late life: antiangiogenic therapy influences and relation with the glandular microenvironment in the transgenic adenocarcinoma of mouse prostate (TRAMP) model, Prostate 75 (2015) 484–499.

[39] F. Montico, L.A. Kido, A.C. Hetzl, R.M. Lorencini, E.M. Candido, V.H. Cagnon, Antiangiogenic therapy effects on age-associated matrix metalloproteinase-9 (MMP-9) and insulin-like growth factor receptor-1 (IGFR-1) responses: a comparative

study of prostate disorders in aged and TRAMP mice, Histochem. Cell Biol. 142 (2014) 269–284.
[40] F. Montico, L.A. Kido, R. San Martin, D.R. Rowley, V.H. Cagnon, Reactive stroma in the prostate during late life: The role of microvasculature and antiangiogenic therapy influences, Prostate 75 (2015) 1643–1661.
[41] J. Moselhy, S. Suman, M. Alghamdi, B. Chandarasekharan, T.P. Das, A. Houda, et al., Withaferin A inhibits prostate carcinogenesis in a PTEN-deficient mouse model of prostate cancer, Neoplasia 19 (2017) 451–459.
[42] S. Nandana, L.W. Chung, Prostate cancer progression and metastasis: potential regulatory pathways for therapeutic targeting, Am. J. Clin. Exp. Urol. 2 (2014) 92–101.
[43] B.A. Narayanan, N.K. Narayanan, B. Pittman, B.S. Reddy, Regression of mouse prostatic intraepithelial neoplasia by nonsteroidal anti-inflammatory drugs in the transgenic adenocarcinoma mouse prostate model, Clin. Cancer Res. 10 (2004) 7727–7737.
[44] Y. Niu, S. Altuwaijri, S. Yeh, K.P. Lai, S. Yu, K.H. Chuang, et al., Targeting the stromal androgen receptor in primary prostate tumors at earlier stages, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 12188–12193.
[45] E. Nogueira-Pangrazi, R.F. da Silva, L.A. Kido, F. Montico, V.H.A. Cagnon, Nintedanib treatment delays prostate dorsolateral lobe cancer progression in the TRAMP model: contribution to the epithelial-stromal interaction balance, Cell Biol. Int. 42 (2018) 153–168.
[46] J. Palmer, V. Venkateswaran, N.E. Fleshner, L.H. Klotz, M.E. Cox, The impact of diet and micronutrient supplements on the expression of neuroendocrine markers in murine lady transgenic prostate, Prostate 68 (2008) 345–353.
[47] M. Parisotto, D. Metzger, Genetically engineered mouse models of prostate cancer, Mol. Oncol. 7 (2013) 190–205.
[48] A.R. Pedrosa, A. Trindade, C. Carvalho, J. Graca, S. Carvalho, M.C. Peleteiro, et al., Endothelial Jagged1 promotes solid tumor growth through both pro-angiogenic and angiocrine functions, Oncotarget 6 (2015) 24404–24423.
[49] L. Persano, L. Moserle, G. Esposito, V. Bronte, V. Barbieri, M. Iafrate, et al., Interferon-alpha counteracts the angiogenic switch and reduces tumor cell proliferation in a spontaneous model of prostatic cancer, Carcinogenesis 30 (2009) 851–860.
[50] A.A. Powolny, A. Bommareddy, E.R. Hahm, D.P. Normolle, J.H. Beumer, J.B. Nelson, et al., Chemopreventative potential of the cruciferous vegetable constituent phenethyl isothiocyanate in a mouse model of prostate cancer, J. Natl. Cancer Inst. 103 (2011) 571–584.
[51] R.S. Pruthi, E.M. Wallen, Cyclooxygenase-2: a therapeutic target for prostate cancer, Clin. Genitourin. Cancer 4 (2005) 203–211.
[52] H. Pu, J. Collazo, E. Jones, D. Gayheart, S. Sakamoto, A. Vogt, et al., Dysfunctional transforming growth factor-beta receptor II accelerates prostate tumorigenesis in the TRAMP mouse model, Cancer Res. 69 (2009) 7366–7374.
[53] K. Raina, S. Rajamanickam, R.P. Singh, G. Deep, M. Chittezhath, R. Agarwal, Stage-specific inhibitory effects and associated mechanisms of silibinin on tumor progression and metastasis in transgenic adenocarcinoma of the mouse prostate model, Cancer Res. 68 (2008) 6822–6830.
[54] P. Sansone, J. Bromberg, Targeting the interleukin-6/Jak/stat pathway in human malignancies, J. Clin. Oncol. 30 (2012) 1005–1014.
[55] S. Shukla, G.T. MacLennan, P. Fu, S. Gupta, Apigenin attenuates insulin-like growth factor-I signaling in an autochthonous mouse prostate cancer model, Pharm. Res. 29 (2012) 1506–1517.
[56] R.F. Silva, E. Nogueira-Pangrazi, L.A. Kido, F. Montico, S. Arana, D. Kumar, et al., Nintedanib antiangiogenic inhibitor effectiveness in delaying adenocarcinoma progression in transgenic adenocarcinoma of the mouse prostate (TRAMP), J. Biomed. Sci. 24 (2017) 31.
[57] R.S. Silva, L.A. Kido, F. Montico, D.B. Vendramini-Costa, R.A. Pilli, V.H.A. Cagnon, Steroidal hormone and morphological responses in the prostate anterior lobe in different cancer grades after celecoxib and goniothalamin treatments in TRAMP mice, Cell Biol. Int. 42 (2018) 1006–1020.
[58] K.B. Singh, E.R. Hahm, L.H. Rigatti, D.P. Normolle, J.M. Yuan, S.V. Singh, Inhibition of glycolysis in prostate cancer chemoprevention by phenethyl isothiocyanate, Cancer Prev. Res. (Phila.) 11 (2018) 337–346.
[59] K.B. Singh, S.H. Kim, E.R. Hahm, S.K. Pore, B.L. Jacobs, S.V. Singh, Prostate cancer chemoprevention by sulforaphane in a preclinical mouse model is associated with inhibition of fatty acid metabolism, Carcinogenesis 39 (2018) 826–837.
[60] R.P. Singh, R. Komal, G. Sharma, R. Agarwal, Silibinin inhibits established prostate tumor growth, progression, invasion and metastasis, and suppresses tumor angiogenesis and EMT in TRAMP mice, Clin. Cancer Res. 14 (2008) 7773–7780.
[61] A. Slusarz, G.A. Jackson, J.K. Day, N.S. Shenouda, J.L. Bogener, J.D. Browning, et al., Aggressive prostate cancer is prevented in ERalphaKO mice and stimulated in ERbetaKO TRAMP mice, Endocrinology 153 (2012) 4160–4170.
[62] W.L. Smith, R.M. Garavito, D.L. Dewitt, Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and −2, J. Biol. Chem. 271 (1996) 33157–33160.
[63] Q. Su, B. Zhang, L. Zhang, T. Dang, D. Rowley, M. Ittmann, et al., Jagged1 upregulation in prostate epithelial cells promotes formation of reactive stroma in the Pten null mouse model for prostate cancer, Oncogene 36 (2017) 618–627.
[64] S. Suman, T.P. Das, J. Moselhy, D. Pal, V. Kolluru, H. Alatassi, et al., Oral administration of withaferin A inhibits carcinogenesis of prostate in TRAMP model, Oncotarget 7 (2016) 53751–53761.
[65] A. Suttie, A. Nyska, J.K. Haseman, G.J. Moser, T.R. Hackett, T.L. Goldsworthy, A grading scheme for the assessment of proliferative lesions of the mouse prostate in the TRAMP model, Toxicol. Pathol. 31 (2003) 31–38.
[66] A.W. Suttie, G.E. Dinse, A. Nyska, G.J. Moser, T.L. Goldsworthy, R.R. Maronpot, An investigation of the effects of late-onset dietary restriction on prostate cancer development in the TRAMP mouse, Toxicol. Pathol. 33 (2005) 386–397.
[67] H.L. Tan, J.M. Thomas-Ahner, N.E. Moran, J.L. Cooperstone, J.W. Erdman Jr., G.S. Young, et al., Beta-carotene 9′,10′ oxygenase modulates the anticancer activity of dietary tomato or lycopene on prostate carcinogenesis in the TRAMP Model, Cancer Prev. Res. (Phila.) 10 (2017) 161–169.
[68] Y. Tani, A. Suttie, G.P. Flake, A. Nyska, R.R. Maronpot, Epithelial-stromal tumor of the seminal vesicles in the transgenic adenocarcinoma mouse prostate model, Vet. Pathol. 42 (2005) 306–314.
[69] D. Thapa, R. Ghosh, Chronic inflammatory mediators enhance prostate cancer development and progression, Biochem. Pharmacol. 94 (2015) 53–62.
[70] D. Tilki, N. Kilic, H. Herbst, O. Reich, M. Seitz, H. Lauke, et al., High level of endostatin in epididymal epithelium: protection against primary malignancies in this organ? Histochem. Cell Biol. 130 (2008) 527–535.
[71] J.A. Tuxhorn, G.E. Ayala, D.R. Rowley, Reactive stroma in prostate cancer progression, J. Urol. 166 (2001) 2472–2483.
[72] J.A. Tuxhorn, G.E. Ayala, M.J. Smith, V.C. Smith, T.D. Dang, D.R. Rowley, Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling, Clin. Cancer Res. 8 (2002) 2912–2923.
[73] M.J. Uddin, B.C. Crews, K. Ghebreselasie, I. Huda, P.J. Kingsley, M.S. Ansari, et al., Fluorinated COX-2 inhibitors as agents in PET imaging of inflammation and cancer, Cancer Prev. Res. (Phila.) 4 (2011) 1536–1545.
[74] K.C. Valkenburg, B.O. Williams, Mouse models of prostate cancer, Prostate Cancer 2011 (2011) 895238.
[75] V. Venkateswaran, N.E. Fleshner, L.M. Sugar, L.H. Klotz, Antioxidants block prostate cancer in lady transgenic mice, Cancer Res. 64 (2004) 5891–5896.
[76] S. Wang, J. Gao, Q. Lei, N. Rozengurt, C. Pritchard, J. Jiao, et al., Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer, Cancer Cell 4 (2003) 209–221.
[77] Z. Weihua, S. Makela, L.C. Andersson, S. Salmi, S. Saji, J.I. Webster, et al., A role for estrogen receptor beta in the regulation of growth of the ventral prostate, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 6330–6335.
[78] X. Wu, S. Gong, P. Roy-Burman, P. Lee, Z. Culig, Current mouse and cell models in prostate cancer research, Endocr. Relat. Cancer 20 (2013) R155–R170.
[79] X. Wu, T. Yang, X. Liu, J.N. Guo, T. Xie, Y. Ding, et al., IL-17 promotes tumor angiogenesis through Stat3 pathway mediated upregulation of VEGF in gastric cancer, Tumour Biol. 37 (2016) 5493–5501.
[80] H. Xu, M.B. Hu, P.D. Bai, W.H. Zhu, S.H. Liu, J.Y. Hou, et al., Proinflammatory cytokines in prostate cancer development and progression promoted by high-fat diet, Biomed. Res. Int. 2015 (2015) 249741.
[81] Z. Yang, Y.C. Peng, A. Gopalan, D. Gao, Y. Chen, A.L. Joyner, Stromal hedgehog signaling maintains smooth muscle and hampers micro-invasive prostate, Cancer 10 (2017) 39–52.
[82] Y. Zhang, D. Wang, M. Li, X. Wei, S. Liu, M. Zhao, et al., Quantitative proteomics of TRAMP mice combined with bioinformatics analysis reveals that PDGF-B regulatory network plays a key role in prostate cancer progression, J. Proteome Res. 17 (2018) 2401–2411.