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Original Article

Early and delayed castrations confer a similar survival advantage in TRAMP mice

Zai-Xian Zhang, Qing-Quan Xu, Xiao-Bo Huang, Ji-Chuan Zhu, Xiao-Feng Wang

Department of Urology, Peking University People's Hospital, Beijing 100044, China

Abstract

The most appropriate time to introduce androgen deprivation therapy for prostate cancer remains controversial. Our aim was to evaluate the effects of early versus delayed surgical castration on prostate cancer progression and survival in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. TRAMP mice were randomly divided into three groups: the early castration group (on which castration was performed at the age of 4 weeks), the delayed castration group (on which castration was performed when abdominal tumours could be palpated), and the sham-castrated group. Mice were monitored daily throughout their lives until cancer-related death or the development of an obviously moribund appearance, at which time the individual mouse was killed. Androgen receptor expression in prostate tumours was also evaluated. The results shows that the average lifespan in early castration, delayed castration and sham-castrated groups were 54.1 weeks, 59.9 weeks and 39.1 weeks, respectively. Both early castration and delayed castration conferred a statistically significant survival advantage when compared with the sham-castrated group ($P < 0.001$). However, the difference in lifespan between the early castration group and the delayed castration group was not statistically significant ($P = 0.85$). The increase in lifespan in the TRAMP mice that received either early or delayed castration correlated with lower G/B value (genitourinary tract weight/body weight) at death than the sham-castrated mice. In conclusion, early and delayed castrations in TRAMP mice prolonged survival to a similar extent. This finding may provide a guide for clinical practice in prostate cancer therapy.

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1 Introduction

Prostate cancer has currently been recognized as the most common malignancy in males and the second leading cause of male cancer death in western countries, and the incidence has increased significantly in the recent years. In 2007, in the United States alone there were a total of 218 890 new prostate cancer cases and 27 050 associated deaths [1,2]. The normal growth, development and function of the prostate gland are dependent on androgens, and accordingly, prostate cancer is believed to be intimately associated with androgens. Androgen ablation, which was first proposed by Huggins and Hodges [3] in the early 1940s, remains the most generally used treatment for recurrent, locally advanced and metastatic prostate cancers. The two main forms of androgen ablation for prostate cancer

Correspondence: Dr Qing-Quan Xu, Department of Urology, Peking University People's Hospital, 11# XiZhiMen, NanDaJie, Beijing 100044, China.
Fax: +86-10-6616-2932 E-mail: xuqingquan@pkuph.edu.cn
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that are used today are medical castration with luteinising hormone-releasing hormone (LHRH) agonists and surgical castration (orchiectomy). It has been shown that medical and surgical castrations are equally efficacious [4, 5]. However, medical management of prostate cancer is more expensive than orchiectomy, which is a more cost-effective androgen suppression treatment [6, 7].

When prostate cancer patients undergo androgen ablation as a first- or second-line treatment, the primary tumour and its metastases usually shrink. Unfortunately, the effect is temporary [8], and after an initial period of response, patients invariably develop androgen-independent prostate cancer, which does not respond to currently available therapies and ultimately leads to death. Therefore, novel treatment strategies are needed to prevent or delay the development of androgen-independent prostate cancer.

Although androgen ablation has been the standard treatment for locally advanced and metastatic prostate cancer for more than 60 years, the best time to perform castration remains controversial. Debates mainly focus on whether androgen ablation should be started early at diagnosis or delayed until there is disease progression with associated symptoms.

Animal models are important tools to study the pathogenesis, development and treatment of cancers [9]. Animal models of prostate cancer for preclinical research have been established and extensively used. One of the best characterised models is the autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which has been well accepted as a relevant mouse model for understanding the early events and the progression of prostate cancer [10, 11]. In this model, a region of the probasin promoter was introduced to target the expression of the simian virus 40 (SV40) early-region tumour genes (T and t, Tag) to the prostate epithelium in a C57BL/6 background strain. The SV40 large T antigen effectively abrogates p53 and retinoblastoma tumour suppressor functions and thus acts as an oncoprotein. As a result, TRAMP mice develop spontaneous progressive prostatic tumours from early lesions of prostatic intraepithelial neoplasia (PIN), to locally invasive carcinoma and finally to metastatic disease, which mimics the whole spectrum of human prostatic carcinoma [11–13].

In TRAMP mice, expression of the transgene (PB-Tag) is hormonally regulated by androgens and temporally correlates with sexual maturity. Several investigators have used the TRAMP model with androgen deprivation for prevention and therapy studies [14–16, 29]. In the course of conducting a preclinical castration study with this model, the short- and long-term effects of castration therapy have been described [14–16], but whether the timing (early, delayed) of castration affects outcome has not been investigated in detail. This study investigated the efficacy of early versus delayed surgical castration on prolonging survival of TRAMP mice.

2 Materials and methods

2.1 Animals

All animal studies were approved by the Peking University People's Hospital Committee on Use and Care of Animals and conducted in accordance with local humane animal care standards. Heterozygous 8-week-old TRAMP mice (C57BL/6-Tg[TRAMP]8247Ng/J), which were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA), were mated with wild-type C57BL/6 mice (Vital river, Beijing, China) to generate both wild-type and heterozygous mice. The progeny was genotyped by PCR as described earlier [10, 17], and the male mice that expressed the transgene were randomly divided into three groups. All mice were maintained under specific pathogen-free (SPF) conditions, allowed free access to drinking water and regular meals and kept under a controlled 12-h light/dark cycle at 22 ± 2°C.

2.2 Castration

TRAMP mice were anaesthetized by intraperitoneal injection of Avertin (1.25% in tertiary amyl alcohol, 250 mg kg^{-1} body weight) (Sigma, Shanghai, China) before the surgical procedure. Bilateral testes were removed through a scrotal midline incision. Early castrations were carried out at 4 weeks of age, whereas delayed castrations were performed only when the abdominal tumours were palpable. The sham-castrated TRAMP mice were used as control. Mice were monitored daily and body weight was measured weekly.

2.3 Tissue preparation

Animals were killed when they became moribund [18]. The criteria included a large palpable tumour, huddled posture, difficulty ambulating, laboured breathing or an obviously moribund appearance. Mice were weighed and then killed with an intraperitoneal injection of a large dose of Avertin. All major organs were inspected for macroscopic evidence of primary tumours

and metastases. The lower genitourinary tract (GU), which included the bladder, seminal vesicles and all prostate lobes, was removed and weighed. The lumbar and sacral lymph nodes were collected and analysed. In addition, intestines, liver, lungs, kidneys and spleen were harvested and examined for metastases. Tissue from all the organs was routinely fixed overnight in 10% neutralized-buffered formalin and then transferred to 70% ethanol before standard tissue processing. Fixed tissues were embedded in paraffin, and 5-μm sections were mounted on slides. Sections were stained with haematoxylin and eosin before histopathological examination.

2.4 Immunohistochemistry

Paraffin-embedded tissue sections were de-paraffinized in xylene and rehydrated in a gradient of ethanol. Antigen retrieval was performed by incubating sections with sodium citrate buffer (0.01 mol L^{-1}, pH 6.0) using a pressure cooker for 2 min at about 120°C and cooling for 30 to 60 min. Sections were washed in phosphate-buffered saline (PBS) for 6 min and then treated with 3% H_2O_2 in methanol for 12 min to inhibit endogenous peroxidase. After the PBS wash, the tissue sections were blocked with normal serum and then incubated with monoclonal mouse anti-human Tag antigen (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and polyclonal rabbit anti-human androgen receptor (AR) (Santa Cruz Biotechnology) at a dilution of 1:50 in PBS for 2 h at room temperature. After the PBS wash, the slides were incubated with the Envision System Anti Mouse solution (Dako, Carpinteria, CA, USA) and the Envision System Anti Rabbit solution (Dako), respectively, for 30 min. Chromogen diaminobenzidine was applied to the samples for 3 min and then followed by a wash in PBS for 5 min. The specimens were counterstained with hematoxylin (Sigma) for 3 min and followed by a wash in tap H_2O. The specimens were then immersed in a series of graded alcohols, placed in xylene and mounted. As a negative control, PBS was used instead of the primary antibody. The stained slides were visualized under a light microscope. Images were captured with an attached camera that was linked to a computer.

2.5 Statistical and survival analysis

All statistical analyses were carried out with SPSS (Chicago, IL, USA) for Windows version 13.0. The data were expressed as mean ± SE. The significance between the control and experimental groups was performed by using the unpaired *t*-test or one-way AVOVA (analysis of variance). The Kaplan–Meier method was used to estimate lifespan and the differences were analysed by the log-rank test. A value of $P < 0.05$ was considered statistically significant in all the tests.

3 Results

3.1 Genitourinary tract and body weight

At the time of necropsy, the GU weight and body weight were determined, as a function of cancer progression [11]. Relative GU weight (genitourinary tract weight/body weight: G/B ratio), which was calculated as (GU weight/body weight) × 100%, was used to evaluate the effect of castration on prostate tumour growth in TRAMP mice. The average G/B ratio of the delayed castration group was 15.86% ± 5.62%, which was significantly less than that of the sham-castrated group, which was 34.49% ± 16.65% ($P = 0.015$). The early castration group showed an average G/B ratio of 22.25% ± 13.67%, which was less than that of the sham-castrated group, but the difference was not statistically significant ($P = 0.104$). There was no statistically significant difference in the G/B ratio between the early castration group and the delayed castration group ($P = 0.383$).

3.2 Survival

Survival benefit is the ultimate goal of any cancer therapy regimen. In this study, we evaluated whether castration at different time points leads to increased lifespan in TRAMP mice. The TRAMP mice that were castrated at early or delayed time points enjoyed a significantly extended lifespan, with an average lifespan of 54.1 and 59.9 weeks, respectively, compared with the sham-castrated group, which had an average lifespan of 39.1 weeks ($P = 0.001$) (Figures 1A, C and D). Mice in the delayed castration group had, on average, a 5.8-week longer lifespan than those in the early castration group, but the difference was not significant ($P = 0.85$) (Figure 1B).

3.3 AR expression

The AR was expressed in the majority of both the epithelial and stromal cells in the tumours from the sham-castrated TRAMP mice (Figure 2A). AR staining was positive in 28.2% ± 1.8% of tumour cells in the delayed castration group, compared with 69.1% ± 2.1% in the sham-castrated group (Figures 2A and 2B). In

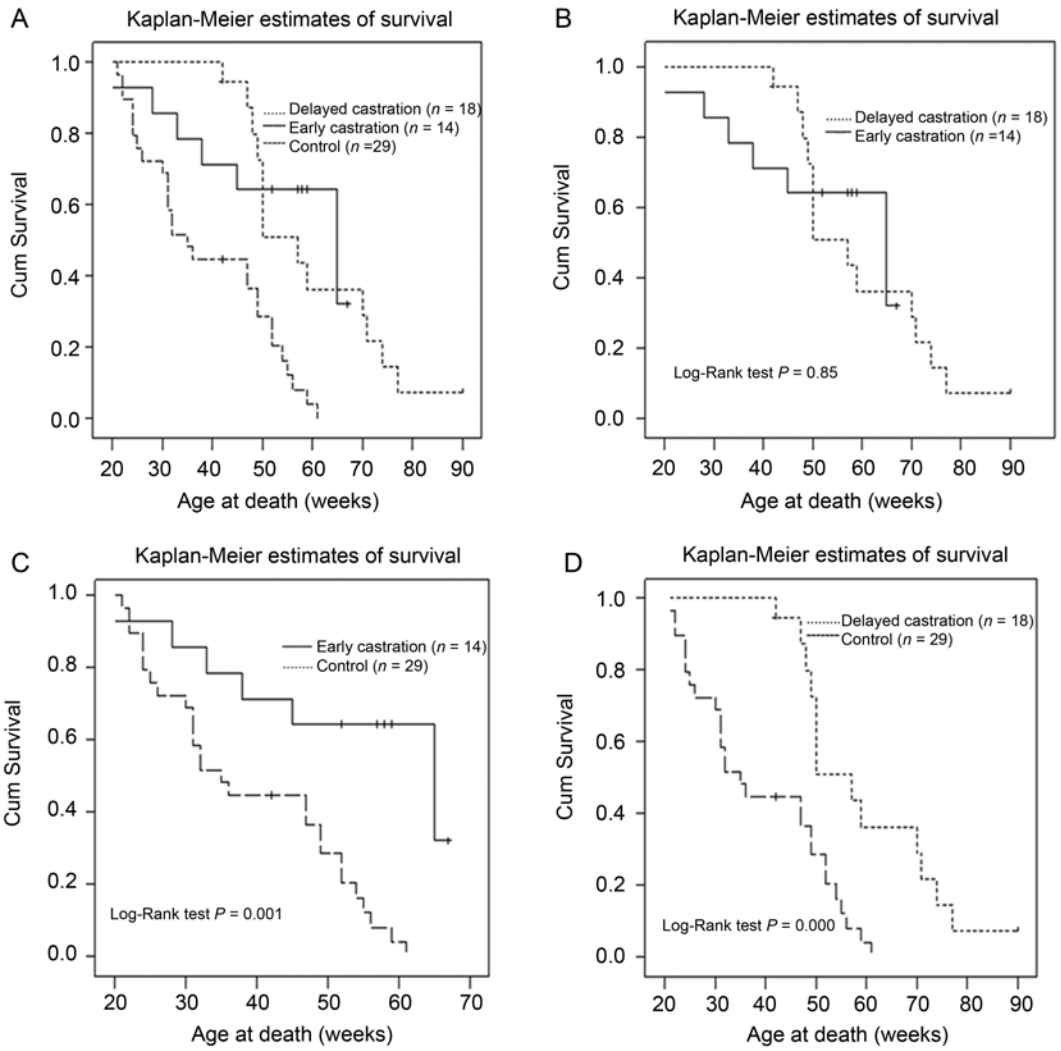

Figure 1. Kaplan–Meier analysis of long-term survival for early castration, delayed castration and control groups in TRAMP mice. (A): Kaplan–Meier analysis of all three groups. (B): Early castration versus delayed castration, in which the difference between the two groups was not statistically significant ($P = 0.85$). (C): Early castration versus control, in which the early castration group had a significantly longer lifespan ($P = 0.001$). (D): Delayed castration versus control, in which the delayed castration group had a significantly longer lifespan ($P = 0.000$).

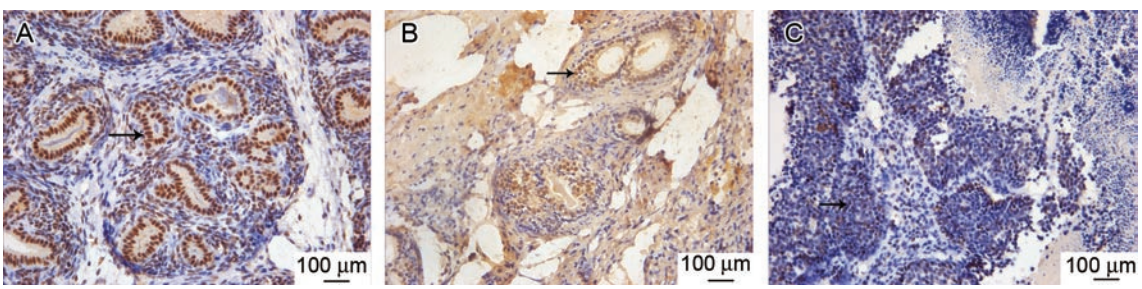

Figure 2. Androgen receptor (AR) staining of tumours for the sham-castrated, delayed castration, and early castration groups. (A): A tumour from a sham-castrated mouse, with arrows pointing to positive staining in the majority of both the epithelial and stromal cells. (B): A tumour from a delayed castration mouse, with arrows pointing to a reduced number of positively stained epithelial and stromal cells. (C): A tumour from an early castration mouse, with only a small number of positively stained cells (arrow).

the early-castrated mice, only 2.5% ± 0.2% of tumour cells showed positive staining (Figure 2C).

We adopted an earlier reported grading system [12] for the TRAMP tumours, which was based on their histological patterns. Well-differentiated prostate cancers were observed in the sham-castrated group (Figure 2A) and moderately well-differentiated prostate cancers were observed in the delayed castration group (Figure 2B), whereas the prostate tumours in the early-castrated group were poorly differentiated (Figure 2C).

4 Discussion

This study was conducted with the well-established TRAMP mouse model to study the effects of early versus delayed surgical castration on prostate tumour progression and survival. Similar to most other cancers, prostate carcinogenesis in TRAMP mice involves a multistep progression from precancerous lesions to localized carcinoma, which is followed by metastatic carcinoma, and this progression closely mimics the progression of prostate cancer in humans [11, 12]. The transgene is androgen-regulated in TRAMP mice, such that removal of androgens will inhibit the development of androgen-dependent prostate cancer in this mouse model. In this study, we performed surgical castration of TRAMP mice either early (at 4 weeks of age, before the onset of prostate cancer) or delayed (at 28 weeks of age or later, by which time the mice displayed large, palpable primary tumours). Mice were monitored daily, until they died from cancer-related causes or were killed according to commonly accepted criteria.

The key observation in this study was that early androgen ablation before the onset of prostate cancer in TRAMP mice had a statistically similar overall survival outcome when compared with delayed surgical castration, and both early and delayed treatments significantly extended lifespan when compared with that of intact TRAMP mice. It is widely believed that the balance between proliferation and apoptosis in prostate is regulated by androgens. However, this balance is lost in prostate cancer [19]. Testosterone, which is mainly produced by Leydig's cells in the testes, is the major active androgen that circulates within the blood of males and is important for the growth of prostate cancer cells. In the absence of testosterone, tumour cells undergo apoptosis, which results in the shrinkage of the primary tumour. Therefore, orchiectomy is an effective therapy for advanced prostate cancer.

In this study, prolonged survival of TRAMP mice in both early and delayed castration groups was correlated with a lower G/B ratio at death than the sham-castrated mice. This suggests that the majority of primary tumours are responsive to androgen ablation and that androgen ablation causes a decrease in prostatic tumour burden and extends overall survival. It was interesting that the castrated mice had a lower G/B ratio at the time of death. That may mean that distant metastasis was actually the cause of death. AR plays an important role in the development of prostate cancer and can be observed in primary prostate cancer and detected throughout the progression of both hormone-sensitive and hormone-refractory cancers [20, 21]. In this study, AR expression was reduced in epithelial and stromal cells 1-week post-castration, which was the normal short-term response to castration in TRAMP mice. Sometimes AR was not detected in tumour cells, which was possibly because of lack of AR expression or the expression of a mutated AR [22, 23]. In this study, AR expression was significantly lower in tumours from the early castration group than in the sham-castrated or delayed castration groups, and the tumours in the delayed castration group showed less AR expression than in the sham-castrated group. In addition, the early castration group showed more poorly differentiated histological features of prostate cancer than the sham-castrated or delayed-castrated groups. These data may suggest a better outcome from delayed castration. The underlying mechanism is unclear and needs further study.

The most appropriate time to introduce androgen deprivation therapy for prostate cancer remains an unresolved issue. Many clinical studies suggest that early androgen ablation may offer a survival benefit in patients with advanced prostate cancer and may prolong the time to the development of androgen-independent prostate cancer [24–26]. Other studies indicate that early castration offers no survival advantage and might not be needed [27,28]. Earlier studies have estimated the effects of surgical castration in TRAMP mice at different time points (4, 12, 15 and 20 weeks of age) [14, 16, 29]. TRAMP mice were usually first observed to develop PIN between 8 and 12 weeks of age, which corresponded to sexual maturation [13]. Therefore, castration at 4 weeks precedes the onset of PIN, primary prostate cancer or metastatic disease. In the study conducted by Eng *et al.* [29], a statistically significant difference was described between the lifespan of the early-castrated mice (at 4 weeks) and the intact mice. In

another study [14], androgen ablation at 12 weeks had a variable impact on tumour progression in TRAMP mice. Some tumours regressed, whereas others continued to grow after castration. Yet another study [16] (in which castration was performed at 15 weeks or 20 weeks) did not unequivocally favour either early or late castration.

One of the most important findings in our study was that the TRAMP mice that received delayed castration had an increased lifespan that was similar to that of the early castration mice. Early castration did not result in a longer lifespan than delayed castration. It seems that early castration cannot prevent prostate cancer genesis in TRAMP mice, which is different from the outcome observed in human studies. Early castration in TRAMP mice probably activated the androgen-independent oncogenic pathway, as the androgen-dependent pathway was shut down. However, standard androgen deprivation did not consistently suppress androgen-dependent gene expression. Many androgen-responsive genes were not suppressed even after 9 months of neoadjuvant androgen deprivation therapy [30]. Androgen-independent prostate cancer may be the consequence of molecular events that are mediated by abnormal androgen receptor signalling. The clinical implication of our finding is that surgical castration, when administrated during the late rather than the early stage of prostate cancer, may actually improve the outcome of the disease and prolong patient survival.

5 Conclusion

This study evaluated the response to early and delayed surgical castration and their effects on overall survival in the TRAMP model. We showed that both early and delayed castration showed an overall survival benefit when compared with the outcome for sham-castrated TRAMP mice. Further research is necessary to study the precise mechanism that underlies the effects of castration at different times on androgen-dependent and androgen-independent prostate cancer.

Conflict of interest

There is no conflict of interest.

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