Nexrutine and exercise similarly prevent high grade prostate tumors in transgenic mouse model

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Abstract

The purpose of this investigation was to compare the antitumorigenic effects of the natural product Nexrutine to voluntary wheel running (VWR) in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Forty-five, 10-week old TRAMP mice were randomized to either receive free access to the running wheel, Nexrutine pelleted into chow at 600 mg/kg or no treatment control. Mice were serially sacrificed at weeks 4, 8,12 and 20 weeks. Palpable tumors, body weight, food consumption and running wheel activity were monitored weekly. At necropsy, tumors and serum were harvested and stored for analysis. Serum was used to quantify circulating cytokines in 4 and 20 week time points. Nexrutine supplementation led to a 66% protection against high grade tumors. Exercise resulted in a 60% protection against high grade tumors. Both interventions reduced concentrations of IL-1α. Exercise also significantly lowered concentrations of eotaxin, IL-5, IL-12(p40) and VEGF. While there were no significant differences at baseline, exercise mice had significantly lower IL-5 and VEGF compared to control at the 20 week time point. Nexrutine also significantly reduced circulating IL-9 concentrations. No significant differences were observed when compared to the control group. Immunohistology of tumor sections showed significantly lower expression of pAkt in Nexrutine fed mice with no visible differences for NFκB. In conclusion, both Nexrutine and exercise suppressed tumor growth. Though similar outcomes were seen in this comparative effectiveness study, the mechanisms by which exercise and Nexrutine exert this benefit may focus on different pathways.

Introduction

Prostate cancer (PCa) is the most common solid-tumor cancer in American males and is the second most common cause of cancer death [1]. Development of PCa spans several decades...
with clinical detection of PCa manifesting in patient in their 60s [2]. This long latency period provides a significant opportunity to implement chemopreventative interventions to inhibit the transition from pre-neoplastic lesions to malignant cells. It has been reported that cancer risk reduces significantly with an active lifestyle and a diet rich with phytochemicals [3, 4]. Lack of healthy activity and diets in the US point to increased incidence rates of several cancers, including PCa, compared to other countries [5].

Exercise has been shown to be beneficial in men with PCa [6–12]. However, the impact of exercise on tumor physiology are not clearly understood. A recent systematic review by Shephard found mixed results on the benefits of physical activity on PCa prevention with 45 of the 83 trials reviewed presenting diminished risk [13]. Prospective cohort studies have also found that lifelong physical activity is directly related to reduced risk of developing high-grade tumors upon biopsy [14–16]. In men diagnosed with PCa, physical activity is associated with lower overall mortality and PCa mortality [17] and reduced risk of recurrence [18, 19]. Though deemed beneficial, the mechanisms by which exercise confer these benefits remain understudied.

Studying the effects of exercise on cancer prevention are difficult and time-consuming requiring years of interventional data. For that reason, relying on exercise interventions in pre-clinical models of cancer are favored. To date, there are few animal studies looking at physical activity and cancer that have reported beneficial outcomes [20, 21]. Voluntary wheel running has been reported to inhibit the formation of chemically induced colon [22] and breast cancer in rats [23], skin cancer [24], Panc-1 pancreatic tumors [25], and androgen-independent PC-2 prostate tumors in mice [26]. Zheng et al. found that voluntary exercise inhibits the growth of prostate tumors in SCID mouse xenograft model and can enhance apoptosis in PCa tumors [20]. Esser et al. reported that 10 weeks of voluntary running at a rate of >5 km/day delayed PCa incidence and progression compared animals that ran <5 km/day [27].

While exercise interventions may be beneficial in preventing PCa, the majority of Americans fail to meet the recommended guidelines of 30 mins of activity per day or 150 mins per week [28]. This is similarly true with cancer patients, as majority of patients with PCa do not exercise regularly [29–32]. Therefore, the investigation of alternative therapies that act as an exercise mimetic can be doubly beneficial.

Nexrutine is a commercially available herbal extract from the Phellodendrom amurense, which is widely used for the treatment of inflammation, gastroenteritis, abdominal pain and diarrhea. [33] The tree is native to Asia and has been reported to contain isoquinoline, alka- loids, phenolic compounds and flavon glycosides. Recently, we have demonstrated that Nexrutine inhibits PCa cells growth implicating a role for Nexrutine in modulating growth factor signaling [34]. Currently, our understanding how Nexrutine compared to other non-toxic, non-pharmaceutical interventions, such as exercise, is missing. To that extent, we conducted a comparative effectiveness study of Nexrutine and exercise on PCa growth in the transgenic adenocarcinoma of mouse prostate (TRAMP) model. We hypothesized that Nexrutine and exercise will have similar inhibitory effect on tumor growth through the modulation of growth and inflammatory signaling. Briefly, the results of this comparative effectiveness study suggest both Nexrutine and exercise inhibit tumor growth with both treatment groups demonstrating significantly fewer high grade tumors compared to the control group.

**Materials and methods**

The primary aim for this comparative effectiveness study was to examine if Nexrutine can serve as an exercise analog in inhibiting tumorigenesis in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Forty-five, 10-week old male TRAMP mice were...
randomized into one of three groups: Nexrutine alone, exercise alone or control. Three animals from each group were scheduled for sacrifice at weeks 4, 8, and 12, with the remaining mice sacrificed at week 20 (Fig 1).

Transgenic mouse model
A total of 45, 10-week old male transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, developed by prostate-specific expression of SV40 large T antigen using the rat probasin promoter, were used in this study. TRAMP mice develop prostate tumors with 100% frequency, in progressive stages that facilitates preclinical studies in the prevention, intervention and regression setting as demonstrated by various groups, including ours [34–37]. TRAMP mice with a pure C57BL/6 background were obtained from Jackson Laboratories (Bar Harbor, ME, USA). SV40 gene expression was verified via western blot (Supplementary Information). Mice were maintained in a climate-controlled environment with a 12-hour light-dark cycle with diet and water provided ad libitum. At necropsy, animals were examined to determine if there are any gross organ abnormalities. Animal care and handling was in accordance with established humane guidelines and protocols approved by the University of Texas Health Science Center at San Antonio’s Institutional Animal Care and Use Committee.

Voluntary wheel running
Mice randomized to the exercise group were given continuous access to a running wheel measuring 11.5 cm in diameter, with wheel revolutions monitored continuously by magnetic sensor (Med Associates, Inc., St. Albans, VT, USA). This modality was chosen for the exercise intervention, as opposed to forced exercise modalities (i.e. forced treadmill running) because it is most reflective of natural mouse locomotion and behavior. In addition, forced exercise has the potential to induce a stress response that may alter the outcomes of our study. Voluntary wheel running is characterized by short period of performance against a low load throughout the entire dark cycle. Therefore, the running wheel modality reflects activity that is consistent with exercise behavior inversely correlated with mortality and closely mimics the capabilities of PCa.

Preparation of Nexrutine diet
Nexrutine was obtained from Interhealth (Benicia, CA, USA). Based on positive results from previously published data, a dose of 600 mg/kg Nexrutine was used [34]. Nexrutine was
pelleted into a standard AIM-93G purified roden diet by Dyets, Inc (Bethlehem, PA, USA). This diet consisted of casein (200 g/kg), Deytrose (132 g/kg) cornstarch (397.5 g/kg), sucrose (100 g/kg), cellulose (50 g/kg), soybean oil (70 g/kg), t-butylhydroquinone (0.014 g/kg), satl mix #210050 (35 g/kg), L-cystine (3 g/kg), and choline bitartrate (2.5 g/kg). Quality assurance and irradiation was performed by the manufacturer. Stability of Nexrutine in the diet pellets was evaluated and reported previously [34–37]. Access to Nexrutine pelleted diet was provided ad lib and food consumption was assessed weekly.

Histology and immunohistochemistry

Genitourinary complex were harvested, weighed and fixed in 10% neutral buffered formalin. Tumors sections were dissected, paraffin embedded, sectioned, placed on poly-lysine slides and stained with hematoxylin and eosin stain to visualize cell nuclei and cytoplasm. Sectioned tumor slides were scored in a blinded fashion using an established grading system for TRAMP mice [35, 38]. Non-cancerous lesions were graded as 1, 2 or 3, indicating normal tissue, low PIN and high PIN, respectively. Grades 4, 5 and 6 are indicative of well-differentiated, moderately differentiated and poorly differentiated cancerous lesions, respectively.

To determine the physiological adaptations within the tumor, 20 week samples were probed with antibody specific to mouse pAkt (Ser473, rabbit monoclonal, 1:50, Cell Signaling, Danver, MA) and p65 NFκB (Ser536, rabbit monoclonal antibody, 1:1000, Cell Signaling, Danver, MA). Secondary and tertiary antibodies were biotinylated and stripped with streptavidin horseradish peroxidase (Biocare 4 plus, Kit, Biocare Medical, Concord, CA). Slides were then reviewed by a pathologist blinded to the treatments. Immunoreactivity was scored based on the percentage of stained cells and graded semi-quantitatively as zero (0% stained cells), 1+ (<10% stained cells), 2+ (10–20% stained cells), 3+ (20–50% stained cells) and 4+ (>50% cells stained). Images were recorded using a light microscope.

Cytokine multiplex

Serum collected from mice sacrificed at the 4 week and 20 week time points were assayed in duplicate using a 32 panel Mouse Cytokine/Chemokine Magnetic Bead Panel Immunology Multiplex Assay (EMD Millipore, Billerica, MA) to determine the changes in cytokine profiles. Proteins analyzed include Eotaxin/CCL11, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, KC-like, LIF, LIX, MCP-1, M-CSF, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, TNF-α, VEGF.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine significant differences between treatment groups. Dichotomous measures, such as the presence or absence of palpable tumors were evaluated for treatment group differences using Fisher’s exact tests. Mann-Whitney U-tests were performed for each intervention duration (weeks 4, 8, 12, and 20) comparing tumor grades of Nexrutine treated animals and exercising animals to control mice. Based on previous studies conducted by our group the effect size of Nexrutine is approximately 1.3, which implies a sample size of 15 mice per group completing the project provides greater than 85% power to detect significant changes between groups with a one-sided alpha of 0.05. All statistical testing were two sided with a significance level of 5%. Statistical Package for the Social Sciences (SPSS) (IBM, Armonk, NY) was used to conduct the analysis. GraphPad Prism (La Jolla, CA) was used to develop the graphs.
Results

Intervention fidelity

On average, Nexrutine fed mice consumed 18.10 ± 1.28 g of chow per week, equivalent to approximately 15 mg of Nexrutine per day (Table 1). Exercising mice ran 14.31 ± 1.8 km/day. Variability of running distance is presented in Fig 2. Mean body weight changes were analyzed with respect to treatment and time point. No significant differences were observed between groups at the respective time points (4, 8, 12, and 20 weeks) nor was a treatment effect observed. Tumor free body mass was also analyzed by taking the difference between total body mass and GU mass. No significant treatment effect was observed across the 20 week study. The only significant time point difference was seen at 12 weeks (f = 4.982; p = 0.045) with Nexrutine fed mice having less tumor free body mass compared to exercise mice (Nexrutine: 23.7 ± 3.76; Exercise: 30.1 ± 1.27; p = 0.04). Since the animals in the Nexrutine group did not demonstrate any significant loss in body mass, these data indicate that Nexrutine is non-toxic in nature (Table 1).

Nexrutine and exercise suppress palpable tumors in TRAMP mice

Tumors were first palpable in the control group with 27% of control mice demonstrating presence at 14 weeks of age (week 4 of the intervention). Tumor were not palpable in the exercise or Nexrutine group until 5 weeks later (19 week old or Week 9 of the intervention). At 19 weeks of age, 8 of the 9 remaining TRAMP mice in each group presented with palpable tumors. Analysis of this data showed that exercise intervention and dietary intervention with Nexrutine suppressed the occurrence of palpable tumors (p = 0.05, control vs. exercise or Nexrutine). These data support the potential for Nexrutine and exercise to reduce tumor incidence and/or increasing the latency of tumor development.

Nexrutine and exercise slow prostate tumor progression in TRAMP mice

No significant pathological changes were observed as a function of time; therefore, data were pooled for analysis. Contrary to our hypothesis, neither exercise nor Nexrutine were able to significantly reduce GU mass (Table 1). However, exercise and Nexrutine were able to reduce the number of high grade tumors. Specifically, Nexrutine fed mice were observed to have a 60% beneficial effect in protecting against high grade tumors with only 5 of the 15 mice in the group presenting with high grade tumors. Exercise had a 60% beneficial effect with only 6 of the 15 mice having high grade tumors (Fig 3). Histopathological evaluation of the prostate tumor from the 3 study groups is shown in Fig 4. Prostate tumors from control mice show poorly differentiated tumor characterized by variable nuclear shape with no gland formation. In comparison, prostate tumor samples from animals fed Nexrutine or exercising TRAMP mice exhibit pathological features consistent with high grade prostatic intraepithelial neoplasia. While 100% of animals developed tumors (varying stages), Nexrutine fed and exercising TRAMP mice had fewer poorly differentiated tumors compared to controls.

Table 1. Animal characteristics.

|                      | Control             | Nexrutine **| Exercise  |
|----------------------|---------------------|-------------|-----------|
| Body Mass (g)        | 33.2 ± 7.28         | 31.5 ± 3.96 | 35.2 ± 8.5|
| GU Mass (g)          | 4.92 ± 7.79         | 4.65 ± 5.95 | 7.18 ± 8.44|
| GU Free Body Mass (g) | 28.28 ± 2.95       | 27.35 ± 4.24 | 28.02 ± 2.13|
| Food Consumption (g/week) | 26.06 ± 3.57 | 18.10 ± 1.28 | 28.22 ± 2.98|

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Nexrutine, but not exercise, reduces pAkt expression in prostate tumors

Consistent with previous work by our group, Nexrutine fed TRAMP mice has significantly lower expression of pAKT (Ser473) compared to control (p = 0.04; Fig 5) and exercising TRAMP mice (p = 0.017), respectively. Contrary to our hypothesis, 20 weeks of exercise did not reduce expression of pAkt (Ser473). Additionally, no differences were observed in p65 expression between the 3 study groups.

Exercise and Nexrutine modulate the cytokine profile of TRAMP mice

Of the 32 cytokines measured in week 4 and week 20 samples, control mice had borderline significant changes only in IL-13 (p = 0.04; Fig 6). Whereas, exercise resulted in lower concentrations
Fig 3. Waterfall Diagram presenting group variability of tumor grades. Individual tumor grades are presented for each group (control, Nexrutine®, Exercise). Data is presented as either low grade (scores 1–3) or high grade (scores 4–6).

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Fig 4. Histology of representative tumors. Prostate lesions were scored in a blinded fashion using an established grading system for TRAMP mice [35, 38]. Non-cancerous lesions were categorized as low grade (graded as 1, 2 or 3), indicating normal tissue, low PIN and high PIN, respectively. Grades 4, 5 and 6 were categorized as high grade, indicative of well-differentiated, moderately differentiated and poorly differentiated cancerous lesions, respectively. Pathology scores were significantly higher in the control group with 71% scoring high compared to Nexrutine® and exercise mice. Representative low magnification (200 μm bar) and high magnification (20 μm bar) images for control, Nexrutine® and exercise mice completing 20 weeks of intervention are presented.

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of eotaxin, IL-1α, IL-5, IL-12(p40) and VEGF p<0.05). While there were no differences at baseline, exercise mice had lower IL-5 and VEGF compared to control at the 20 week time point (p<0.05). Nexrutine significantly reduced circulating IL-1α and IL-9 concentrations however, no significant differences were observed when compared to the control group (Fig 6).

**Discussion**

The purpose of this study was to compare the effectiveness of exercise and Nexrutine interventions in delaying tumor progression in TRAMP mice. Combining the time points through the
Fig 6. Significant differences observed in cytokines associated with tumor progression. Samples from week 4 (black bars) and week 20 (gray bars) from each group were assayed using a 32 cytokine multiplex. Though there is variability in cytokines that were observed to be significant, both exercise and Nexrutine similarly reduced concentrations of IL-1α, a cytokine associated with cachexia, anorexia and bone loss. * indicates significant at p<0.05. ** indicates significance at p<0.01. *** indicates significance at p<0.001.

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20 week intervention, we found that both exercise and Nexrutine were able to reduce the number of high grade tumors at necropsy. The outcomes in the Nexrutine group support work previously published by our group and others on the antitumorigenic capabilities of this natural product [39–41]. Similarly, the results seen in the exercise group support previous work suggesting exercise has cancer preventative traits. Here we report that the anti-inflammatory effects of exercise may be a mechanism by which exercise exerts its benefits. We also aimed to identify the role of Nexrutine as an exercise analog. Our results represent the first of its kind to report comparing a natural product to exercise. The outcomes of our study suggest that both Nexrutine and exercise can protect against aggressive tumors, thought it appears that they do so through different mechanisms.

It is well established that there is a negative association of PCa with consumption of fruits and vegetables [42]. Nexrutine is a commercially available herbal extract from the P. amurense, a tree native to Asia widely used for the treatment of inflammation, gastroenteritis, abdominal pain and diarrhea [36]. Recently, our group published a review of the benefits of Nexrutine as a chemopreventative agent [39]. The results of this study provide preliminary evidence that this herbal extract inhibits tumor development similarly to exercise.

The translational potential of Nexrutine was recently demonstrated in a first of its kind study by our group demonstrating the safety of Nexrutine supplementation in a phase I clinical trial [43]. In this study, men diagnosed with PCa received Nexrutine orally (500 mg three times a day) either 1 to 2 months preoperatively or 1 to 2 months prior to or concurrently with radiation therapy. By the end of the neoadjuvant treatment, 81% of the patients had a decline in prostate specific antigen (PSA). More importantly, this study revealed minimal and self-limited toxicities.

The current context of exercise as a chemopreventative intervention is mixed with many studies reporting on the benefits of physical activity [44], but some reporting physical activity is unfavorable to reducing PCa risk [45]. Although the precise biological mechanisms linking physical activity to reduced PCa risk are debated, McTiernan and colleagues suggest the role of reduced inflammation and improved immune function as key contributors [46]. The results of our investigation suggest that aerobic exercise can reduce key cytokines associated with tumor progression.

Growth signaling through Akt has been associated with PCa development. Therefore, altering Akt activity/phosphorylation may inhibit tumor cell proliferation and gene transcription [47]. In this study, exercise reduced high grade tumors; however, exercise did not alter intratumoral pAkt (Ser473). Standard and colleagues (2009) reported previously that 12 weeks of ad-lib aerobic exercise did not affect Akt gene expression [48]. Exercise is a strong promoter of growth factor signaling [49], which may be counterintuitive as an intervention to reduce tumor mass. However, the reduction in high grade tumors reported in this publication provide valuable rationalization for the use of aerobic exercise as a preventative intervention. Nexrutine on the other hand was able to reduce tumor expression of pAkt (Ser473), supporting previous work by our group [35, 39, 41]. Continued research in the area of growth factor signaling in relation to tumor growth and aggressiveness in the context of aerobic exercise is needed.

Aerobic exercise has been reported to have significant anti-inflammatory effects [46]. This led us to the hypothesis that exercise would exert its effect by modulating cytokine signaling evident by reduced p65 NFκB. This hypothesis was partially supported with lower concentrations of circulating eotaxin, IL-1α, IL-5, IL-12 (p40) and VEGF. Nonetheless, the decreased inflammatory profile in exercise TRAMP mice was not associated with changes in p65 NFκB in the tumor.

The results of our study found that both Nexrutine and exercise reduced circulating concentrations of IL-1α. IL-1α is a regulatory cytokine that can induce the activation of
transcription factors and promotes the expression of genes involved in cell survival, proliferation, and angiogenesis [50]. IL-1α has also been implicated in cachexia and anorexia [51, 52]. IL-1α, as well as other cytokines, such as IL-6, IL-11 and TNF-α, activate the RANK pathway that controls bone remodeling [53]. While bone density was not measured in this study, our group has reported previously that Nexrutine preserves bone density in TRAMP mice [37]. In cases of advanced cancers, bone mineral density loss is often observed and may be mediated via IL-1α. Therefore, a reduction in IL-1α concentrations could suppress osteoclastogenesis and osteoclast activity [54]. Others have also reported exercise prevents bone loss in PCa patients [55]. Future research is needed to elucidate the mechanism by which exercise and Nexrutine decrease IL-1α concentrations and the role of IL-1α on cachexia and bone loss seen with cancer.

The decreased circulating concentrations of cytokines provides insight into the benefits of exercise in suppressing tumor progression. Eotaxin is of significant interest as an exercise target due to its role as a potential prognostic biomarker for PCa [56]. Even though little is known regarding the role of eotaxin in tumor pathology, eotaxin is known to activate matrix metalloproteinases that are implicated in the PCa progression [57]. Others have also reported that eotaxin is able to promote cell migration in vitro and induce angiogenesis in vivo [58]. This reduction in eotaxin may account for the reduced VEGF concentration observed in our study.

In our study, exercise resulted in lower circulating VEGF concentrations. The suppression of VEGF is significant because of the vasculature remodeling that occurs in solid tumors. Tumor blood vessels are structurally and functionally abnormal [59]. Greater than 50% of tumor vessels are non-functional [60]. Reducing VEGF has the potential of inhibiting vessel sprouting and normalize tumor vasculature [61]. VEGF can be implicated in the proliferation, survival, adhesion, migration and chemotaxis of tumor cells [62]. Thus, reducing VEGF will likely delay tumor progression as seen in this study. Further, the discovery of the multiple isoforms of VEGF raise the possibility that they each have different functions [62–64].

Our study isn’t without limitations. First, the study design with multiple timepoint sacrifices decreased statistical power for our study. Based on previous studies conducted by our group, a sample size of 15 mice per group provides 80% power to detect significance changes between groups. Additionally, the exercise modality of leads to great variability in exercise durations. Future research should consider the variance between forced exercise compared to voluntary exercise interventions. Similarly, consumption of Nexrutine through the diet is variable as well. Alternatives would include daily gavage or injection of suspended Nexrutine. While it may provide consistent dosing of Nexrutine, these protocols may also stress animals which may impact outcomes measures.

The results of our study indicate that both exercise and Nexrutine can independently reduce tumor aggressiveness. The mechanisms by which these interventions exert their positive effects different, suggesting that aerobic exercise prevents tumor aggressiveness by modulating circulating cytokines while Nexrutine exerts its benefit by reducing intratumoral growth factor signaling. Tumor growth and proliferation are managed through a number of different signaling pathways. The results of our study suggest that future research considering combining the two interventions to investigate an additive/synergistic effect that may target tumor grown through multiple mechanistic pathways.

In conclusion, the results of our study suggest that the natural produce Nexrutine and exercise similarly reduce PCa risk and severity. Future research is necessary to identify commonalities and differences in the mechanisms by which exercise and Nexrutine exert this beneficial effect on prostate cancer.
Supporting information

S1 Table. Study database.
(XLSX)

S2 Table. Absolute values of cytokine concentrations with means ± standard deviations.
(XLSX)

S1 Fig. SV40 transgene expression PCR protocol.
(JPEG)

S2 Fig. SV40 expression western blot results. The ladder in lane 1 is New England BioLabs Quick-Load 100 bp DNA Ladder (catalog number N0467). Lanes 2–5 represent samples from animals carrying the transgene. Lanes 6–8 are from animals that are not carrying the transgene. (JPG)

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016; 66(1):7–30.
2. Haas GP, Delongchamps N, Brawley OW, Wang CY, de la Roza G. The Worldwide Epidemiology of Prostate Cancer: Perspectives from Autopsy Studies. The Canadian journal of urology. 2008; 15(1):3866–71. PMID: 18304396
3. Norat T, Aune D, Chan D, Romaguera D. Fruits and vegetables: updating the epidemiologic evidence for the WCRF/AICR lifestyle recommendations for cancer prevention. Advances in nutrition and cancer: Springer; 2014. p. 35–50.
4. Kushi LH, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV, et al. American Cancer Society guidelines on nutrition and physical activity for cancer prevention. CA: a cancer journal for clinicians. 2012; 62(1):30–67.
5. Yang M, Kenfield SA, Van Blarigan EL, Batista JL, Sesso HD, Ma J, et al. Dietary patterns after prostate cancer diagnosis in relation to disease-specific and total mortality. Cancer prevention research. 2015; 8(6):545–51. https://doi.org/10.1158/1940-6207.CAPR-14-0442 PMID: 26031631
6. Beydoun N, Bucci JA, Chin YS, Spry N, Newton R, Galvao DA. Prospective study of exercise intervention in prostate cancer patients on androgen deprivation therapy. J Med Imaging Radiat Oncol. 2013. Epub 2013/10/15. https://doi.org/10.1111/jjmr.12115 PMID: 24118798.
7. Galvao DA, Nosaka K, Taaffe DR, Spry N, Kristjansson LJ, McGuigan MR, et al. Resistance training and reduction of treatment side effects in prostate cancer patients. Medicine and science in sports and exercise. 2006; 38(12):2045–52. Epub 2006/12/06. https://doi.org/10.1249/01.mss.0000233803.48691.8b PMID: 17146309.
8. Galvao DA, Taaffe DR, Spry N, Joseph D, Newton RU. Combined resistance and aerobic exercise program reverses muscle loss in men undergoing androgen suppression therapy for prostate cancer without bone metastases: a randomized controlled trial. J Clin Oncol. 2010; 28(2):340–7. Epub 2009/12/02. https://doi.org/10.1200/JCO.2009.23.2488 PMID: 19949016.
9. Greiwe JS, Cheng B, Rubin DC, Yarasheski KE, Semenkovich CF. Resistance exercise decreases skeletal muscle tumor necrosis factor α in frail elderly humans. The FASEB Journal. 2001; 15(2):475–82. https://doi.org/10.1096/fj.00-0274com PMID: 11156963
10. Monga U, Garber SL, Thornby J, Vaillcona B, Kerrigan AJ, Monga TN, et al. Exercise Prevents Fatigue and Improves Quality of Life in Prostate Cancer Patients Undergoing Radiotherapy. Archives of physical medicine and rehabilitation. 2007; 88(11):1416–22. https://doi.org/10.1016/j.apmr.2007.08.110 PMID: 17964881
11. Segal RJ, Reid RD, Courneya KS, Malone SC, Parliament MB, Scott CG, et al. Resistance exercise in men receiving androgen deprivation therapy for prostate cancer. J Clin Oncol. 2003; 21(9):1653–9. Epub 2003/05/02. https://doi.org/10.1200/JCO.2003.09.534 PMID: 12721238.
12. Windsor PM, Nicol KF, Potter J. A randomized, controlled trial of aerobic exercise for treatment-related fatigue in men receiving radical external beam radiotherapy for localized prostate carcinoma. Cancer. 2004; 101(3):550–7. Epub 2004/07/27. https://doi.org/10.1002/cncr.20378 PMID: 15274068.
13. Shephard RJ. Physical activity and prostate cancer: an updated review. Sports Medicine. 2017; 47(6):1055–73. https://doi.org/10.1007/s40279-016-0648-0 PMID: 27844337
14. Singh AA, Jones LW, Antonelli JA, Gerber L, Calloway EE, Shuler KH, et al. Association between exercise and primary incidence of prostate cancer. Cancer. 2013; 119(7):1338–43. https://doi.org/10.1002/cncr.27791 PMID: 23401030
15. Antonelli JA, Jones LW, Baez LL, Thomas J-A, Anderson K, Taylor LA, et al. Exercise and prostate cancer risk in a cohort of veterans undergoing prostate needle biopsy. The Journal of urology. 2009; 182(5):2226–31. https://doi.org/10.1016/j.juro.2009.07.028 PMID: 19758620
16. De Nunzio C, Presicce F, Lombardo R, Cancrini F, Petta S, Trucchi A, et al. Physical activity as a risk factor for prostate cancer diagnosis: a prospective biopsy cohort analysis. BJU International. 2016; 117(6B).
17. Kenfield SA, Stampfer MJ, Giovannucci E, Chan JM. Physical Activity and Survival After Prostate Cancer Diagnosis in the Health Professionals Follow-Up Study. Journal of Clinical Oncology. 2011; 29(6):726–32. https://doi.org/10.1200/JCO.2010.31.5226 PMID: 21205749
18. Richman EL, Kenfield SA, Stampfer MJ, Paciorek A, Carroll PR, Chan JM. Physical activity after diagnosis and risk of prostate cancer progression: data from the cancer of the prostate strategic urologic research endeavor. Cancer research. 2011.

19. Friedenreich CM, Wang Q, Neilson HK, Kopciuk KA, McGregor SE, Courmeya KS. Physical activity and survival after prostate cancer. European urology. 2016; 70(4):576–85. https://doi.org/10.1016/j.euro.2015.12.032 PMID: 26774959

20. Zheng X, Cui XX, Huang MT, Liu Y, Shih WJ, Lin Y, et al. Inhibitory effect of voluntary running wheel exercise on the growth of human pancreatic Panc-1 and prostate PC-3 xenograft tumors in immunodeficient mice. Oncol Rep. 2008; 19(6):1583–8. Epub 2008/05/24. PMID: 18497989

21. Zheng X, Cui XX, Huang MT, Liu Y, Wagner GC, Lin Y, et al. Inhibition of progression of androgen-dependent prostate LNCaP tumors to androgen independence in SCID mice by oral caffeine and voluntary exercise. Nutr Cancer. 2012; 64(7):1029–37. Epub 2012/10/16. https://doi.org/10.1080/01635581.2012.716899 PMID: 23061906.

22. Andrianopoulos G, Nelson RL, Bombeck CT, Souza G. The influence of physical activity in 1,2 dimethylhydrazine induced colon carcinogenesis in the rat. Anticancer Res. 1987; 7(4b):849–52. Epub 1987/07/01. PMID: 3674772.

23. Cohen LA, Choi K, Backlund JY, Harris R, Wang CX. Modulation of N-nitrosomethylurea induced mammary tumorigenesis by dietary fat and voluntary exercise. In Vivo. 1991; 5(4):333–44. Epub 1991/07/01. PMID: 1810418.

24. Wannamethee SG, Shaper AG, Walker M. Physical activity and risk of cancer in middle-aged men. British journal of cancer. 2001; 85(9):1311–6. Epub 2001/11/27. https://doi.org/10.1054/bjoc.2001.2096 PMID: 11720466.

25. Craven-Giles T, Tagliaferro AR, Ronan AM, Baumgartner KJ, Roebuck BD. Dietary modulation of pancreatic carcinogenesis: calories and energy expenditure. Cancer Res. 1994; 54(7 Suppl):1964s–8s. Epub 1994/04/01. PMID: 8137321.

26. Roebuck BD, McCaffrey J, Baumgartner KJ. Protective effects of voluntary exercise during the postinitiation phase of pancreatic carcinogenesis in the rat. Cancer research. 1990; 50(21):6811–6. Epub 1990/11/01. PMID: 2208145.

27. Esser KA, Harpole CE, Prins GS, Diamond AM. Physical activity reduces prostate carcinogenesis in a transgenic model. The Prostate. 2009; 69(13):1372–7. Epub 2009/06/03. https://doi.org/10.1002/pros.20987 PMID: 19489028.

28. Blackwell DL, Clark TC. State variation in meeting the 2008 federal guidelines for both aerobic and muscle-strengthening activities through leisure-time physical activity among adults aged 18–64: United States 2010–2015. National Health Statistics Report. 2018;(112).

29. Barnard RJ, Leung PS, Aronson WJ, Cohen P, Golding LA. A mechanism to explain how regular exercise might reduce the risk for clinical prostate cancer. European Journal of Cancer Prevention. 2007; 16 (5):415–21. https://doi.org/10.1097/01.cej.0000243851.66985.e4 PMID: 17923812

30. Keogh JW, Shepherd D, Krageloh CU, Ryan C, Masters J, Shepherd G, et al. Predictors of physical activity and quality of life in New Zealand prostate cancer survivors undergoing androgen-deprivation therapy. N Z Med J. 2010; 123(1325):20–9. Epub 2011/02/15. PMID: 21317957.

31. Bellizzi KM, Rowland JH, Jeffery DD, McNeel T. Health behaviors of cancer survivors: examining opportunities for cancer control intervention. J Clin Oncol. 2005; 23(34):8884–93. Epub 2005/11/30. https://doi.org/10.1200/JCO.2005.02.2343 PMID: 16314649.

32. Coupes EJ, Ostroff JS. A population-based estimate of the prevalence of behavioral risk factors among adult cancer survivors and noncancer controls. Prev Med. 2005; 40(6):702–11. Epub 2005/04/27. https://doi.org/10.1016/j.ypmed.2004.09.011 PMID: 15850888.

33. Kumar AP, Graham H, Robson C, Thompson IM, Ghosh R. Natural products: potential for developing Phellodendron amurense bark extract for prostate cancer management. Mini Rev Med Chem. 2010; 10 (5):388–97. Epub 2010/04/08. https://doi.org/10.2174/138955710791330936 PMID: 20370708.

34. Kumar AP, Bhaskaran S, Ganapathy M, Crosby K, Davis MD, Kochunov P, et al. Akt/cAMP-responsive element binding protein/cyclin D1 network: a novel target for prostate cancer inhibition in transgenic adenocarcinoma of mouse prostate model mediated by Nexrutine, a Phellodendron amurense bark extract. Clinical cancer research: an official journal of the American Association for Cancer Research. 2007; 13 (9):2784–94. Epub 2007/05/03. https://doi.org/10.1158/1078-0432.ccr-06-2974 PMID: 17473212

35. Garcia GE, NICOLE A, Bhaskaran S, Gupta A, Kyprianou N, Kumar AP. Akt-and CREB-mediated prostate cancer cell proliferation inhibition by Nexrutine, a Phellodendron amurense extract. Neoplasia. 2006; 8(6):523–33. Epub 2006/07/06. https://doi.org/10.1593/neo.050745 PMID: 16820098

36. Ghosh R, Garcia GE, Crosby K, Inoue H, Thompson IM, Troyer DA, et al. Regulation of Cox-2 by cyclic AMP response element binding protein in prostate cancer: potential role for nextrutine. Neoplasia. 2007; 9(11):893–9. https://doi.org/10.1593/neo.07502 PMID: 18030357
Nexrutine and exercise independently slow prostate cancer aggressiveness

37. Ghosh R, Graham H, Rivas P, Tan XJ, Crosby K, Bhaskaran S, et al. Phellodendron amurense Bark Extract Prevents Progression of Prostate Tumors in Transgenic Adenocarcinoma of Mouse Prostate: Potential for Prostate Cancer Management. Anticancer Research. 2010; 30(3):857–65. PMID: 20393007

38. Gingrich JR, Barrios RJ, Foster BA, Greenberg NM. Pathologic progression of autochthonous prostate cancer in the TRAMP model. Prostate Cancer Prostatic Dis. 1999; 2(2):70–5. Epub 2002/12/24. https://doi.org/10.1038/sj.pcan.4500296 PMID: 12496841.

39. Hussain S, Patel D, Ghosh R, Kumar A. Extracting the Benefit of Nexrutine® for Cancer Prevention. Current Pharmacology Reports. 2015; 1:1–8. https://doi.org/10.1007/s40495-015-0029-7 PMID: 26539341

40. Kumar A, Graham H, Robson C, Thompson I, Ghosh R. Natural products: potential for developing Phellodendron amurense bark extract for prostate cancer management. Mini reviews in medicinal chemistry. 2010; 10(5):388–97. https://doi.org/10.2174/138955710791330936 PMID: 20370708

41. Kumar AP, Bhaskaran S, Ganapathy M, Crosby K, Davis MD, Kochunov P, et al. Akt/CREB/Cyclin D1 network: a novel target for prostate cancer inhibition in transgenic adenocarcinoma of mouse prostate (TRAMP) model mediated by Nexrutine®, a Phellodendron amurense bark extract. Clinical cancer research: an official journal of the American Association for Cancer Research. 2007; 13(9):2784–94.

42. Glade MJ. World Cancer Research Fund/American Institute for Cancer ResearchFood, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective2007American Institute for Cancer ResearchWashington, D. CISBN: 978-0-9722522-2-5. Elsevier; 2008.

43. Swanson GP, Jones WE, Ha CS, Jenkins CA, Kumar AP, Basler J. Tolerance of Phellodendron amurense Bark Extract (Nexrutine®) in Patients with Human Prostate Cancer. Phytotherapy Research. 2015; 29(1):40–2. https://doi.org/10.1002/ptr.5221 PMID: 25205619

44. Wekesa A, Harrison M, Watson R. Physical activity and its mechanistic effects on prostate cancer. Prostate cancer and prostatic diseases. 2015; 18(3):197–207. https://doi.org/10.1038/pcan.2015.9 PMID: 25800589

45. Friedenreich C, McGregor S, Courmey A, Angyalfi S, Elliott F. Case-control study of lifetime total physical activity and prostate cancer risk. American journal of epidemiology. 2004; 159(8):740–9. https://doi.org/10.1093/aje/kwh106 PMID: 15051583

46. McTiernan A. Mechanisms linking physical activity with cancer. Nature Reviews Cancer. 2008; 8(3):205–11. https://doi.org/10.1038/ncc2325 PMID: 18235448

47. Gao J, Tian J, Lu Y, Shi F, Kong F, Shi H, et al. Leptin induces functional activation of cyclooxygenase-2 through JAK2/STAT3, MAPK/ERK, and PI3K/AKT pathways in human endometrial cancer cells. Cancer science. 2009; 100(3):389–95. Epub 2009/01/22. https://doi.org/10.1111/j.1349-7006.2008.01053.x PMID: 19154413.

48. Standard J, Jiang Y, Yu M, Su X, Zhao Z, Xu J, et al. Reduced signaling of PI3K-Akt and RAS-MAPK pathways is the key target for weight-loss-induced cancer prevention by dietary calorie restriction and/ or physical activity. The Journal of nutritional biochemistry. 2014; 25(12):1317–23. Epub 2014/10/07. https://doi.org/10.1016/j.jnutbio.2014.07.010 PMID: 25283328

49. Sakamoto K, Aschenbach WG, Hirshman MF, Goodyear LJ. Akt signaling in skeletal muscle: regulation by exercise and passive stretch. American journal of physiology-Endocrinology and metabolism. 2003; 285(5):E1081–E8. https://doi.org/10.1152/ajpendo.00228.2003 PMID: 12837686

50. Vornov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. Proceedings of the National Academy of Sciences. 2003; 100(5):2645–50.

51. Steeke KT, Marc P, Sandrine T, Dominique H, Yannick F. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine & growth factor reviews. 2004; 15(1):49–60.

52. Fong Y, Moldawer LL, Marano M, Wei H, Barber A, Manogue K, et al. Cachectin/TNF or IL-1 alpha induces cachexia with redistribution of body proteins. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1989; 256(3):R659–R65.

53. Jagielska B, Poniatsowska G, Talasiewicz K, Demkow T, Wiechno P. Systemic complications in the hormonal treatment of prostate and breast cancer. Nowotwory Journal of Oncology. 2017; 67(3):206–14.

54. Lee YM, Fujikado N, Manaka H, Yasuda H, Iwakura Y. IL-1 plays an important role in the bone metabolism under physiological conditions. International immunology. 2010; 22(10):805–16. Epub 2010/08/04. https://doi.org/10.1093/intimm/dxq431 PMID: 20679512.

55. Winters-Stone KM, Dobek JC, Bennett JA, Maddalozzo GF, Ryan CW, Beer TM. Skeletal Response to Resistance and Impact Training in Prostate Cancer Survivors. Medicine and science in sports and exercise. 2014; 46(8):1482–8. https://doi.org/10.1249/MSS.0000000000000265 PMID: 24500540

56. Agarwal M, He C, Siddiqui J, Wei JT, Macoska JA. CCL11 (eotaxin-1): A new diagnostic serum marker for prostate cancer. The Prostate. 2013; 73(6):573–81. https://doi.org/10.1002/pros.22597 PMID: 23059958
57. Zhu F, Liu P, Li J, Zhang Y. Eotaxin-1 promotes prostate cancer cell invasion via activation of the CCR3-ERK pathway and upregulation of MMP-3 expression. Oncology reports. 2014; 31(5):2049–54. https://doi.org/10.3892/or.2014.3060 PMID: 24604010

58. Salcedo R, Young HA, Ponce ML, Ward JM, Kleinman HK, Murphy WJ, et al. Eotaxin (CCL11) induces in vivo angiogenic responses by human CCR3+ endothelial cells. The Journal of Immunology. 2001; 166(12):7571–8. https://doi.org/10.4049/jimmunol.166.12.7571 PMID: 11390513

59. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nature Reviews Cancer. 2008; 8(6):425. https://doi.org/10.1038/nrc2397 PMID: 18500244

60. Jain RK. Determinants of tumor blood flow: a review. Cancer research. 1988; 48(10):2641–58. Epub 1988/05/15. PMID: 3282647.

61. Ma J, Waxman DJ. Combination of antiangiogenesis with chemotherapy for more effective cancer treatment. Molecular cancer therapeutics. 2008; 7(12):3670–84. Epub 2008/12/17. https://doi.org/10.1158/1535-7163.MCT-08-0715 PMID: 19074844

62. Perrot-Aplanat M, Di Benedetto M. Autocrine functions of VEGF in breast tumor cells: Adhesion, survival, migration and invasion. Cell Adhesion & Migration. 2012; 6(6):547–53. https://doi.org/10.4161/cam.23332 PMID: 23257828

63. Breuss JM, Uhrin P. VEGF-initiated angiogenesis and the uPA/uPAR system. Cell Adhesion & Migration. 2012; 6(6):535–40. https://doi.org/10.4161/cam.22243 PMID: 23076133

64. Goel HL, Mercurio AM. Enhancing integrin function by VEGF/neuropilin signaling: Implications for tumor biology. Cell Adhesion & Migration. 2012; 6(6):554–60. https://doi.org/10.4161/cam.22419 PMID: 23076131