Androgen Receptor– and E2F-1–Targeted Thymoquinone Therapy for Hormone-Refractory Prostate Cancer

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Abstract

Relapse of prostate cancer after androgen ablation therapy is hormone-refractory, with continued tumor growth being dependent on the androgen receptor (AR). E2F-1, a regulator of cell proliferation and viability, reportedly plays a role in the development of hormone-refractory prostate cancer. Thymoquinone is a component of Nigella sativa, an herb used for thousands of years for culinary and medicinal purposes in Asian and Middle Eastern countries and has been reported to have an antineoplastic effect both in vitro and in vivo. We observed that thymoquinone inhibited DNA synthesis, proliferation, and viability of cancerous (LNCaP, C-4, DU145, and PC-3) but not noncancerous (BPH-1) prostate epithelial cells by down-regulating AR and E2F-1. In LNCaP cells, this was associated with a dramatic increase in p21Cip1, p27Kip1, and Bax. Thymoquinone blunted progression of synchronized LNCaP cells from G1 to S phase, with a concomitant decrease in AR and E2F-1 as well as the E2F-1-regulated proteins necessary for cell cycle progression. In a xenograft prostate tumor model, thymoquinone inhibited growth of C-4-2B–derived tumors in nude mice. This in vivo suppression of tumor growth, as with C-4-2B cell growth in culture, was associated with a dramatic decrease in AR, E2F-1, and cyclin A as determined by Western blot of tissue extracts. Tissue immunohistochemical staining confirmed a marked reduction in E2F-1 and showed induction of apoptosis on terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling assay. These findings show that thymoquinone suppresses the expression of AR and E2F-1 necessary for proliferation and viability of androgen-sensitive as well as androgen-independent prostate cancer cells both in vitro and in vivo and, moreover, produced no noticeable side effects in mice. We conclude that thymoquinone, a naturally occurring herbal product, may prove to be effective in treating hormone-sensitive as well as hormone-refractory prostate cancer. Furthermore, because of its selective effect on cancer cells, we believe that thymoquinone can also be used safely to help prevent the development of prostate cancer. [Cancer Res 2007;67(16):7782–8]

Introduction

Prostate cancer is the second most frequently diagnosed neoplasm after skin cancer and is the second leading cause of cancer deaths in men, with an estimated 218,890 new cases and 27,050 deaths in the United States during 2007 (1). Whereas state-of-the-art treatment of prostate cancer provides prolonged disease-free survival for many patients with localized disease, it is rarely curative in patients with locally advanced or metastatic hormone-refractory disease. Androgen ablation therapy is the mainstream strategy for treatment of hormone-sensitive prostate cancer; however, hormonal therapy leads to remissions typically lasting 2 to 3 years, whereas in most men, metastatic prostate cancer eventually progresses to an androgen-independent state resulting in hormone-refractory prostate cancer, for which there is presently no known effective treatment (2, 3). Androgen receptor (AR) is expressed in nearly all prostate cancers (4–6), and growth of hormone-refractory disease continues to depend on AR that is either overexpressed or activated promiscuously (7). Therefore, developing a curative strategy should involve finding effective ways of not just inactivating AR but eliminating it altogether from cancer cells (8).

E2F-1 is an essential transcription factor required for expression of a number of proteins involved in G1-S transition and DNA synthesis (9). Tight regulation of E2F-1 is critical for cell proliferation and viability (10). E2F-1 seems to be an oncogene at lower levels and induces apoptosis at higher levels (11, 12). Because of its ability to repress AR transcription, elevated levels of E2F-1 may contribute to the progression of hormone-refractory prostate cancer (13). Many human cancers involve pRB mutations that result in E2F-1 activation (14). Disruption of the pRb-E2F1 complex may predispose the prostatic epithelium to hormonal carcinogenesis (15) and lead to the development of hormone-refractory prostate tumors (16). Therefore, targeting E2F-1 could be a viable and effective means of treating prostate cancer. Furthermore, because prostate cancer is predominantly a tumor of older men, who frequently have coexisting medical illnesses that usually limit treatment options, and because androgen deprivation therapy can cause osteoporosis and bone fractures (17), it is important to focus on agents that lack the toxic side effects of currently used therapies.

Herbal remedies have been used for thousands of years with very minimal side effects and clearly merit extended research for their ability to selectively kill prostate cancer cells. Several herbal products have recently been incorporated into cancer research (18–21), among them is Nigella sativa whose seeds have been used medicinally for centuries in a variety of diseases (22). Extracts prepared from N. sativa seeds, called black seeds, reportedly exhibit significant in vitro and in vivo antineoplastic activity (22–24). Chemotherapeutic and chemopreventive effects of black seed extracts are attributed to quinines, mainly thymoquinone, which is present in the volatile oil of the seed (25) and reportedly induces antitumor effects in a variety of cancer cell lines (26, 27). However, despite knowledge of these potential antineoplastic effects, the molecular pathways involved are not clear, and the potential
benefits of thymoquinone for treatment and/or prevention of prostate cancer have received very little attention (28).

We investigated the molecular events involved in the anti-neoplastic effects of thymoquinone in prostate cancer cells. We observed a dose-dependent increase in the inhibitory effect of thymoquinone on DNA synthesis, proliferation, and viability of cancerous (LNCaP, C4-2B, PC-3, and DU145) but not noncancerous prostate (BPH-1) epithelial cells. In AR-positive LNCaP and C4-2B cells, this growth suppression was associated with a noticeable decrease in AR and E2F-1 and an increase in proapoptotic proteins. Furthermore, thymoquinone inhibited the growth of tumors derived from androgen-independent C4-2B prostate cancer cells in nude mice, and, as in cultured cells, this effect was associated with a dramatic loss of AR and E2F-1 expression and induction of apoptosis. Thus, we believe that thymoquinone may prove to be effective in treating prostate cancer, particularly in hormone-refractory cases.

Materials and Methods

Cell culture. LNCaP, DU145, and PC-3 cells were purchased from American Type Culture Collection. C4-2B cells were generously provided by Dr. Fazul H. Sarkar (Wayne State University School of Medicine, Detroit, MI) and BPH-1 cells by Dr. Simon W. Hayward (Vanderbilt University Medical Center, Nashville, TN). LNCaP, C4-2B, and BPH-1 cells in RPMI medium (Life Technologies, Inc.) and DU145 and PC-3 cells in DMEM were supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 units/ml penicillin, and grown in a humidified incubator with 5% CO2 and 95% air at 37°C. The medium for LNCaP and C4-2B cells also contained 10 mM/L testosterone and 10 mM/L HEPES, respectively.

Cell synchronization. LNCaP cells in early passages (passages 3–12) were synchronized by isoleucine deprivation as described before (29). Synchronized G2–G1 phase cells were released from isoleucine blockade in the absence or presence of 50 μM/L thymoquinone. The ability of cells to enter S phase was determined at regular intervals following release from isoleucine blockade by pulse labeling the cells with 2 μCi/ml [3H]thymidine (ICN Biomedicals) for 30 min at 37°C in a humidified incubator and measuring the radioactivity incorporated into DNA as described before (29).

Cell proliferation and viability assays. LNCaP, DU145, PC-3, and BPH-1 cells were seeded at a density of 5,000 per well in 96-well plates in appropriate medium, allowed to grow for 24 to 48 h, and then treated with increasing concentrations of thymoquinone (40–100 μM/L; Sigma-Aldrich). Colorimetric 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was used to detect live cells in each well as well as manufacturer’s instructions. This involved adding 20 μl/well CellTiter96 AQ One Solution (Promega) and incubating the cells for 1 h in a humidified incubator with 5% CO2 and 95% air at 37°C. Absorbance at 450 nm was recorded using a μQuant microplate spectrophotometer equipped with KCJunior Data Analysis Software (Bio-Tek Instruments). The effect of thymoquinone on DNA synthesis was measured by [3H]thymidine incorporation into DNA. For this, LNCaP cells were treated with increasing concentrations of thymoquinone for 48 h as described above and pulse labeled with [3H]thymidine (2 μCi/ml) for 30 min, measuring the radioactivity incorporated into DNA as described before (29).

Preparation of cell and tissue extracts. Exponentially growing or synchronized cancerous cells in 150-mm cell culture dishes were collected by trypsinization. They were first washed with and then suspended in buffer A (50 mM/L Tris-HCl (pH 7.4), 0.1% Triton X-100, 5 mM/L EDTA, 250 mM/L NaCl, 50 mM/L NaN3, and 0.1 mM/L Na2VO4) supplemented with protease inhibitor cocktail (P-8340, Sigma Chemical) at a density of 2 × 107 cells/ml. Cells were freeze-thawed and then thrice passed in and out of a syringe with a 27-gauge needle (1.25 in.). For tissue extracts, tumors were dissected in PBS to remove stromal contamination, cut into small pieces (1 mm3) using a scalpel, washed, and finally suspended in buffer A containing the same protease inhibitor cocktail. The tissue suspension was homogenized in a top-driven Wheaton Overhead Stirrer (Wheaton Instruments) until the tissue was completely dispersed (usually six strokes at a speed setting of 3). Soluble cell and tissue extracts were cleared by centrifugation at 3,800 rpm for 15 min in a Sorvall RT7 centrifuge equipped with an RTH-750 rotor at 4°C. Protein concentration in soluble extracts was assessed using Bio-Rad Protein Assay reagent (Bio-Rad Laboratories).

Western blot. Equal amounts of protein in individual fractions were subjected to denaturing 10% or 12% SDS-PAGE and then transferred to nitrocellulose membranes. Individual membranes were probed with rabbit polyclonal antibodies against AR, E2F-1, p21Waf1, and cyclin A (Santa Cruz Biotechnology); mouse monoclonal antibodies against p27Kip1, cyclin-dependent kinase (Cdk)-2, Cdk-4, Bax, and pRB (Transduction Labs, BD Biosciences); or goat polyclonal antibodies against β-actin (Santa Cruz Biotechnology). Immunoreactive bands were developed using horseradish peroxidase–conjugated secondary antibodies (Pierce) and SuperSignal WestPico chemiluminescent substrate (Pierce) and visualized using X-ray film.

Human prostate tumor xenograft mouse model. Five-week-old male athymic nude mice (NCR Nu-M) were purchased from Taconic Research Animal Services and housed under pathogen-free conditions according to Wayne State University animal care guidelines. The animal protocols were reviewed and approved by the Institutional Laboratory Animal Care and Use Committee of Wayne State University. C4-2B cells (5 × 105) were injected s.c. into one flank. Tumor size was measured with calipers every other day. Tumor volume (V) was determined by the equation $V = \frac{1}{2} \times L \times W^2 \times 0.5$, where $L$ is the length and $W$ is the width of the tumor. When xenografts reached a volume of ~100 mm3, the tumor-bearing mice were randomly assigned to a control (solvent-treated) or thymoquinone-treated group and given either solvent (mixture of PBS/cremophor/ethanol, 5:3:2) or 20 mg/kg/d thymoquinone. When the control tumors reached ~1,100 mm3 (on day 31), the experiment was terminated and the mice were sacrificed. Part of each tumor was used to prepare the tissue extracts and the remainder was fixed and paraffin embedded.

H&E staining, E2F-1 immunohistochemistry, and terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling assay. Paraffin-embedded tissue sections were deparaffinized, hydrated, and stained with hematoxylin for 1 min. After rinsing, the slides were stained with eosin in 1 min, rinsed thoroughly, and mounted with Permount. For E2F-1 immunohistochemistry, after deparaffinization and hydration, the tissue sections were blocked with 3% hydrogen peroxide, incubated first with a primary antibody to E2F-1 (1:50; Santa Cruz Biotechnology) and then with a biotinylated secondary antibody or antimouse immunoglobulin G (H + L), incubated in ABC reagent (avidin-biotinylated horseradish peroxidase complex), mounted, and visualized under a microscope. Terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay was done using an Apoptag Peroxidase In-Situ Apoptosis Detection Kit from Chemicon as per manufacturer’s instructions. Briefly, after deparaffinization and hydration, the tissue sections were incubated with working solution terminal deoxyribonucleotidyl transferase enzyme and working solution stop/wash buffer, conjugated with anti-digoxigenin, and then stained with peroxidase substrate. The tissue sections were mounted under a glass coverslip in Permount and viewed under a microscope.

Results

Thymoquinone inhibits proliferation and viability of cancerous but not noncancerous prostate epithelial cells. Because thymoquinone is a product of an herb used both in cooking and medicinally in many parts of the world for centuries, we predicted it would have very little cytotoxicity. To test this, we studied the effect of thymoquinone on proliferation and viability of noncancerous and cancerous prostate epithelial cells. As shown in Fig. 1A, thymoquinone had very little effect on the morphology of
BPH-1 cells, which are considered noncancerous (30). However, under similar treatment conditions, thymoquinone caused significant rounding of prostate cancer LNCaP cells. On [3H]thymidine incorporation assay, thymoquinone inhibited DNA synthesis in AR-positive LNCaP as well as AR-negative DU145 and PC-3 prostate cancer cells, but not in BPH-1 cells (Fig. 1B). The inhibitory effect of thymoquinone on DNA synthesis in prostate cancer cells was both dose and time dependent, with cells treated with thymoquinone exhibiting an IC_{50} for [3H]thymidine incorporation of ~75 μmol/L after 48 h (Fig. 1B) as against a relatively lesser effect at 24 h and a greater effect at 72 h (data not shown).

Consistent with the inhibitory effect of thymoquinone on DNA synthesis, MTS assay showed a dose-dependent decrease in viable prostate cancer cells at 48 h (Fig. 1C). This decrease in viability was likewise observed in prostate cancer cells but not in BPH-1 cells. Thus, thymoquinone had a selective antiproliferative effect on cancerous prostate epithelial cells.

**Inhibitory effect of thymoquinone on proliferation and viability of prostate cancer cells is associated with suppression of AR and E2F-1.** To clarify the molecular events involved in the inhibitory effect of thymoquinone on proliferation and viability of prostate cancer cells, we examined quantitative changes in specific antiproliferative and proapoptotic proteins (including AR and E2F-1) in LNCaP and BPH-1 cells following thymoquinone treatment. We found a noticeable decrease in both AR and E2F-1 in LNCaP cells within 6 h after treatment with low doses of thymoquinone (Fig. 2A), associated with increased levels of the antiproliferative proteins p21\textsuperscript{Cip1} and p27\textsuperscript{Kip1} and the proapoptotic protein Bax. By comparison, in similarly treated BPH-1 cells that lacked AR, there was no change in either E2F-1 or its target proteins such as Cdk-2 (Fig. 2A), cyclin E, and cyclin A (data not shown) even at 24 h after treatment. Furthermore, thymoquinone had very little effect on the Cdk inhibitors p21\textsuperscript{Cip1} and p27\textsuperscript{Kip1} (Fig. 2A). These observations are consistent with the antiproliferative and proapoptotic effects of thymoquinone on LNCaP but not BPH-1 cells.

Because thymoquinone suppressed AR and E2F-1 in androgen-sensitive LNCaP cells, we examined whether similar effects could be seen in androgen-independent C4-2B cells. As shown in Fig. 2C, thymoquinone down-regulated both AR and E2F-1 in C4-2B cells, just as it did in LNCaP cells. Interestingly, whereas thymoquinone suppressed phospho-pRb (which results in E2F-1 activation) in LNCaP cells, it down-regulated both phospho-pRb and non-phospho-pRb in C4-2B cells. Thus, in both cell lines thymoquinone seemed to affect not only the level but also the activity of E2F-1. Decreased E2F-1 activity was also evident from down-regulation of one of its target proteins, cyclin A, which showed a substantial decrease in LNCaP and C4-2B cells treated with 25 μmol/L thymoquinone even when significant residual E2F-1 was present (Fig. 2B).

**Thymoquinone inhibits progression of prostate cancer cells from G\textsubscript{1} to S phase.** Because E2F-1 and AR play an important role in cell cycle progression from G\textsubscript{1} to S phase (29), we examined the effect of the thymoquinone-induced decrease in E2F-1 and AR on the ability of LNCaP cells to progress from G\textsubscript{0}–G\textsubscript{1} to S phase. We synchronized LNCaP cells by isoleucine deprivation in G\textsubscript{0}–G\textsubscript{1} as described before (29) and examined their ability to enter S phase after release from isoleucine blockade in the absence (control) and presence of thymoquinone. We used a concentration of thymoquinone (50 μmol/L) that was below the IC_{50} (75 μmol/L) required for inhibition of DNA synthesis (Fig. 1B) and decreased viability (Fig. 1C) of exponentially growing LNCaP cells. As shown in Fig. 3A,
we observed that whereas control cells released from isoleucine blockade could progress synchronously from G1 to S, thymoquinone-treated cells failed to enter S phase as determined by their inability to incorporate [3H]thymidine. Thus, down-regulation of AR and E2F-1 in thymoquinone-treated LNCaP cells seems to contribute to their failure to enter S phase. We further validated this possibility by measuring AR and E2F-1 in thymoquinone-treated G1 phase LNCaP cells progressing from G1 to S. AR levels are reported to fluctuate with the cell cycle (31) and increased AR in late G1 (8–12 h after release from isoleucine blockade) is required for synchronized LNCaP cells to enter S phase (32). As shown in Fig. 3B, this increase in AR in late G1 phase was noticeable in control but not in thymoquinone-treated LNCaP cells. In control cells, there was an increase in E2F-1 starting 12 h after release from isoleucine blockade (late-G1 phase), whereas in thymoquinone-treated cells this increase in E2F-1 was blunted. This dramatic decrease of E2F-1 in thymoquinone-treated cells as compared with control LNCaP cells was also reflected in down-regulation of several cell cycle regulatory proteins, including Cdk-4, Cdk-2, and cyclin A (Fig. 3B), all of which are regulated by E2F-1 (9). We also observed that Cdc6, an essential component of the prereplication complex whose expression is regulated by E2F-1 (33), was also attenuated in thymoquinone-treated LNCaP cells (data not shown). Thus, thymoquinone prevents cell growth by blocking expression of the AR, E2F-1, and E2F-1-target cell cycle regulatory proteins necessary for transition of prostate cancer cells from G1 to S phase.

Thymoquinone suppresses the growth of tumors derived from androgen-independent prostate cancer C4-2B cells in nude mice. Because thymoquinone inhibited both the growth and viability of prostate cancer cells under in vitro culture conditions, we examined whether these antiproliferative and proapoptotic effects of thymoquinone could be observed in vivo in nude mice that had prostate tumors growing from androgen-independent C4-2B cells implanted in flank. To test the effect of thymoquinone on tumor growth, thymoquinone (20 mg/kg) was administered s.c. on a daily basis starting from the day when C4-2B tumors reached ~100 mm³. As shown in Fig. 4A, thymoquinone...
administration suppressed growth of C4-2B tumors to half the size of those found in solvent-treated controls at 31 days after the start of treatment. Daily administration of thymoquinone had very little effect on average body weight [26.6 ± 2.86 g for thymoquinone-treated mice (n = 3) versus 27.3 ± 0.75 g for the controls (n = 3)]. Thus, thymoquinone showed no discernible side effects in mice at a dose that prevented growth of C4-2B tumors.

We next tested whether thymoquinone suppression of C4-2B growth in mice was associated with down-regulation of AR and E2F-1 in tumors, as in cultured cells. As shown in Fig. 4B, AR and E2F-1 could be readily detected in tissue extracts of tumors from solvent-treated controls but not thymoquinone-treated mice. There was also a dramatic decrease in pRb and cyclin A expression in C4-2B tumors of thymoquinone-treated mice compared with the controls. These changes in AR, E2F-1, pRb, and cyclin A in thymoquinone-treated C4-2B tumors in nude mice (Fig. 4B) are consistent with the observed changes in these proteins in cultured thymoquinone-treated cells (Fig. 2B). Immunohistochemical staining of tumor tissue sections also revealed a dramatic decrease in E2F-1 in tumors from thymoquinone-treated mice compared with the controls (Fig. 4C). There were fewer live tumor cells and increased infiltrating lymphocytes in tumors from thymoquinone-treated mice compared with the controls as determined by H&E staining of tumor sections. Furthermore, TUNEL staining of these tissue sections indicated massive apoptosis in tumors from thymoquinone-treated mice compared with the controls (Fig. 4C), consistent with the increase in Bax observed in thymoquinone-treated LNCaP cells (Fig. 2A). Taken together, these observations show that thymoquinone prevents growth of prostate cancer cells both in vitro and in vivo by suppressing the expression of AR and E2F-1.

Discussion

Our study shows that growth of prostate cancer cells is highly sensitive to the inhibitory effect of thymoquinone, a natural compound present in the seeds of the annual plant *N. sativa*. This inhibitory action is highly selective, exhibiting very little effect on growth of noncancerous prostate epithelial cells in culture and preventing growth of human prostate tumors in nude mice without causing any discernible side effects or changes in body weight. Our studies show for the first time that thymoquinone induces antiproliferative and proapoptotic effects in prostate cancer cells by down-regulating AR and E2F-1, which play an important role not only in the proliferation and survival of prostate cancer cells (6–8, 10, 11) but also in the development of hormone-refractory disease (13, 34) for which there is no cure. Based on our observation that thymoquinone inhibits proliferation and viability of cancerous but not noncancerous cells, we propose that this herbal agent be used not only for treatment but also for prevention of hormone-sensitive as well as hormone-refractory prostate cancer.

*N. sativa* has been used medicinally for thousands of years to treat a variety of diseases including cancer. Oil extracted from *N. sativa* seeds has been shown to have an antineoplastic effect both in vitro and in vivo (23, 24, 35). Thymoquinone, a major...
constituent in <i>N. sativa</i> seed oil, was reported to arrest papilloma cells, canine osteosarcoma cells (COS31), and human colon cancer cells (HCT-116) in the G<sub>1</sub> phase (26, 27, 36). In papilloma cells, such an arrest is associated with an increase in p16 and a decrease in cyclin D1, whereas in HCT-116 human colon cancer cells, it is associated with an increase in p21 and p53 (27) as well as a significant decrease in Bcl-2 (antiapoptotic) protein (26). In this study, we observed no significant change in Bcl-2 protein levels (data not shown); instead, there was a noticeable increase in Bax, a proapoptotic protein (Fig. 2B). Thymoquinone also caused G<sub>M</sub>-phase arrest in spindle carcinoma cells (27) associated with an increase in p53 and a decrease in cyclin B1. In p53-null myeloplastic leukemia HL-60 cells, thymoquinone induced apoptosis by activating caspase-3, caspase-8, and caspase-9 (37). Thymoquinone inhibited proliferation of canine osteosarcoma (COS31) and its cisplatin-resistant variant (COS31/rCDDP), human breast adenocarcinoma (MCF7), and human ovarian adenocarcinoma (BG-1) cells, but had very little effect on normal kidney cells (Madin-Darby canine kidney cells; ref. 36). Thus, we concluded that thymoquinone can offer a better treatment strategy in which thymoquinone exerts an antiproliferative effect on prostate cancer cells. AR is also necessary for viability of prostate cancer cells (8), and AR down-regulation can trigger proapoptotic events. Accordingly, thymoquinone treatment resulted in induction of p21<sup>Cip1</sup> and p27<sup>Kip1</sup>, which cause cell cycle arrest, and an increase in Bax, which promotes apoptosis (Fig. 2A). E2F-1 overexpression has been associated with AR down-regulation in hormone-resistant prostate cancer (13). We now observed that thymoquinone not only down-regulated E2F-1 but also suppressed AR. Thus, thymoquinone seems to induce antiproliferative and proapoptotic effects by suppressing expression of both E2F-1 and AR in prostate cancer cells.

In summary, natural agents are advantageous for application to humans because of their mild mechanism of action and their ability to spare normal cells. Thymoquinone, a component of the herb <i>N. sativa</i>, has gained attention in the last decade for its notable antiproliferative effect on a variety of cancer but not normal cell lines. This is the first study examining the effect of thymoquinone on cell cycle regulatory and proapoptotic proteins in synchronized prostate cancer cells progressing from G<sub>1</sub> to S phase. These studies show the effectiveness of thymoquinone in down-regulating AR and E2F-1 and inducing proapoptotic proteins such as p53, p21<sup>Cip1</sup>, p27<sup>Kip1</sup>, and Bax in androgen-sensitive prostate cancer cells. Together, these observations warrant clinical trials to examine the effectiveness of thymoquinone as an adjuvant therapy for androgen-sensitive and hormone-refractory prostate cancer.

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Androgen Receptor– and E2F-1–Targeted Thymoquinone Therapy for Hormone-Refractory Prostate Cancer


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