The Mutant Androgen Receptor T877A Mediates the Proliferative but Not the Cytotoxic Dose-Dependent Effects of Genistein and Quercetin on Human LNCaP Prostate Cancer Cells

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Received April 8, 2002; accepted July 31, 2002 This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

High consumption of soybean products, such as phytoestrogens, has been hypothesized to contribute to a reduced incidence of prostate cancer in Southeast Asian people, although there have been inconsistent results among studies. Human LNCaP cells, extensively used as a model for androgen-dependent prostate tumor, express the androgen receptor (AR) mutant T877A promiscuously transactivated by estrogens and other ligands, which may further facilitate cancer progression. Here, for the first time to our knowledge, we demonstrate that genistein and quercetin, two phytoestrogens abundantly present in soybeans, activate either the AR mutant T877A in LNCaP or in transfected Chinese hamster ovary cells. This observation is supported by their capability to induce AR accumulation in the nuclear compartment of LNCaP together with mRNA down-regulation of the androgen target genes AR and PAP, and PSA up-regulation. Of interest, at concentrations eliciting transcriptional activity, both genistein and quercetin stimulate LNCaP cell growth, whereas at high levels, they become cytotoxic independently of AR expression, as ascertained in steroid receptor-negative Hela cells. The results of our study provide evidence that phytoestrogens may regulate several signaling processes in LNCaP cells; however, further studies are needed to assess their potential capability to restrain prostate tumor progression.

Prostate cancer is the most common malignancy among men in the United States, where about 190,000 new cases were diagnosed in the last year and approximately 32,000 men died from the disease (Taplin and Ho, 2001). The incidence is much lower in the peoples of Southeast Asia compared with Americans but it substantially increases when low-risk populations migrate to the United States, thereby highlighting the role of dietary factors on tumor development (Lopez-Otin and Diamandis, 1998).

Epidemiological studies have hinted at the association between the intake of soy products and the reduction in prostate cancer risk; however, insufficient data are available to draw definitive conclusions about the protective effects of soy consumption against the tumor (Barnes, 2001 and references therein).

The phytoestrogens genistein and quercetin (Fig. 1), abundantly present in soybeans, vegetables, and fruits (Price and Fenwick, 1985), have recently received a great deal of attention because at high concentrations they are able to restrain the process of carcinogenesis in the hormone-dependent breast tumor (Adlercreutz, 1995; Kurzer and Xu, 1997; Griffiths et al., 1998; Zhou et al., 1999; Messina and Loprinzi, 2001 and references therein). However, these bioflavonoids exert estrogenic effects through direct binding and activation of the estrogen receptor (ER) α and β, influencing breast cancer cell proliferation in a dose-dependent fashion (Maggiolini et al., 2001, and references therein).

The androgen receptor (AR) wild-type and the AR mutant T877A expressed in human LNCaP prostate cancer cells are both transactivated by 17β-estradiol (E2) and other ligands, which may induce an androgen-like stimulation of prostate tumor cells (Elo et al., 1995; Tan et al., 1997; Yeh et al., 1998; Grigoryev et al., 2000). Interestingly, estrogen has a multitude of effects on prostate growth and differentiation (Small

Supported by grants from Ministero dell’Istruzione, dell’Università, e della Ricerca and Consiglio Nazionale delle Ricerche (L. 95/95), and from the Swiss National Science Foundation, the Krebsforschung Schweiz, and the Canton de Genève.

ABBREVIATIONS: ER, estrogen receptor; AR, androgen receptor; E2, 17β-estradiol; CHO, Chinese hamster ovary; PSA, prostate-specific antigen; DHT, 5α-dihydrotestosterone; ICI 182,780, fulvestrant; PCR, polymerase chain reaction; FCS, fetal calf serum; DMEM, Dulbecco’s modified Eagle’s medium; CS, charcoal-stripped; RT, reverse transcriptase.
and Prins, 1995) and the phytoestrogens bind to ERs α and β, but with higher affinity to the latter, which is predominantly expressed in prostate tissue and tumor cells (Chang and Prins, 1999). When considering the possible role of natural estrogenic compounds, such as genistein and quercetin, on the reduction of prostate cancer risk or progression, either the activity exerted by pharmacological doses or physiologically achievable levels from dietary intake should be taken into account.

In this work, we used as model systems the androgen-dependent LNCaP prostate tumor cells together with steroid receptor negative CHO and HeLa cells to provide new insight into the action of phytoestrogens on prostate malignancy. We ascertained the ability of genistein and quercetin: 1) to trans-activate the AR mutant T877A and 2) to induce its nuclear localization, 3) to modulate the mRNA of AR and other target genes such as prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP), 4) to up-regulate the AR protein levels, 5) to exert either growth stimulatory or antiproliferative effects over a wide concentration range that has been reported (Hargreaves et al., 1999; Strom et al., 1999) to be potentially supplied by normal processed food and soy-based products.

Materials and Methods

Reagents. 5α-Dihydrotestosterone (DHT), E2, genistein, and quercetin were purchased from Sigma (St. Louis, MO). Casodex and ICI 182,780 were gifts from Zeneca (Milan, Italy). All compounds were dissolved in ethanol except genistein and quercetin, which were solubilized in dimethyl sulfoxide.

Plasmids. Firefly luciferase reporter plasmids used were XG46TL and XETL for the AR and the ER, respectively (Bunone et al., 1996). The Renilla reniformis luciferase expression vector pRL-CMV (Promega, Madison, WI) was used as a transfection standard. The AR mutant T877A (a gift from F. S. French, Department of Pediatrics, University of North Carolina Medical School, Chapel Hill, NC) was constructed by two-step PCR method replacing the HindIII/BamHI fragment from the wild-type in pCMVhAR (Tan et al., 1997).

Cell Culture. Human prostate cancer LNCaP cells (a gift from R. Baserga, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA) were grown in RPMI 1640 medium without phenol red supplemented with l-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 U/ml), and 10% FCS. CHO and HeLa cells were maintained in DMEM without phenol red supplemented with l-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 U/ml), and 10% FCS. Cells to be processed for immunocytochemical staining, RT-PCR, or immunoblot were switched to medium supplemented with 1% charcoal stripped (CS)-FCS four days before treatments.

Transfections and Luciferase Assays. Cells were transferred into 24-well plates with 500 μl of regular growth medium per well on the day before transfection. The medium was replaced with RPMI 1640 medium or DMEM lacking phenol red as well as serum on the day of transfection, which was performed using the Fugene6 Reagent as recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany) with a mixture containing 0.5 μg of reporter plasmid, 5 ng of pRL-CMV, and 0.5 μg of mutant AR T877A plasmid, where applicable. After 8 to 9 h, the medium was replaced again with RPMI 1640 medium or DMEM lacking phenol red supplemented with 0.5% CS-FCS; ligands were added at this point, and cells were incubated for 48 h. Luciferase activity was then measured with the Dual Luciferase Kit (Promega, Milan, Italy) according to the manufacturer’s recommendations. Firefly luciferase activity was normalized to the internal transfection control provided by the Renilla reniformis luciferase activity.

Immunocytochemical Staining. Cultured LNCaP cells were fixed in fresh paraformaldehyde (2% for 30 min). After paraformaldehyde removal, hydrogen peroxide (3% in methanol for 30 min) was used to inhibit endogenous peroxidase activity. Cells were then incubated with normal horse serum (10% for 30 min) to block the nonspecific binding sites. Immunocytochemical staining was performed using as the primary antibody a mouse monoclonal IgG (Santa Cruz Biotechnology, Santa Cruz, CA) against human androgen receptor (1:50 overnight at 4°C). A biotinylated horse-anti-mouse IgG (1:600 for 60 min. at room temperature) was applied as the secondary antibody (Vector Laboratories, Burlingame, CA). Subsequently, the amplification of avidin-biotin-horseradish peroxidase complex was carried out (avidin-biotinylated enzyme complex/horseradish peroxidase, 1:100 for 30 min. at room temperature; Vector Laboratories) and the 3-3-diaminobenzidine tetra-chloride dihydroxide (Vector Laboratories) was used as a detection system. Cells were rinsed after each step with Tris-buffered saline (0.05 M Tris-HCl plus 0.15 M NaCl, pH 7.6) containing 0.05% Triton X-100. In control experiments, cells were processed replacing the primary antibody by mouse serum (Dako S.P.A., Milan, Italy) or using a primary antibody preabsorbed (48 h at 4°C) with an excess of purified AR protein (M-Medical, Florence, Italy).

RT-PCR. The evaluation of gene expression was performed by semiquantitative RT-PCR as described previously (Maggiolini et al., 1999b). For AR, PSA, PAP, and the internal control gene 36B4, the primers were: AR: forward, 5’-TGCCATTTAGACTATTACCTTTCC-3’; reverse, 5’-TGCCACACAGACTACACCC-3’; PSA: forward, 5’-GAGGTCACACACTGAAGTT-3’; reverse, 5’-CCTCCGGAGAAGATGATT-3’; PAP: forward, 5’-CCTGGATCCCGGAGAGGCTGTAACCGCTCC-3’; reverse, 5’-CGGGATCCCGCTAATCTGTACTGTC-3’; and 36B4: forward, 5’-TCGATTCCT-3’; reverse, 5’-CGGGATCCCGCTAATCTGTACTGTC-3’; to yield products of 407, 214, 1100, and 408 base pairs, respectively, with 20, 15, 20, and 15 PCR cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C.

Immunoblotting. LNCaP cells were grown in 10-cm dishes and exposed to ligands for 24 h before lysis in 500 μl of 50 mM HEPEs, pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, 10% glycerol, 1% Triton X-100, a mixture of protease inhibitors (Aprotinin, phenylmethylsulfonyl fluoride), and Na-orthovanadate. Equal amounts of total protein were resolved on a 10% SDS-polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane, probed with the antibodies against human AR and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA), and revealed using the ECL System (Amer sham Bioscience, Piscataway, NJ).
**ATP Bioluminescence Assay.** To evaluate cell proliferation and cytotoxicity by ATP bioluminescence (Crouch et al., 1993) we used the ATP Bioluminescence Assay Kit as recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany). LNCaP and HeLa cells (1 x 10^4) were seeded in 24-well plates in regular growth medium. Cells were washed extensively once they had attached and further incubated in medium without serum for 24 h. On the second day, the LNCaP medium was changed and supplemented with 1% CS-FCS, whereas the growth medium of HeLa cells was supplemented with 5% CS-FCS. Ligands diluted in increasing concentrations were added at this point using the same volume of solvent (0.2%) in either control or treated cells. Thereafter, the medium was changed every day and treatments were renewed following the procedure mentioned above. On day 6, cells were lysed for ATP bioluminescence detection by Berthold luminometer.

**Results**

**Genistein and Quercetin Activate the AR Mutant T877A Expressed in LNCaP Cells.** The human prostate cancer cell line LNCaP, which expresses the mutant AR T877A (Hoszowszic et al., 1983; Trapman et al., 1990), has been extensively used as a model of androgen-dependent prostate cancer (Webber et al., 1997). Such mutation influences the AR ligand-binding specificity (Veldscholte et al., 1990; Sack et al., 2001) enabling the receptor transactivation by steroids and antiandrogens (Elo et al., 1995; Kemppainen and Wilson, 1996; Tan et al., 1997; Yeh et al., 1998; Grigoryev et al., 2000).

In the same vein, we aimed to assess whether an AR reporter gene transiently transfected in LNCaP is able to respond to genistein and quercetin. The reporter plasmid XG46TL carries firefly luciferase sequences under the control of an androgen response element upstream of the thymidine kinase promoter. As an internal transfection control, we co-transfected a plasmid that expresses *R. reniformis* luciferase, which is enzymatically distinguishable from firefly luciferase, from the strong cytomegalovirus enhancer/promoter. Luciferase activity of cells receiving vehicle was set as 1-fold induction, upon which the results of treatments were calculated.

Figure 2A shows that both phytoestrogens transactivate the endogenous AR in LNCaP cells, increasing the transcriptional potential with a higher receptor expression (Fig. 2, A and B). Next, genistein and quercetin elicit agonistic activity exclusively through the AR mutant T877A as assessed in steroid receptor negative CHO cells (Fig. 2C).

To verify whether ERβ expressed in LNCaP (Chang and Prins, 1999) could also be activated by phytoestrogens, which have up to 10-fold higher affinity for this ER isoform compared with ERα (Kuiper et al., 1997), we transfected cells with the ER reporter gene XETL. Treatments were not capable of inducing transcriptional activity either at a concentration of 10 nM (Fig. 2A) or up to 10 μM (data not shown).

**Genistein and Quercetin Induce Nuclear Localization of AR in LNCaP Cells.** It has been reported that AR, upon ligand activation, undergoes conformational changes leading to homodimerization, nuclear translocation and target gene regulation (Jenster et al., 1991; Simental et al., 1991). To provide further evidence that phytoestrogens are able to transactivate the mutated AR expressed in LNCaP cells, we attempted to evaluate its nuclear accumulation after 1 h of exposure to phytoestrogens.

In this endeavor, we set basal experimental conditions to provide no detectable AR immunoreactivity (Fig. 3A). Notably, the addition of DHT, E2, genistein, and quercetin produced in all cells a strong staining intensity exclusively in the nuclear compartment, as demonstrated by the representative immunoreactions in Fig. 3, B-E. In contrast, no signals were observed either by replacing the anti-AR antibody by irrelevant rabbit IgG (Fig. 3, insets) or by using the primary antibody preabsorbed with an excess of receptor protein (data not shown). Thus, the ability of the natural compounds genistein and quercetin to transactivate AR was clearly confirmed by its nuclear accumulation after treating LNCaP cells with physiological achievable concentrations (10 nM) of these phytoestrogens.

**Genistein and Quercetin Modulate the mRNA of AR, PSA, and PAP.** AR is a ligand-dependent transcription factor that belongs to nuclear hormone receptor superfamily (Zhou et al., 1994). AR regulates gene expression through interaction with DNA sequences termed androgen response elements, which are located within the regulatory regions of such target genes as PSA and PAP (Riegman et al., 1991; Lin et al., 1992).

In tumor cell lines such as LNCaP, androgens down-regulate AR mRNA at the transcription level, as demonstrated in different model systems for other steroid hormone receptors (Wolf et al., 1993, and references therein). Thus, the receptor mediated autologous down-regulation of AR mRNA represents an additional hallmark of AR activation by an agonist. Moreover, the androgen responsiveness of PSA and PAP further confirm the functional activity of AR in prostate cancer cells (Young et al., 1991; Lin et al., 1998). Taken together, the aforementioned findings prompted us to investigate whether in LNCaP cells the expression of AR, PSA, and PAP are also sensitive to phytoestrogens. The mRNA levels were compared by semiquantitative RT-PCR and standardized using the mRNA levels of the house-keeping gene 36B4 (Fig. 4). Similarly to DHT, a 24-h exposure to E2, genistein, and quercetin down-regulated the mRNA of AR as well as PAP and up-regulated the expression of PSA with the following rank order of efficacy: DHT > E2 > genistein > quercetin.

**Genistein and Quercetin Up-Regulate AR Protein Expression.** Having determined that E2 and phytoestrogens repress AR mRNA mimicking DHT action, we next examined the effects of the same treatments on AR protein expression, which has been reported either increased or not affected by androgens (Yeap et al., 1999, and references therein).

Our results confirmed and extended to E2 and phytoestrogens (Fig. 5) the complicated scenario emerging from studies in LNCaP cells, where the androgen-dependent negative modulation of AR mRNA is associated with an up-regulation of AR protein levels (Krongard et al., 1991; Yeap et al., 1999).

A large body of evidence supports the notion that reduced protein content of different steroid receptors parallels decreased mRNA levels induced by agonists (Maggiolini et al., 2001, and references therein). On the contrary, upon all treatments AR protein expression showed a divergent pattern respect to mRNA in LNCaP cells, even though the AR protein increase occurred to a lesser extent with genistein and quercetin (Fig. 5).
Fig. 2. Genistein and quercetin activate the AR mutant T877A in LNCaP and CHO cells. A, transfections were performed with the indicated plasmids to evaluate the response of the endogenous and overexpressed AR mutant T877A or endogenous ERβ to 10 nM of ligands. Luciferase activity of cells receiving vehicle alone (−) was set as 1-fold induction, upon which treatments were calculated. B, protein expression of AR in LNCaP cells and in LNCaP transfected with the AR mutant T877A. β-actin serves as loading control. C, transfections were performed and ligands added as in A. Data in A and C are from the same experiment and each data point represents the mean of triplicate samples of a representative experiment.
Both Genistein and Quercetin Display a Biphasic Effect on Proliferation of LNCaP cells. Given the efficacy of phytoestrogens to either transactivate the mutated AR expressed in LNCaP cells or modulate the expression of AR target genes, we attempted to analyze a more complex physiological response like cell proliferation. LNCaP cells were

![Image of Genistein and Quercetin inducing nuclear compartmentalization of AR in LNCaP cells](image)

**Fig. 3.** Genistein and quercetin induce nuclear compartmentalization of AR in LNCaP. Cells were incubated in serum-free medium and treated 1 h with vehicle (A) or 10 nM of DHT (B), E2 (C), genistein (D), and quercetin (E). No immunodetection was observed replacing the anti-AR antibody with an irrelevant rabbit IgG (insets). Each experiment is representative of at least 10 tests. Bar, 5 μM.

![Image of mRNA expression in LNCaP cells](image)

**A**

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![Image of quantification of mRNA expression](image)

**B**

![Bar graph of optical density](image)

**Fig. 4.** Genistein and quercetin modulate AR, PSA, and PAP mRNA expression. A, LNCaP cells were stimulated for 24 h with vehicle or with 10 nM of ligands to evaluate by semi-quantitative RT-PCR the mRNA expression of AR, PSA, and PAP. The level of 36B4 mRNA was determined as a control. B, quantitative representation of data of two independent experiments including that of A after densitometry and correction for 36B4 expression.
treated for 5 days with the different compounds and the next day lysed to measure ATP bioluminescence as a marker of cell proliferation and cytotoxicity (Crouch et al., 1993). The results reported in Fig. 6A are expressed as percentage of ATP production in cells upon treatments with respect to those receiving vehicle alone.

DHT showed a bell-shaped dose-response curve with maximal luciferase response (>250% of untreated control) at a concentration of 0.1 nM (Fig. 6A). Higher doses resulted in a progressive ATP reduction that was similar to control cells from 100 nM to 100 μM. E2 enhanced the signal until 10 nM (>200% of untreated control), thereafter mirroring the DHT drop. Surprisingly, both genistein and quercetin displayed a biphasic effect increasing the bioluminescence up to 100 nM (about 250% of untreated control), but promoting subsequently its severe reduction as a consequence of massive cell death (Fig. 6A). The luciferase signal obtained upon 10 nM of each treatment was severely reduced by 1 μM of the antiandrogen casodex and to a lesser extent by the same amount of the antiestrogen ICI (Fig. 6C), suggesting that the proliferative effects are mainly mediated by an AR-dependent mechanism, although we cannot rule out a functional interaction between ERβ and transductional pathways involved in cell proliferation. Steroid receptor negative HeLa cells exhibited only the inhibitory effects of high concentrations of phytoestrogens (fig. 6B), as similarly observed in ERβ-positive but hormone-insensitive DU145 prostate tumor cells (data not shown).

Our data demonstrate for the first time to our knowledge that genistein and quercetin at low concentrations induce the proliferation of LNCaP cells acting as agonists for the mutant AR T877A. However, at high levels they may result in severe cytotoxic effects independently of steroid receptor expression.

Recent epidemiological, genetic, and biochemical findings support the view that hormone-dependent tumors, such as prostate and breast, exhibit many similar features (Lopez-Otin and Diamandis, 1998, and references therein). Here, we unravel another parallel between two cell lines of these malignancies, presenting evidence for a common sensitivity to dietary factors such as phytoestrogens. Thus, the present study extends to LNCaP prostate cancer cells our previous work (Maggiolini et al., 2001), in which a biphasic action of genistein and quercetin was also observed on viability of MCF7 breast cancer cells in relation to levels of exposure.

The ability of the T877A mutant, unlike wild-type AR, to respond to different ligands has largely been reported (Yeh et al., 1998, and references therein) and substantiated by crystallographic structures of AR ligand-binding domains complexed with the natural agonist DHT (Sack et al., 2001). In this study Sack et al. (2001) interestingly demonstrated that the replacement of threonine 877 by alanine leaves additional space off of the 17-position of DHT to accommodate a larger substituent on position 17, providing an explanation for the promiscuity of this AR mutant to bind a variety of compounds, which includes some antagonists.

Genistein and quercetin may now be considered agonists for the AR mutant T877A, because they are able 1) to activate the receptor inducing its nuclear accumulation in LNCaP cells, 2) to autoregulate AR mRNA and protein levels, 3) to modulate the expression of androgen target genes such as PSA and PAP, and 4) to induce LNCaP cell proliferation as a counterpart of the aforementioned action.

In transfection assays, both genistein and quercetin substantially activated the endogenous AR in LNCaP cells, acquiring a further efficacy in presence of a higher receptor expression. The latter finding interestingly confirmed that the transcriptional potential of ligands may also include levels of receptor content, according to our previous observation (Maggiolini et al., 1999a) in a variant of MCF-7 breast cancer cell line overexpressing ERα (Kalkhoven et al., 1996). It is well known that the ligand-dependent nuclear import of steroid receptors is a complex phenomenon consequent to the transactivational activity of different modulators (Tyagi et al., 2000; Avancés et al., 2001; Tomura et al., 2001). Both genistein and quercetin were capable of inducing a complete nuclear compartmentalization of AR similarly to DHT and E2, further sustaining a direct effect on AR signaling processes.

It has been reported (Yeap et al., 1999, and references therein) that in LNCaP cells the transactivation of AR by androgens promotes a reduced transcription rate of its own gene, whereas increased levels of AR protein occur as a consequence of ligand-receptor complex stabilization. Low amounts of phytoestrogens (10 nM) were able to follow this complex pattern consistent with a divergent behavior of AR mRNA and protein as we more impressively observed with DHT and E2. A recent study (Xing et al., 2001) suggested that quercetin inhibits both expression and function of AR in
Fig. 6. Dose-dependent biphasic effects of genistein and quercetin on proliferation of LNCaP cells. A, phytoestrogens increase LNCaP cell viability in the nanomolar range but became cytotoxic at micromolar concentrations even in HeLa cells (B). Low numbers of cells were seeded in 24-multiwell plates, treated with increasing levels of ligands (logarithmic scale) and lysed on day 6. The ATP bioluminescence production is expressed as percentage of cells upon treatments with respect to those treated with vehicle alone. C, proliferation of LNCaP was assayed as above except that cells were treated with 10 nM of ligands plus 1 μM of the antiandrogen casodex or the antiestrogen ICI 182,780. The antihormones did not modify cell viability when used alone (data not shown). Each data point is the average of several independent experiments.
LNCAp cells, but this conclusion was dictated by the use of pharmacological doses that in our proliferation assays interfere with cell viability. Moreover, the aforementioned functional outcome was also obtained with two other androgen-responsive genes named PSA and PAP (Rieger et al., 1991; Lin et al., 1992). Notably, we confirmed that the mRNA expression of such important prostate biomarkers in LNCAp cells follows an inverse pattern upon DHT and E2 (Henttu et al., 1992), extending for the first time a comparable responsiveness to phytoestrogens.

Interestingly, the agonistic activity of genistein and quercetin for AR was recapitulated in a more complex biological system, such as cell proliferation. The DHT exposure overlapped the bell-shaped growth response curve reported previously (Lee et al., 1995), whereas both phytoestrogens displayed a biphasic action. In fact, they increased the ATP bioluminescence production in a concentration range able to elicit transcriptional activity, resulting thereafter in a sharp reduction of luciferase signals consequent to marked cytotoxicity (Kumi-Diaka et al., 2000). Because the steroid-receptor negative HEla cells and ERp positive DU145 prostate cancer cells were also killed by the highest levels of phytoestrogens, nonspecific inhibitory effects of such compounds may be responsible (see below). The pure androgen antagonist casodex substantially blocked the proliferative effects of genistein and quercetin, confirming that an AR-mediated mechanism plays a primary role on cell growth. The E2 antagonist ICI was also effective albeit to a lesser extent, reminiscent of previous data (Migliaccio et al., 2000) on the ability shared by phytoestrogens and phytoecdysteroids to inhibit the growth of several tumor cell lines. Further investigations are required to demonstrate whether their addition to the diet of men or high consumption through habitual intake may reduce prostate cancer risk or progression.

Acknowledgments

We are grateful to Dr. V. Izzo and P. Ciarelli for technical support.

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Androgen Receptor, Phytoestrogens, and Prostate Cancer

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