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Four Stages of Prostate Cancer: Suppression and Eradication by Androgen and Green Tea Epigallocatechin Gallate

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Introduction

We have shown that prostate cancer (PCA) cells can exist in four stages of progression in culture media or in experimental animals based on their androgen (A) dependency or sensitivity. This is in contrast with the common characterization of prostate tumors as occurring in two forms, simply based on whether the tumors are A-dependent or A-independent. Recognition of four different forms of PCA strongly suggests that the current design of hormonal therapy by anti-androgenic agents requires revision; otherwise the practice may be very harmful to patients. In this chapter, we summarize our effort in understanding the four forms of PCA cells and show that their growth or proliferation can be suppressed or eradicated in culture or in athymic mice by selectively utilizing anti-androgen, A, or green tea (-)epigallocatechin-3-gallate (EGCG).

Androgens and Green Tea Catechins are Two Groups of Ancient Medicines

As and green tea are ancient medicines that have been in use for many thousands years. More than 2,000 years ago, in China, androgenic crystals were prepared from urine and organs by sublimation and used to treat individuals lacking 'maleness activity'. In oriental culture, tea beverage was used for prevention and treatment of many diseases for over 3,000 years, although scientific and medical evaluation of tea started only very recently (1).

In this chapter, we summarize our effort in establishing models of human PCA progression in culture and in athymic mice to understand how the process of the PCA progression may occur in patients. We have used these model systems to explore novel methods for control and eradication of PCA by As and the green tea catechin, epigallocatechingallate (EGCG).

Control of Androgen Action and Medicinal Applications

In the early 1960s, we found that As can rapidly enhance RNA synthesis in target organs, such as the ventral prostate of rats, suggesting that As act by modulating gene expression in target cell nuclei (2-4). Subsequently, we (5, 6) and Bruchovsky and Wilson (7) showed that, in many target organs, testosterone, the major A produced by testis and circulating in blood, is converted by 5α-reductase to 5α-dihydrotestosterone (5α-DHT). 5α-DHT is the active A that binds to a specific nuclear androgen receptor (AR) (8-12). The 5α-DHT-AR complex, apparently in conjunction with other chromosomal proteins (13), then regulates specific transcription of genes and production of specific proteins that modulate cellular activities and organ functions. Cloning and sequence determination of the genes for AR (10, 11) and 5α-reductase (14) have shown that mutations of these genes are responsible for A-insensitivity syndromes, including pseudo-hermaphroditism.

The molecular steps required for A action in target cells provide two effective methods for control of testosterone-regulated responses: (a) the use of a 5α-reductase inhibitor to suppress 5α-DHT production, and (b) the use of anti-androgens to block the interaction of 5α-DHT with AR (12). Both methods are now being utilized as therapies for A-related disorders including PCA, prostate enlargement, as well as male pattern baldness. As will be described below, both 5α-reductase inhibitors and anti-androgens are useful in the study of PCA progression and treatment.

5α-Reductase Inhibitors

Since testosterone activation is dependent on 5α-reductase, synthetic inhibitors of the reductase have been prepared by pharmaceutical companies. The synthetic 4-aza-steroid, finasteride, is now prescribed as Proscar for benign prostate hyperplasia (BPH), and as Propecia for male pattern baldness.

Many natural compounds that inhibit 5α-reductase have been described. In 1992 (15), we demonstrated that γ-linolenic acid [C18:3 (cis-9,12,15)] (GLA) (Figure 1), an essential fatty acid in many plant oils was an inhibitor of 5α-reductase. We also found that EGCG (Figure 1) was a 5α-reductase inhibitor (16, 17). Other gallated catechins are also active, but non-gallated catechins are inactive. The gallate group is important for the inhibitory activity. However, gallic acid and the methyl ester of gallic acid are inactive. Curcumin and alizarin (Figure 1) are also 5α-reductase inhibitors (17). The biological activity of these inhibitors has been tested *in-vivo* using flank organs of male hamsters as an animal model. Topical application of these inhibitors suppressed testosterone-dependent growth of the flank organ (18, 19).

Two isozymes of 5α-reductase have been identified (14). The specific roles of the individual isozymes are not well understood. Finasteride is a selective inhibitor of the type 2 isozyme of 5α-reductase whereas GLA, curcumin, alizarin, can inhibit both the type 1 and type 2 isozymes. EGCG was a better inhibitor of the type 1 isozyme than of type 2. The biomedical significance of this difference is unclear. While oral Proscar has been shown to be effective in treating BPH, the effectiveness of natural inhibitors for benign or cancerous prostate growth has not been demonstrated.

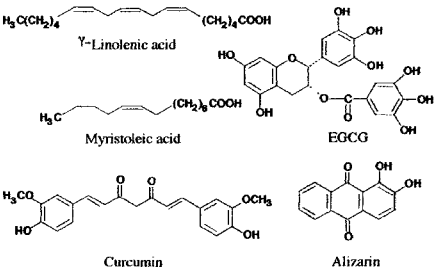

Figure 1. Structures of natural inhibitors of 5α-reductases.

Establishment of Models for Studying Prostate Cancer Cell Progression

Because PCA is initially dependent on As for its growth, hormonal therapy (A deprivation), pioneered by Charles Huggins 60 years ago (20) using castration or more recently anti-androgens, has been a standard PCA therapy. Although over 70% of patients may benefit from this therapy, PCA recurs in most of these patients in one to three years as tumors that do not need A for growth (A-independent). For lack of effective therapy, patients die from this A-independent PCA.

We believe that a better understanding of the process of PCA cell progression is very important for establishing better methods for PCA treatment. To investigate PCA progression, we established a model system (Figure 2) using a clone derived from the human PCA cell line, LNCaP, whose growth was dependent on the presence of nanomolar concentrations of testosterone, 5α-DHT or 17β-hydroxy-17-methyl-estra-4,9,11-trien-3-one (R1881) (21-23). The original cancer cell (named LNCaP 104-S) population was cultured through weekly passages and after about 40-70 passages in A-depleted culture medium, these cells progressed to A-independent cancer cells that we named LNCaP 104-R1 cells. These cells can grow well in culture without A. After continuous culture of 104-R1 cells in A-depleted medium for 60-120 additional passages, these 104-R1 cells were transformed into faster-growing cells, named 104-R2 cells. This transition of A-dependent 104-S cells to A-independent 104-R1 and 104-R2 cells is accompanied by dramatically increased AR expression apparently without new mutations in the ligand binding domain of AR gene. Despite an increase in cellular levels of AR, we found that the growth of both 104-R1 and 104-R2 cells in culture are suppressed by physiological concentrations of A. In

the presence of a high A concentration (>20 nM), some 104-R1, but not 104-R2 cells, can revert back to A-dependent cells (104-R1Ad) that behave like 104-S cells.

Recently, we found an alternative method to generate A-repressed cells from 104-S cells by using the anti-androgen, Casodex (bicalutamide), to suppress the growth of 104-S cells and isolate cells that can grow in the presence of anti-androgen. These cells, called CDXR cells, behave like 104-R2 cells and can not revert back to A-dependent cells. These A-independent cells also contain high levels of AR and their growth is suppressed by low A concentrations (<1 nM). When CDXR cells were cultured further in the presence of A, we were able to isolate A-insensitive cells, named IS cells. IS cells express very low levels of AR and are not stimulated or suppressed by A in the culture medium (Figure 2). These cells resemble human prostate PC-3 cells, since they do not have AR and can grow in the absence or presence of A.

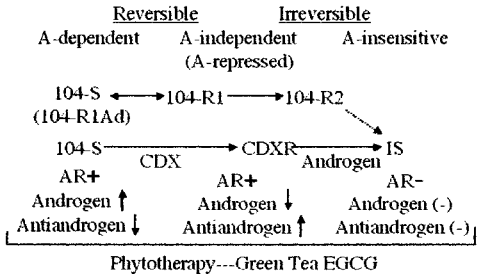

Figure 2. Four stages of PCA progression and treatment.

Growth Suppression of Prostate Cancer Cells in Culture by Androgen

The cellular level of AR mRNA was 2-3 fold higher in the LNCaP 104-R1 and 104-R2 cells than in LNCaP 104-S cells. AR protein level increased 10-20 fold during this transition from A-dependent 104-S cells to A-independent 104-R1 or 104-R2 cells. The growth of both 104-R1 and 104-R2 cells, as well as CDXR cells in culture was suppressed by physiological concentrations (<1 nM) of testosterone, 5α-DHT, or R-1881. Non-androgenic steroids, such as 5β-DHT, 17β-estradiol, medroxyprogesterone, and cortisol, did not suppress 104-R tumor growth (24). AR in 104-R (R1 and R2) cells was functional, since A induction of prostate-specific antigen (PSA) mRNA increased up to 20 times in these 104-R cells.

A suppression of 104-R cells is apparently due to a G1 arrest during cell cycling. In 104-R cells, R1881 at 0.1-1 nM repressed cell growth and induced the cyclin-dependent kinase (cdk) inhibitor, $p27^{kip1}$ (23). The same concentrations of R1881 that promote the growth of 104-S cells, reduced the cellular level of $p27^{kip1}$ in 104-S cells. CDXR cells also behave like 104-R cells; A increased $p27^{kip1}$ level in CDXR cells, and caused G1 arrest and suppress CDXR cell proliferation.

The effect of testosterone on c-*myc* gene expression correlated well with the proliferative activity of both 104-S and 104-R cells. R1881, at 0.1 nM, induced c-*myc* mRNA level in 104-S cells but repressed the mRNA level to less than 20% of the control value in 104-R cells. At high concentrations (~20 nM), R1881 inhibited both proliferation and c-*myc* expression in these cells. The retroviral overexpression of c-*myc* could block the A repression of LNCaP cells (21). A also suppresses c-*myc* in CDXR cells.

Androgen Specific Suppression and Eradication of Androgen-independent Prostate Tumors in Athymic Mice

A-dependent 104-S tumors grew very well in normal but not in castrated athymic mice. In contrast, A-independent LNCaP 104-R cells (24) or CDXR cells grew as tumors in castrated athymic mice but not in normal athymic male mice. Administration of testosterone, 5α-DHT, or R1881 to castrated mice prevented the growth of these A-independent tumors, and suppresses 104-R prostate tumors already present in these animals. As in cell culture, non-androgenic steroid hormones (progesterone, cortisol and 17β-estradiol) did not suppress the growth of these tumors in athymic mice. In many animals, testosterone caused regression of 104-R1, 104-R2, and CDXR tumors to less than 10% of the original tumor size within 30 days. In fact, in some mice, testosterone administration actually eradicated 104-R or CDXR tumors, and no tumor re-growth was observed thereafter for more than five mo. Testosterone did not affect the growth of A-insensitive human PCA cell PC-3 tumors or IS tumors in athymic mice.

Testosterone treatment of mice bearing 104-R tumors reduced c-*myc* mRNA in the tumors, but increased PSA mRNA in tumors and PSA level in serum before tumor regression (24). The 5α-reductase inhibitor, finasteride (Proscar) or anti-androgens, such as Casodex, blocked the repressive effect of testosterone on these xenografts and stimulated the tumor growth, suggesting that the growth suppression required conversion of testosterone to 5α-DHT and binding of 5α-DHT to AR (24). This observation suggested that if, in patients, testosterone can suppress PCA growth, the use of these drugs may enhance the growth of A-independent PCAs.

As in the cell culture system, A-independent 104-R1 cells can adapt to the presence of A and become A-dependent cells in normal mice (24). Adapted tumors behaved like A-dependent tumors. These cells in culture and tumors grown in mice not castrated can then be again controlled by anti-androgens or 5α-reductase inhibitors, such as Proscar. Adaptation to A was not observed with 104-R2 or CDXR tumors in mice.

EGCG Suppression of Prostate and Breast Tumors

Green tea consumption has been linked to lower incidence of some cancers in humans and animals. Epidemiological studies, however, have not provided consistent evidence about the anti-tumorigenic effect of green tea in humans.

For a better understanding of the ability of green tea to control different forms of prostate tumors, we produced tumors in athymic mice by subcutaneously inoculating athymic mice with AR positive and A-dependent LNCaP 104-S cells, AR positive LNCaP 104-R2, 104-R1 or CDXR cells whose growth is repressed by A, or AR negative PC-3 or IS cells whose growth is neither stimulated or repressed by A. We found that green tea EGCG (>98% pure, 1 mg/20g body weight daily), injected intraperitoneally (ip), significantly inhibited the growth and rapidly (in 1-2 week) reduced the size of all types of human prostate tumors in athymic mice. Structurally-related catechins, such as epicatechin gallate (ECG) that lacks only one of the eight hydroxyl groups in EGCG, were inactive. Epicatechin (EC) and epigallocatechin (EGC) were also inactive (25).

Since both A-dependent and A-independent prostate tumors respond to tumor suppression by EGCG. EGCG action was not related to modulation of A activity or due to 5α-reductase inhibition. In addition, the growth of human breast tumors in athymic mice produced by human breast cancer MCF-7 cells, were also clearly inhibited by ip injection of EGCG.

It is possible that the low clinical incidence of prostate and breast cancer in some Asian countries is, in part, related to high green tea consumption. The frequency of the latent, localized PCA does not vary significantly among geographically different populations, but the clinical incidence of metastatic PCAr varies considerably among countries (low in Japan and high in the USA). If consumption of green tea beverage is related to this difference, EGCG may play an important role in preventing the progression or metastasis of PCA cells.

EGCG Modulation of Food Intake and Endocrine Systems

The mechanism by which EGCG suppresses prostate tumor growth may be very complex. Many *in-vitro* effects of EGCG, including inhibition of cancer cell mobility, inhibition of key enzymes and protein factors, induction of apoptosis, and inhibition of angiogenesis, have been shown (1). It is very difficult to assess whether these *in-vitro* observations are related to *in-vivo* effects because EGCG and other catechins can interact non-specifically with enzymes or other macromolecules as well as cellular membranes.

We have studied the effects of EGCG on endocrine systems (1, 26, 27) We found that EGCG, given to rats by ip injection, could within one week reduce body weight by about 20%. Other structurally related catechins, such as EC, EGC, or ECG were not effective at the same dose. Reduction of body weight appeared

to be due to EGCG-induced reduction in food intake. EGCG, therefore, may influence neuropeptides and cause the loss of appetite.

After 7 days of daily ip treatment with EGCG, circulating levels of testosterone are reduced by about 75% in male rats and 17β-estradiol levels by 34% in female rats. The weights of A-sensitive organs, such as ventral prostate and seminal vesicles and estrogen-sensitive organs, such as the uterus and ovary were reduced by about 50%. Other catechins were not as effective as EGCG. We also found that the serum level of LH is reduced by 40-50%, suggesting that low LH production led to the reduced blood levels of sex hormones. In both male and female rats, we observed significant reduction in blood levels of leptin, IGF-I, and insulin (1, 26). Some of these peptide hormones may modulate the levels of sex hormone and indirectly alter tumor growth in the animals.

In male rats treated with EGCG for one week, the serum level of protein, fatty acids, and glycerol were not altered, but significant reductions in serum glucose (-32%), lipids (-15%), triglycerides (-46%) and cholesterol (-20%) were observed. Based on proximate composition analysis, there was no change in % water and protein content, a moderate decrease in carbohydrate content, but a very large reduction in fat content, decreasing from 4.1% in control to 1.4% in EGCG-treated group. EGCG treatment also decreases subcutaneous fat by 40 to 70%, and abdominal fat by 20 to 35% in male rats (1, 26). Reduction of body fat may also influence the hormonal levels in animals and influence tumor growth.

Although orally administered EGCG is not as effective as ip injected EGCG (26), probably due to poor intestinal EGCG absorption, long-term oral use of green tea beverage or EGCG-containing drinks may mimic the effects of ip injected EGCG. This was clearly shown by Gupta, *et. al.* (28) who used a transgenic adenocarcinoma of the mouse prostate (TRAMP) model that mimics progressive forms of human PCA. When these mice were orally infused with green tea polyphenols, in an amount equivalent to six cups of green tea per day in humans, PCA development was significantly inhibited and mice survival was increased. The green tea polyphenol infusion almost completely inhibited distant site metastasis.

Concluding Remarks

Our studies revealed that our current understanding of PCA progression is very inadequate, especially for designing clinical treatment of PCA after the initial hormonal therapy has failed and A-independent tumors reappeared. The four stages we have identified take a long period of time, over years, for progression, which resembles the PCA patient situation. Based on our findings, at the initial stage after recurrence of A-independent tumors (R1-form), As at physiological concentration can suppress the growth of R1-tumors or promote some of these cells to revert back to A-dependent tumors (S-form).

These tumors may then be treated again with anti-androgenic agents including natural or synthetic 5α-reductase inhibitors and anti-androgens. Repetitive cyclic treatment by an A and an anti-androgen may be appropriate at this stage and may delay and reduce the number of cancer cells progressing to cancer cells (R2-form) that cannot revert back to the S or R-1 form. R1 and R2-form tumors can be treated with As, but 5α-reductase inhibitors or anti-androgens should not be employed for treatment of the R1 or R2-form tumors since they can interfere with the A suppression of these tumors. R2-tumors after A treatment eventually lose the ability to produce AR and become IS-form tumors that cannot be stimulated or suppressed by As. Fortunately, green tea EGCG is an effective treatment for the A-insensitive tumors and may be used at this final stage of PCA progression. In fact EGCG is effective for suppression of all forms of prostate tumors regardless of their A sensitivity. Therefore, infusion of EGCG-rich green tea beverage or polyphenol products may be advisable for patients with prostate tumors at any stage. Since we have shown that rodents may gradually adapt to the continuous use of EGCG (1), possibly due to induction of enzymes or proteins that can degrade EGCG or increase its excretion, an intermittent use of EGCG may be advisable for PCA treatment.

Our studies suggest that the use of 5α-reductase inhibitors and anti-androgens for treatment of PCA patients needs careful evaluation of individual patients. These drugs may stimulate tumor growth if the patient's prostate tumors are at the A-repressed stage and behave like 104-R or CDXR tumors. This might be the reason that, in a recent PCA prevention trial, finasteride was shown to reduce the risk of PCA by about 25%, but increased the chance of acquiring more aggressive tumors (29).

It is also important to note that our studies also suggest that, only physiological doses of A are needed for suppression of the growth of R1, R2, or CDXR-like tumors in patients. The amount of green tea EGCG required may also be achievable. Many chemotherapeutic agents for PCA treatment are very cytotoxic and have not been very effective. The use of two groups of ancient natural medicines, A and green tea EGCG, may provide a novel approach for PCA control and cure.

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