

Review

Prevention of Prostate Cancer by Androgens: Experimental Paradox or Clinical Reality

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Abstract

Androgen replacement therapy in the aging male with partial androgen deficiency improved quality of life. However, such treatment is prohibited for men with a preexisting prostate cancer. The possibility of an increased risk of prostate cancer for healthy men has also been suggested on theoretical basis but recent experimental data showed that androgens may act in prevention of prostate cancer. In this review, we try to evaluate benefits and risks associated to a hormonal replacement therapy in regard to recent data. Several studies analyzing the role of testosterone for prostatic epithelial cells evidenced that testosterone acts in prostatic cell differentiation but does not have a direct role for induction of cell proliferation. Moreover, clinical studies have shown that low free testosterone levels in serum is associated with aggressive prostate cancer, like that has been observed in men with prostate cancer under prostate cancer chemoprevention by finasteride. These data suggest that an androgen pathway disruption in prostate is responsible of cell deregulations that may be associated not only with apoptosis of differentiated prostatic cells but also with potential cell transformation. The effects of androgens withdrawal for prostate cancer therapy induced in a short time the tumor arrest growth. However with time, cells adapt to low levels of androgens leading to the evolution of an androgen-independent tumor, which is more aggressive and most often fatal. The molecular mechanisms of this evolution begin to merge. A hypothesis is that such mechanisms could be initiated in elderly men with an androgen deficiency. The question is raised of whether hormonal replacement therapy could prevent prostate cancer. An encouraging recent study performed on rats demonstrated a protective effect of DHEA for prostate cancer. However, the putative role of the normalization of DHEA or other androgen levels in prevention of prostate cancer should be evaluated in clinical trials.

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1. Introduction

Testosterone levels decrease with age and elderly men present a partial androgen deficiency. The evolution of androgen deficiency has been estimated to be 16.2% (40–49 years), 20% (50–59 years), 22.6% (60–69 years) and 26% (80 years and more). Testosterone deficiency is associated with multiple deregulations that can lead to

symptoms as decreased libido, loss of muscle mass, osteoporosis, decreased cognitive ability and depression. Multiple studies have demonstrated that the reestablishment of normal hormonal levels improves quality of life and decreases symptoms associated with the loss of androgens. However, it has been reported that androgen replacement therapy increased the growth of already existing prostate carcinoma, meaning that before prescription, patients should be carefully screened to detect the presence of a preexisting tumor. However, for healthy patients, the question is raised of whether androgen replacement therapy could induce

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prostate tumor. Regarding to recent data, we made a tentative to evaluate benefits and risks of hormonal replacement therapy for men presenting a decrease in androgens. We reported several studies that described the role of androgens for normal epithelial prostatic cells. Concerning the levels of circulating testosterone in the serum of patients with prostate cancer, we analyzed several studies that evidenced a correlation between low levels of testosterone for patients with a prostate tumor. We also described new studies analyzing the molecular mechanisms involved in response to a decrease of androgens. Such mechanisms induced cell deregulations and are responsible for the evolution of tumors to androgen-independent tumors. However, a question is whether such mechanism could be initiated in elderly men with a deficiency in androgens, suggesting that hormone normalization may prevent prostate cancer. The potential role of hormonal replacement therapy in the prevention of prostate cancer is also evaluated in regard to new data with animals treated with DHEA.

2. The role of androgens on epithelial cells proliferation and differentiation

Testosterone is the most abundant hormone circulating in males. Through the androgen receptor, testoster-

one plays a key role in the development and maintaining of prostate. The androgen receptor is activated by two ligands; testosterone and dihydrotestosterone, this latter one binding the androgen receptor with a higher affinity [1]. The interaction of steroids with their specific receptors induces the binding of such activating receptors to promoter region of genes. Steroid receptors participate directly to the induction of transcription of genes (Fig. 1). However, the activation of cells by steroids can also induce a so-called “non-genomic” (second messenger mediated) action of steroid receptors via the MAP kinase [2] or a “non-genomic” action of non-classic steroid receptors; G protein coupled receptors (GPCR) increasing intracellular concentration of Ca^{2+} (Fig. 1). Such mechanisms have been described for hormones as glucocorticoids, progesterone, estrogens, androgens, neurosteroids, mineralcorticoids, vitamin D3 and the thyroid hormones T3 and T4 [3]. Testosterone has been described to act through or independently of intracellular androgen receptor in cells of the immune system as described in studies performed on macrophages and T lymphocytes. Bente et al. described testosterone signaling in macrophages IC-21 lacking intracellular androgen receptor but exhibiting a membrane form of testosterone receptor. Treatment of such cells by testosterone induces a rapid and transient increase of intracellular

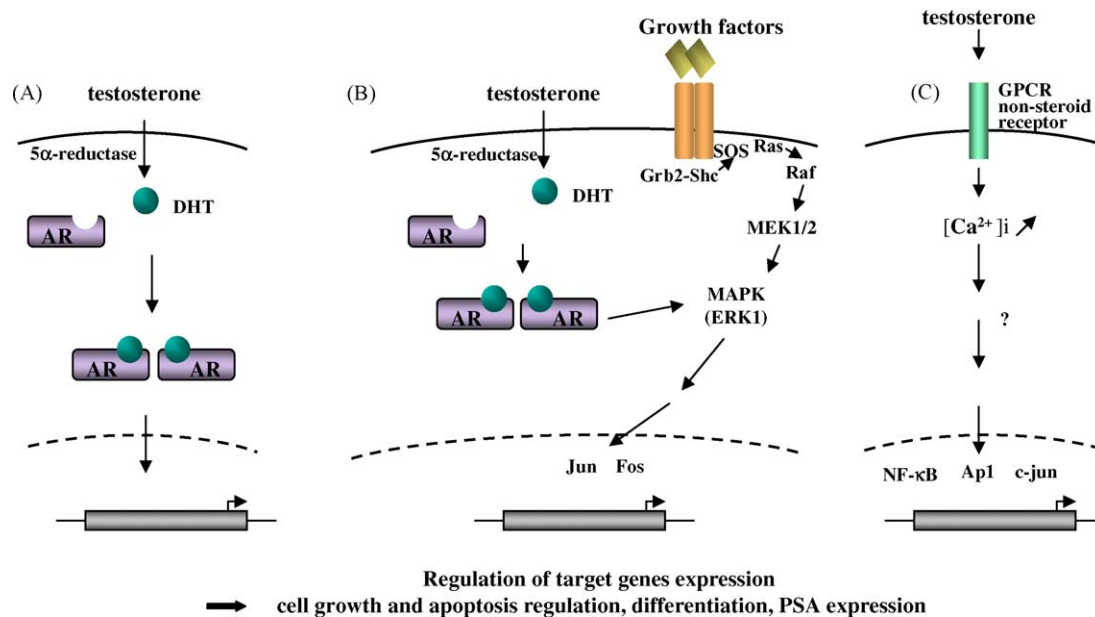


Fig. 1. Summary of testosterone mechanisms for cell induction. “Genomic” action of androgens: interaction of DHT (after testosterone conversion by 5 α reductase) with androgen receptors (AR) induces the binding of such activating receptors to promoter region of genes (A). “Non-genomic” action of androgen receptor: interaction of DHT with androgen receptors induces activation of MAPK. MAPK activates second messengers and transcription factors to regulate specific gene expression (B). “Non-genomic” action of non-steroid receptors: AR receptor induces G protein coupled receptors (GPCR), leading to increase of intracellular Ca^{2+} release from intracellular Ca^{2+} stores. The link between GPCR activation by testosterone and induction of transcription factors has to be determined (C).

concentration of Ca^{2+} . It is well documented that the intracellular elevation of calcium in cells of the immune system induces the activation of transcription factors such as NF-AT, Jun kinase and NF- κ B. Activated transcription factors participate to the regulation (activation or inhibition) of the transcription of specific genes. However, activation of transcription factors in response to testosterone binding GPCR has not yet been demonstrated. The physiological significance of the signal triggered by testosterone via a membrane receptor in remains to be elucidated [4].

Many studies have demonstrated the role of androgens in prostate growth and differentiation. However these functions involved complex interactions between multiple growth factors and receptors with the different cell type constituting the prostate. The paracrine role of growth factors expressed by stromal, epithelial and neuroendocrine cells regulates differentiation and growth of the prostate [5].

The role of growth factors in prostate development and maintaining has been largely studied; however, the exact contribution of androgens still remains unclear. Several studies analyzed the role of androgens on proliferation and differentiation for isolated epithelial cells from prostate. To analyze the effect of androgens on isolated cells, human prostatic epithelial cells (HEPC) derived from normal, benign (BPH), primary cancer and metastatic cells were treated with increasing concentrations of dihydrotestosterone (10^{-11} to 10^{-7} M). The treatment including different doses of DHT had no effect on the proliferation of normal cells or cells from benign or metastatic prostate cancer. Furthermore, adding the anti-androgen Casodex[®] to cell media did not induce growth inhibition of cell proliferation. The absence of effects of androgens for metastatic cell lines is likely to be related to androgen independent growth of tumoral cells. For normal and BPH primary cells, androgens have no direct effects on proliferation, however primary cells may have decreased expression of androgen receptor in culture [6]. Also, several studies have demonstrated the absence of direct mitogenic effects of DHT on rodent prostatic epithelial cells. A systematic analysis of normal rat prostate epithelial cells demonstrated a direct mitogenic effect of several hormones, including insulin, epidermal growth factor, glucocorticoids and prolactin. However this analysis didn't reveal a mitogenic effect of DHT [7]. A second study submitted primary cultured epithelial cells derived from the rat dorso-lateral prostate to a treatment of DHT (10^{-10} to 10^{-6} M). Interestingly, cell proliferation was significantly inhibited at the physiological dose of 10^{-9} M [8]. These data suggest that DHT is not directly

involved in the proliferation of isolated epithelial cells. The discrepancy between experiments performed on isolated epithelial cells demonstrating that DHT had no effect on isolated epithelial cells and in vivo studies suggesting a role for androgens in proliferation and differentiation [9,10] may reflect an indirect action of androgens on prostate epithelium for proliferation. In the prostate, growth of epithelial cells may be the consequence of a multi-hormonal contribution.

However, a role for androgens in prostate differentiation has been largely described [10]. The differentiated functions of primary cultures of human prostate epithelial cells have been analyzed. Such isolated cells in culture lose secretory differentiated functions and androgen responsiveness, as well as the capacity of re-aggregation in the first 2 or 3 weeks. The authors showed that a combined treatment of androgens and retinoid is able to preserve low level of PSA (Prostatic Specific Antigen) for at least 40 days. Cells also exhibited the capacity of re-aggregation and still express androgen receptors [11]. The participation of androgens to prostate differentiation is supported by multiple studies and is mediated by the high range of genes activated in response to the binding of testosterone or dihydrotestosterone to the androgen receptor. Recently, a cDNA microarray analysis identified two dozen of androgen responsive genes, including proteins involved in metabolism, chaperoning, trafficking, cell cycle apoptosis, protein synthesis, structural and extracellular matrix proteins. Also novel proteins were induced, which functions remain to be elucidated [12]. These data suggest a role of androgens in epithelial cell differentiation, without a direct mitogenic effect.

Interactions between stromal and epithelial cells are essential for prostate differentiation and growth. In normal prostate, growth of epithelial cells is dependent of growth factors secreted by stromal cells and exerting paracrine effects. However, epithelial cells derived to malignant tumor growth independently from stroma. Several growth factors have been identified to be secreted by stromal cells and to stimulate proliferation of epithelial cells. Studies performed on prostate development or on isolated cells suggest that Fibroblast growth factor, FGF-7 (KGF), acts as an andromedin that mediates the indirect control of epithelial cells by androgens. Androgens induced expression of FGF-7 by stromal cells and, through paracrine control, FGF-7 induces proliferation of epithelial cells [13]. Regulation of FGF-7 by androgens was not identified in vivo, as demonstrated by RNase protection assays experiments. However, experiments suggest that FGF-7 and androgen receptor pathway may interact since antiandrogens can block FGF-7 stimulated development [14].

FGF-10 is the second candidate identified to act as an andromedin. FGF-10 is secreted by stromal cells and induced proliferation of epithelial cells. As for FGF-7, androgens induced expression of FGF-10 from stromal isolated cells, however *in vivo* experiments should be performed to determine if androgens directly induced FGF-10 gene expression or if androgen receptor pathway may interact with FGF-10 [15]. Also, androgens action on epithelial cells may be linked to several pathways, including FGF-7 and FGF-10.

3. Low levels of testosterone are detected in the serum of patients with prostate cancer

Testosterone levels decrease with age and elderly men present a partial androgen deficiency, while prostate cancer incidence increases with the age [16]. Several studies analyzing the level of total testosterone in patients with a prostate cancer showed controversial results. Recently, reports that discriminated free and total testosterone evidenced that a low level of free testosterone in the serum of patients can be associated with a prostate cancer compared with healthy men in the same range of age. Interestingly, a decrease in free testosterone level has been observed on 14% of elderly men belonging to a group with a cancer of the prostate (Gleason score 6 or 7) undetectable with digito-rectal examination and presenting normal PSA level [17]. The same group investigated whether the level of free testosterone could have an incidence on prostate cancer by analyzing biopsy from men with low versus normal free testosterone level. The study showed that patients with low free testosterone have an increased mean percent of biopsies that revealed cancer (43% versus 22%) with higher Gleason score of 8 or greater (7 of 64 versus 0 of 48). Moreover the authors did not evidence changes in the level of total testosterone. In agreement with these results, the authors raised the possibility that level of free testosterone in the sera of patients could be a marker of aggressive prostate cancer [18].

The levels of both total and free testosterone have been compared in the serum of patients exhibiting a moderate or high grade prostate cancer; it appear that a low level of testosterone was detected for patients with a high grade tumor, and normal levels are observed in patients presenting only a moderate grade of tumor. Testosterone levels have also been followed in a group of patients presenting a high grade prostate cancer. After radical prostatectomy patients exhibit a higher level of both total and free testosterone. Different hypotheses have been proposed to explain the fluctua-

tion of testosterone from patients presenting a prostate tumor. However, the fact that the authors of this study observed an increase of testosterone level after prostatectomy suggests that factors secreted by the cells from the tumoral prostate may influence testosterone levels [19].

The serum androgen bioactivity (ABA) was quantified by using a recombinant cell-based bioassay. This functional assay included the association of testosterone to the androgen receptor followed by the binding of this complex to the promoter of a reporter gene. The results showed a decrease in androgen bioactivity for patients with low Gleason score (≤ 5) whereas the level of free testosterone was not changed, as described above. This bioactivity is more decreased in the serum of patients with a high Gleason score (≥ 8). One of the explanations for the discrepancy between the level of free testosterone and the bioactivity detected may reflect the presence of androgen antibodies in the serum of patients presenting a prostate cancer. However, the androgen bioactivity is inversely proportional to the tumor grade and volume [20].

Finally, a study performed on a large cohort of 879 patients was realized, following different parameters during several years. Pretreatment total testosterone was measured before prostatectomy and this analysis could be used as prognostic in patients with prostate cancer. This analysis confirmed that low levels of testosterone can predict aggressive disease. The level of this hormone appeared inversely proportional to the tumor grade [21].

A recent study (Prostate Cancer Prevention Trial) [22] performed on a large cohort of 18882 men within 7 years analyzed the potential effect of finasteride, a 5α -reductase type 2 inhibitor, on prostate cancer prevention. In the trial, men chosen were 55 years and presented a normal digital rectal examination and a PSA level not exceeding 3.0 ng per millimeter. Men received finasteride (5 mg per day) treatment or placebo. Men underwent annual digital rectal examination and PSA measurements, and when abnormal examinations were observed a biopsy was performed. The rate of prostate cancer diagnosed was higher in the placebo group (24%) compared to the group receiving finasteride (18%). However, patients receiving finasteride exhibited a higher proportion (37%) of tumors with worse Gleason score (7–10), compared to the placebo group (22%). The difference of cancer incidence which appears early in the trial suggests that finasteride could inhibit the development of emerging cancer. As the difference between the finasteride and placebo groups increases with time, this suggests that finasteride postpones the clinical emerging of prostate cancer.

However, the emergence of prostate cancer with a higher grade for patients treated with finasteride may be in favor of the hypothesis that decrease of DHT is associated with more aggressive tumors [22]. Even if a wrongly pejorative interpretation of the Gleason score, induced by Finasteride, has been suggested.

4. Deregulations of tumorigenic prostate cells induced by the decrease of steroid levels leading to hormone independence

At early stages of prostate cancer, cells are dependent of androgens for growth and survival. One of the first treatments for patients with advanced prostate cancer is the androgen ablation, hence inducing at early stages of the treatment a tumor regression by the induction of apoptosis for epithelial cells. However, tumors still progress in an androgen-independent way, more aggressive, leading to metastasis and in death. Multiple studies try to elucidate whether the androgen ablation is responsible for the evolution of the prostate cancer to androgen-independent disease.

One of the effects of steroid ablation is the selection of prostatic cells sensitive to low levels of androgens. The prostatic cancer cell line LNCaP (obtained from a lymph node metastatic prostate cancer), which expresses an androgen receptor mutated in the ligand domain, has been cultured in conditions with a steroid free medium in long term. Such treated cells respond strongly to low levels of androgens compared to LNCaP cells cultured in the presence of steroid. The level of response is evaluated by measuring the fold induction of a reporter gene controlled by a promoter region containing androgen responsive elements [23].

The molecular mechanisms of androgen independence are largely studied, several modifications have been evidenced, such as amplification of the androgen receptor gene in approximately 30% of the tumors or missens mutations in the androgen binding domain of the androgen receptor [24] which led to an hypersensitive receptor activated by a wide range of steroid hormones or antiandrogen molecules. Also, some growth factors as insulin-like growth factor (IGF), keratinocyte growth factor (KGF), and epidermal growth factors (EGF) can activate the androgen receptor in the absence of androgens and induced genes normally regulated by steroid hormones. Intracellular molecules involved in signal transduction such as AKT and MAPK, generally upregulated in cancer, also bypass the androgen pathway and induce multiple cellular deregulations. The amplification of the recep-

tor gene has also been reported in prostate tumorigenic cells deprived for steroids [25]. Hormone receptors, following activation, bind DNA to activate gene transcription together with co-activator complex having histone acetyltransferase activity [26]. Several co-activators or repressor molecules have been identified to interact with androgen receptors for optimal activation of transcription. Brady et al. showed the role of co-activator Tip60 which is a histone acetyltransferase that interacts directly with the androgen receptor to enhance the transcriptional activity of this molecule. Tip60 is a co-activator specific for class I nuclear hormone receptor, also including estrogen and progesterone [27]. These authors also evidenced, following androgen withdrawal, the increased expression of both mRNA and protein of Tip60 with a major localization in the nucleus in androgen-independent tumors. Also, experiments using the prostate tumor cell line LNCaP demonstrated the same result; up-regulation and nuclear localization of Tip60. In such cells Tip60 functionally binds the promoter region of PSA. These studies demonstrated that in conditions of deprivation of androgens, cells adapt the regulation of molecules to bypass the signals regulated by testosterone. However the upregulation of functional co-activators, less specific than hormones, may have other unexpected effects affecting cell proliferation or differentiation [27,28].

Another characteristic of androgen-independent tumors is the expression of neuroendocrine patterns of differentiation. By secretion of neuropeptide, neuroendocrine cells exert auto/paracrine activities involved in proliferation, transformation and metastasis [29,30]. Several studies demonstrated that withdrawal of androgens is responsible for the differentiation of prostatic epithelial cells in neuroendocrine cells [31,32]. However the mechanisms of this change in cell population still remained unclear. Inhibition of androgen receptor expression, by the method of silencing RNA (siRNA), induced biochemical and morphological changes associated with the neuroendocrine differentiation process on LNCaP cells. Thus, the androgen receptor is directly responsible for the repression of an intrinsic neuroendocrine differentiation process in androgen responsive tumoral cells. The alteration of androgen receptor or the withdrawal of testosterone cells may be linked to the neuroendocrine differentiation in prostate cancer [33].

These studies analyzed the molecular mechanisms that may be involved in the androgen-independent evolution of a prostate tumor. It appears that a withdrawal in androgens induced the adaptation and/or selection of cells responsive to very low levels

of hormones. Mutations or increased expression of androgen receptor, upregulation of androgen co-activators or enrichment in the population of neuroendocrine cells have been analyzed in tumor cells deprived in androgens. However, the question is raised of whether such phenomenon could be initiated in patients with a prostate cancer at early stages and presenting low level of testosterone or in elderly men with a decrease of androgens.

To explore the mechanisms of androgen-independent growth, the effects of hormone deprivation, which induce G1 arrest, were observed on cell cycle regulators. As a consequence of androgen withdrawal on LNCaP cells, increase of the cell cycle inhibitor p27^{kip1} and decrease of p21^{waf/cip1} were observed [34]. However the decrease of p21^{waf/cip1}, which has been described to inhibit cyclin E/CDK2 functions, represents a paradox in cells that are blocked in the progression of S phase. Studies have also described a role for p21^{waf/cip1} in the promotion of cyclin D1/CDK4 association, targeting to the nucleus and kinase activity. Thus, the decrease of p21^{waf/cip1} may contribute to the inhibition of cell cycle progression [35]. To elucidate the mechanism of independence growth linked to the cell cycle, Liao's group developed LNCaP sublines in conditions of androgen deprivation in long-term culture. The androgen-dependent clonal subline LNCaP 104-S derived in androgen deprivation to the cell sublines 104-R1 which is slow growing and 104-R2 which is fast growing. 104-R1 and 104-R2 cells are androgen-independent for proliferation. Cell growth is not affected by Casodex[®] treatment and, moreover, proliferation of 104-R1 and 104-R2 cells is repressed by androgens. The molecular analysis of androgen repression revealed that androgens induced the transient expression of the cyclin-dependent kinase (cdk) inhibitor p21^{waf/cip1} in 104-R1 cells and expression of the cyclin-dependent kinase (cdk) inhibitor p27^{kip1} in 104-R1 and 104-R2 cells, which led to cell cycle G1 arrest [36,37]. Moreover, in normal secretory epithelial compartment of prostate gland, androgens have been shown to be involved in up-regulation of p27^{kip1} [38]. Interactions of androgens or androgen receptor with cell cycle regulators is not yet clearly understood, however it appears that in conditions of androgen withdrawal, modifications of androgen regulation induce changes in cell cycle and consequently affect progression of cell cycle (Fig. 2). Androgen receptor has also been identified to interact directly with cell cycle regulators. Interestingly, cyclin D1 that can be induced by androgens [34] has also been shown to bind and inhibit the transactivation functions of the androgen receptor in LNCaP cells [39]. Thus, androgens may

act through cyclin D1 as an autoregulator by inhibiting transactivation of its own receptor. These raised the question of how such autoregulation can be driven in conditions of androgen withdrawal, or in carcinoma cells exhibiting mutations of androgen receptor. In contrast, cyclin E has been shown to acts as a co-activator of androgen receptor [40].

Beside direct interactions of androgens with proteins regulating the cell cycle, androgens have been shown to regulate cell growth via the interaction with the analog of vitamin D; 1,25-dihydroxyvitamin D(3) (1,25-(OH)(2)D(3)) (Fig. 3). The literature is well documented on the action of 1,25-(OH)(2)D(3) on growth inhibition of various cells, including prostate carcinoma cells. Several mechanisms of the cell proliferation inhibition by 1,25-(OH)(2)D(3), depending of cell type, have been described including interactions with cell cycle regulators, and growth factors, apoptosis, differentiation and androgen receptor regulation (for review [41]). Cell growth inhibition by 1,25-(OH)(2)D(3) was demonstrated to be androgen-dependent for MDA PCa 2a, MDA PCa 2b which expresses low affinity AR [42], primary culture of prostatic epithelial cells, PC3 and DU145 cells lacking AR [43]. However, inhibition of cell proliferation induced by 1,25-(OH)(2)D(3) is androgen independent for LNCaP cells expressing AR with high affinity [44], as demonstrated by treatment with Casodex[®]. Also, in vivo experiments performed on rats demonstrated that prostate growth inhibition induced by 1,25-(OH)(2)D(3) is dependent of androgens [45]; 1,25-(OH)(2)D(3) induce cell cycle arrest of LNCaP cells in the G1 phase by increasing protein level of p21^{waf/cip1} and p27^{kip1}. The mechanisms by which 1,25-(OH)(2)D(3) induced cell cycle inhibitors are indirect and have been elucidated. It has been shown that 1,25-(OH)(2)D(3) reduce nuclear Cdk2 availability. As Cdk2 phosphorylate p27^{kip1} on Thr¹⁸⁷ residue and this phosphorylation target the protein to degradation, cell treatment by 1,25-(OH)(2)D(3) resulted in the increase of p27^{kip1} level and cell cycle is blocked G1 phase [46]. Also, 1,25-(OH)(2)D(3) upregulate Insulin Growth Factor Binding Protein 3 (IGFBP-3) at the mRNA and protein levels. IGFBP-3 induce p21^{waf/cip1}; this results to cell cycle arrest [47]. Interestingly, it has been demonstrated that DHT treatment increases both AR and vitamin D receptor (VDR) [48,49] and reciprocally, 1,25-(OH)(2)D(3) up-regulate AR expression [50]. In consequence, it appears that androgen, 1,25-(OH)(2)D(3) and receptors are closely linked to contribute to cell cycle regulation.

Interactions of androgens and androgen receptor with cell cycle regulators acts as a balance for regula-

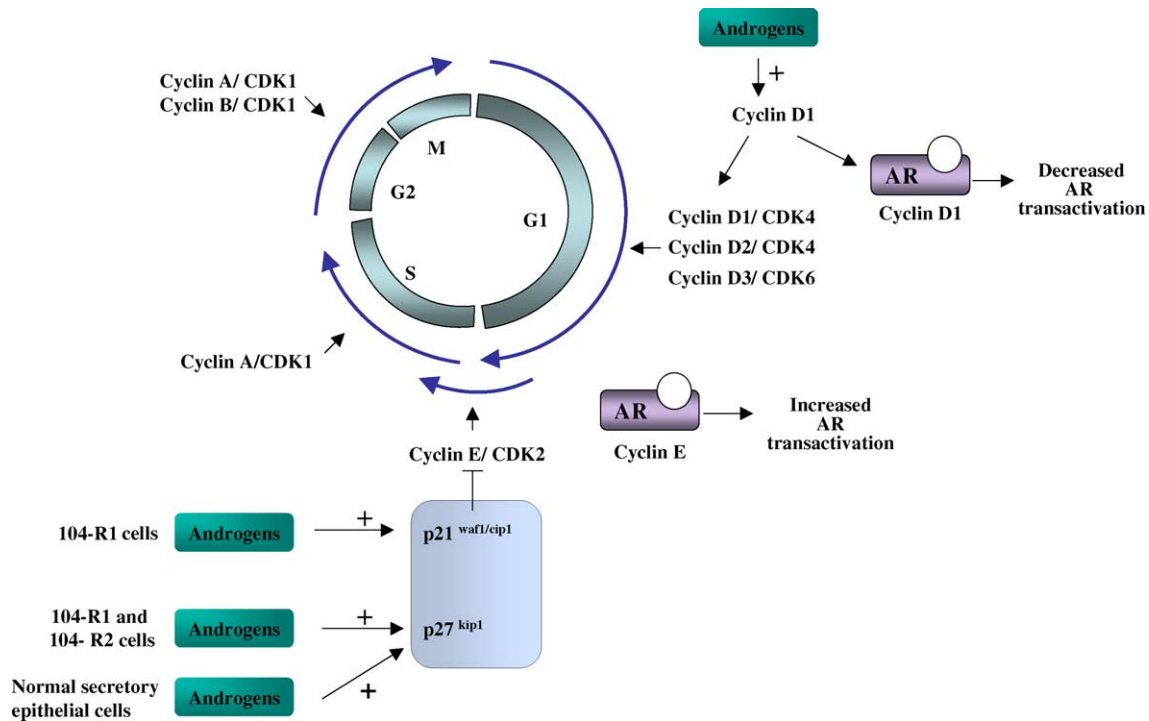


Fig. 2. Summary of androgen and androgen receptor (AR) interactions with the cell cycle regulators. In normal secretory epithelial cells, androgens increase the cyclin-dependent kinase (Cdk) inhibitor p27^{kip1} level and block the cell cycle at transition G1 to S. 104-R1 is a slow growing subline and 104-R2 is fast growing subline derived from an LNCaP subclone maintain in conditions of androgen deprivation in long-term culture. In 104-R1 cells and 104-R2 cells, p27^{kip1} level is increased in response to androgen treatment, this correlate with cell cycle arrest and inhibition of cell proliferation. In 104-R1 cells p21^{waf1/kip} level is increased in response to androgen treatment and cell cycle progression is inhibited. Androgens can induce cyclin D1, this protein can associate with cyclin dependent kinase 4 (Cdk4) to promote the G1 phase of the cell cycle. Cyclin D1 also associate with the androgen receptor (AR) and decrease the transactivation function of this receptor. Cyclin E was shown to interact and to increase the transactivation activity of the androgen receptor.

tion of cell proliferation, suggesting that androgen withdrawal or androgen receptor disruption may contribute, like abnormal androgen receptor upregulation,

to the carcinogenesis process and androgen-independent growth.

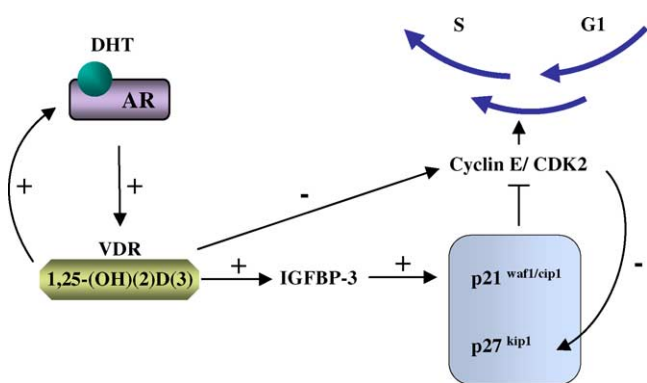


Fig. 3. Mechanisms described for 1,25-dihydroxyvitamin D(3) (1,25-(OH)(2)D(3)) and vitamin D Receptor (VDR) inducing cell cycle arrest. 1,25-(OH)(2)D(3) increase Insulin Growth Factor Binding Protein 3 (IGFBP-3) at the mRNA and protein levels. IGFBP-3 induce the cell cycle inhibitor p21^{waf1/cip1}, leading to cell cycle arrest. 1,25-(OH)(2)D(3) decrease nuclear level of Cdk2 available. As Cdk2 is responsible to the phosphorylation that target p27^{kip1} to degradation, 1,25-(OH)(2)D(3) indirectly increase p27^{kip1} level and inhibit cell cycle progression. Androgens induce 1,25-(OH)(2)D(3) which increase AR and 1,25-(OH)(2)D(3) up-regulate AR expression.

5. Androgen level normalization may prevent prostate cancer

DHEA (dehydroepiandrosterone) is the primary steroid precursor of both androgens and estrogens and represents an abundant circulating steroid hormone. The progressive decrease of this hormone with age has been related to multiple diseases as cancer, diabetes, arteriosclerosis, obesity and Alzheimer's. Beneficial effect of additional DHEA treatment in the elderly population is currently under study and seems to prevent these disorders. Prostate cancer progression is slow and can be extended to decade. A hypothesis is that low levels of testosterone induce changes in molecular balance of epithelial prostate cells as we described above. It is possible that accumulation of changes during years in the cells may induce deregulations that lead to tumorigenesis.

Several studies have demonstrated a protective role of DHEA in the development of cancer, particularly

mammary gland in rodents [51,52], skin [53], lung [54], liver and thyroid [55]. Concerning prostate cancer, DHEA has been shown to inhibit the development of adenocarcinoma in rats [56] and to inhibit the proliferation of tumorigenic cells [57]. Recently, Rao et al. described a protocol based on the administration of low doses of DHEA prior or post-inducing prostate cancer in rats. To induce prostate adenocarcinomas rats were submitted to a sequential regimen of cyproterone acetate and cyproterone propionate, followed by a single injection of N-methyl-N-nitrosourea (MNU) and chronic androgen stimulation. For the assay, three groups of animal received different treatments. The first group received DHEA (1000 or 2000 mg/kg diet), which was continuously administrated to rats beginning one week before MNU exposure. The second group received DHEA (2000 mg/kg diet) continuously either one week before or 20 or 40 weeks after MNU exposure. The third group, as control, received basal diet without DHEA. Then, 13 months after MNU exposure prostate cancer was evaluated by histopathology analyses. The group I exhibited DHEA dose related inhibition of prostate cancer. The group II, interestingly presented a comparable reduction of prostate cancer for animals treated by DHEA one week before 20 or 40 weeks after MNU treatments. This study demonstrates first, that DHEA at physiological doses was able to prevent prostate cancer development, and second that DHEA is also able to suppress the progression of prostate cancer [58]. The same authors in a second study which compares the effects of DHEA and 9-cis-retinoic acids described similar results. Both of these compounds prevent or suppress progression of prostate cancer induced in rats [59]. These impressive data need to be confirmed for humans, to analyze the putative preventive or curative role of DHEA in prostate cancer. Cancer prevention by androgen normalization should be evaluated by clinical trials, with men followed during several year. As the levels of androgens decrease with age dependently of each people, clinical trials should be performed on a large randomized population. Different forms of androgens should be tested as well of doses administration. However, if these effects for cancer prostate prevention and/or treatment were confirmed, administration of DHEA would be a great advantage for patients with a prostate cancer and as a cancer prostate prevention for elderly people.

6. Conclusions

Androgen replacement therapy provides benefits to elderly men as increasing libido, muscle mass and

prevention of osteoporosis. Patients with prostate cancer should not receive androgen supplementation, as additional androgens are likely to increase prostate cancer progression. However for aging men without prostate cancer, studies of the roles of androgens demonstrated no evidence that this hormone could trigger prostate carcinogenesis. As we previously described, several studies, performed on prostatic isolated epithelial cells, showed that testosterone or dihydrotestosterone didn't have any direct effects on cell proliferation.

Multiple analyses evaluated the level of testosterone in serum of patients with a prostate cancer. Results appeared conflicted and controversial; equivalent, increased or lower levels of testosterone were measured in patients presenting prostate cancer at different stages compared to control patients in the same range of age. Finally, interesting results emerged from new studies discriminating free and total testosterone. Low free testosterone level appeared to be correlated with prostate cancer. Moreover, a study measuring the bioactivity of testosterone present in the serum evidenced that low level of bioactive testosterone is correlated with the aggressiveness of prostate cancer. Such observations suggested that patients with low levels of testosterone present a risk for prostate cancer, and this analysis may be used as prognostic.

The prostatic specific antigen (PSA) is one of the genes regulated by the androgen receptor, in response to testosterone or DHT binding to the receptor [60–62]. The measurement of PSA present of the serum is one of the elements for prostate cancer diagnosis. However cases have been reported of patients with advanced prostate cancer having low serum PSA levels [17,63]. As PSA gene is directly regulated by testosterone, low free testosterone levels may be responsible of the decrease of PSA expression. Also, treatments for testosterone normalization could be an advantage to detect prostate cancer at an earlier stage using PSA test screening.

As in elderly men, testosterone levels decreased with the age, it may not be excluded that such low levels of hormone initiated deregulations of prostatic cells. Furthermore, as low levels of free or bioactive testosterone are likely to be associated with prostate cancer, hormonal replacement therapy in aging men would normalize the testosterone levels and may prevent deregulations of prostate cells. The role of DHEA in the prevention and also in the treatment of prostate cancer demonstrated in rats should be confirmed for humans. If such advantages are conserved for men, DHEA or normalization of other androgens may be a direct prevention for prostate cancer.

Molecular analysis of prostate tumor tends to consider testosterone as a regulator of prostate cell maintaining and differentiation without direct mitogenic effect. Moreover recent data suggests that the maintaining physiological levels of testosterone may prevent prostate cancer. Also, hormones regulated multiple physiological functions, including the immune system. Although additional steroid hormones remain a contraindication for patients with clinical

prostate cancer, it represents a benefice for aging men and a potential thought for prostate cancer prevention development.

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Editorial Comment

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There are continuing efforts to improve understanding of androgen action in prostate cancer. It is widely

accepted that androgenic hormones regulate in different ways proliferation, apoptosis, angiogenesis, metastasis and differentiation. In this review, Algarté-Génin and colleagues discuss some effects of androgens which in general did not receive sufficient attention. The fact that

high doses of androgen cause a progressive decline of proliferation of LNCaP cells was further investigated and it became clear that this effect is mediated through increased expression of the tumour suppressor p27 [1]. Regulation of proliferation of prostate cancer cells and expression of cell cycle regulatory genes become even more complex during long-term androgen ablation, a process characterized by increased expression and activity of the androgen receptor [2]. Therefore, it is difficult to decide on proper timing of androgen ablation therapy keeping in mind that multiple changes occur in the androgen signalling pathway.

Another important issue addressed in this review is a prodifferentiation effect mediated through up-regulation of the androgen receptor after vitamin D treatment [3]. In addition, interleukin-6 and phenylbutyrate inhibit cellular proliferation, increase androgen receptor activity and prostate-specific antigen expression [4,5]. The basis for these variant effects of androgens is not understood; one possibility could be differential recruitment of androgen receptor coactivator/corepressor complexes. Although up-regulation of the androgen receptor is a consistent change associated with prostate cancer progression observed in several tumor models,

there is still lack of therapy approach based on differential regulation of target genes responsible for either proliferation or differentiation [6].

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