J. Endocrinol. Invest. 34: 232-243, 2011
DOI: 10.3275/7501

REVIEW ARTICLE

Androgen regulation of prostate cancer: Where are we now?

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ABSTRACT. ***Background:*** **Androgens play an essential role in the development and differentiation of the prostate gland; their contribution to pathological conditions, such as benign prostatic hyperplasia and prostate cancer (PC), remains unclear.** ***Aim:*** **We reviewed relationships between androgens and the prostate both in physiological and pathological conditions.** ***Material and methods:*** **A systematic search of published evidence was performed using Medline (1969 to September 2010).** ***Results:*** **Androgen-dependency of prostate growth is evident only in the hypogonadal condition, but not in the eugonadal state (the "saturation hypothesis"). There is unequivocal evidence that reducing androgen signaling to the hypogonadal range can reduce PC growth and patient symptoms. At physiological testosterone concentration there is no link between androgen levels and PC risk. In addition, different strategies of androgen deprivation (ADT) for advanced PC are only palliative and rarely cure patients. Preliminary evidence indicates that a low androgen milieu is associated with tumor aggressiveness. Transition to androgen-independence is complex and involves both selection and outgrowth of pre-existing androgen resistant clones, as well as adaptative up-regulation of genes that help the cancer cells to survive and grow after ADT. Because androgens are essential for the regulation of fat distribution, insulin sensitivity, and lipid and bone metabolism, recent publications have highlighted the concept that ADT may also be involved with an increase in overall, as well as cardiovascular, morbidity and mortality.** ***Conclusions:*** **While ADT still represents a cornerstone for the palliative therapy of a small fraction of aggressive PC, a "misuse and/or abuse" of ADT should be avoided.**
(J. Endocrinol. Invest. 34: 232-243, 2011)

INTRODUCTION

The human prostate is a zinc accumulating, citrate producing urogenital accessory organ of the male reproductive tract. It surrounds the urethra below the bladder and produces prostatic fluid, a secretion that makes up 30% of the total ejaculate. Prostatic fluid provides nutrients and optimal pH for sperm survival and is rich in fibrinolytic enzymes, such as prostatic-specific antigen (PSA). PSA is a 34 kD protein, which liquefies the seminal coagulum, allowing sperms to swim freely and facilitating their entry into the cervical mucous cap (1, 2). PSA is synthesized almost exclusively by the prostate and, in clinical practice, it is considered a reasonable proxy for prostate volume. Morphological structure of the prostate gland includes 40 to 50 ducts distributed in three anatomically distinct zones, essentially: peripheral, central and transitional or periurethral. The prostate weight is only a few grams at birth and it increases consistently during puberty. At this particular stage in life, the prostate gland undergoes extensive remodeling, characterized by branching of ducts and development of new gland buds, followed by acini formation within fibromuscular stroma, reaching a volume of approximately 20 ml (1, 2). In contrast to the pubertal growth phase, which involves the entire gland, in about 75% of men, during the fifth decade of life, there is a second growth phase involving only the periurethral zone (1, 2). Conversely, the peripheral and central zones, which constitute up to 95% of the entire prostate volume, are usually unaffected. While cell growth in the periurethral zone leads to the most common age-related disease of the male: benign prostatic hyperplasia (BPH), cell transformation in the peripheral zone gives rise to prostate cancer (PC) (1, 2). PC develops primarily in men over fifty and it is the most common type of cancer in men in the United States, with 186,000 new cases in 2008 and 28,600 deaths (3). It is the second leading cause of cancer death in U.S. men after lung cancer. Although it is one of the most prevalent types of cancer in men, many never have symptoms, undergo no therapy, and eventually die of other causes. This is because PC is, in most cases (about 2/3 of cases), slow-growing, symptom-free, and, since men with the condition are older, they often die of causes unrelated to it. Only 30% of cases are aggressive and develop quickly. Current treatment options for clinically localized or locally advanced cancer of the prostate include radical prostatectomy, radiation therapy, brachytherapy, cryotherapy, or "watchful waiting". Depending on a man's age, slow-growing cancer can often be left alone and monitored, while aggressive cancers demand urgent action. Increased levels of PSA may suggest the presence of PC. Gleason score is currently used to grade PC and it is based on the sum of the two most common histologic patterns, each of which is graded on a scale of 1 to 5, 5 being the most aggressive. The PSA rate of increase may also be valuable in PC prognosis: men with PC whose PSA level increased by more than 2 ng per milliliter during the year

Key-words: Androgen deprivation therapy, cardiovascular risk, osteoporosis, prostate, testosterone.

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Accepted September 29, 2010.

First published online February 4, 2011.

before the diagnosis of PC have a higher risk of having an aggressive form of PC (4).

ANDROGEN REGULATION OF PROSTATE GROWTH IN PHYSIOLOGICAL AND CANCER CONDITIONS

During fetal and adult life, prostate development, growth and differentiation are regulated both directly and indirectly by androgens (1, 2, 5). Behre et al. (6) clearly demonstrated that prostate volume is age- and androgen-dependent in hypogonadal subjects, while T replacement therapy (TRT) restored prostate volumes to the control (eugonadal) level. The pioneering work of Huggins of almost 70 years ago (7) gave rise to the clinical concept of the androgen dependence of PC, with the demonstration that castration caused regression of the PC. This finding began a new era in the management of metastatic PC. Androgen deprivation therapy (ADT) can be obtained by orchidectomy (surgical castration), or gonadotrophin-releasing hormone (GnRH) agonists and antagonists, which by way of different modalities decrease gonadotrophin secretion by the pituitary gland (medical castration). Following castration, however, a residual androgen synthesis in the adrenal gland is present (about 10% of the total androgen pool) and more potent androgen receptor (AR) ligands can be sensitized directly in the PC (non-gonadal androgens) (8). Hence, a combination with AR competitive antagonists (such as bicalutamide) is often used (combined androgen blockade, CAB) and blockers of androgen formation, such as abiraterone acetate, are being developed; abiraterone acetate is a specific inhibitor of CYP17A1, a rate-limiting enzyme in androgen biosynthesis (see below, 8, 9). The majority of PC subjects have an initial response to ADT, as derived by a fall in PSA concentration, however, different strategies of ADT for advanced PC are only palliative and, in fact, rarely cure patients (10). The combined or primary use of ADT results in a 94% fall in serum T (T), but intra-prostatic androgen concentrations only decline by 70-80% (11, 12). After a few years of treatment, PC cells often acquired an androgen-independent phenotype and the majority of subjects became refractory to this therapy (see below).

5-alpha reductase and prostate growth

Wilson (13) first hypothesized that the main androgen inducing prostate growth was not T, but its highly biologically active metabolite dihydro-T (DHT) which is formed locally from T by the action of two 5α-reducing iso-enzymes, 5α-reductase type 1 (5AR1) and 2 (5AR2), the latter being predominant. According to Wilson's original hypothesis, blocking DHT formation with type 2 selective (finasteride) or unselective (dutasteride) inhibitors of 5α-reductase isoforms is an effective strategy in the medical treatment of BPH.

The fact that eunuchs and men with genetic deficiencies in 5α-reductase do not typically experience PC, along with the fact that androgen ablation causes PC regression, has long been cited to support a causal role of high androgen levels in PC development (14, 15). Accordingly, the use of 5α-reductase inhibitors (5ARI) has been studied not only in BPH, but also as a chemopreventive strategy in PC. Both 5α-reductase isoenzymes are expressed in normal prostatic tissue, but in PC cells 5AR1 expression is increased and 5AR2 expression is decreased or unchanged, as compared to BPH tissue (16). Both 5ARI have been shown to decrease the risk of PC. Data from the PC Prevention Trial (PCPT) showed a 24.8% reduction in PC prevalence during the 7-yr period between the finasteride (18.4%) and the placebo group (24.4%) (17). The results of the REDUCE (Reduction by Dutasteride of PC Events) trial using dutasteride, has recently been published in a large, randomized study to determine its ability to prevent PC (18). Over the 4 years of the trial, dutasteride, as compared to a placebo, reduced the relative risk of biopsy-detected PC by 23%. A total of 1516 cancers were seen, with 659 in the dutasteride section and 857 in the placebo one. Over the course of the study, 6.8% of men in the placebo group and 6.7% of men in the dutasteride group were found to have aggressive, high-grade tumors, defined as a Gleason score of 7-10. The investigators found that there was no greater risk for the men who developed PC of having aggressive tumors. This outcome was closely watched because an earlier trial of a similar BPH drug – finasteride – produced controversial results with regard to the risk of more aggressive tumors in those men who developed PC while on finasteride (17). An initial analysis of the finasteride data suggested that there was an increased risk for men to have PC with higher Gleason scores but a later re-analysis suggested that there is probably no greater risk for these men to develop higher grade PC (19). From these studies it can be concluded that both finasteride and dutasteride not only improve the outcomes related to BPH but also they might reduce the risk of incident PC detected with a biopsy. However, caution is needed in interpreting these results because, by suppressing PSA, this class of drugs can delay the diagnosis and they do not really prevent PC but merely temporarily shrink tumors with low aggressive potential, without affecting the outcome of the most aggressive ones (20).

The "saturation" hypothesis

Androgens promote prostate cell growth and differentiation by way of two different mechanisms: the ligation of the AR expressed by epithelial and stromal cells, and the induction of stromal synthesis of growth factors, which act on epithelial and stromal compartments in a paracrine and an autocrine manner, respectively (5, 21). Figure 1 shows results from our laboratory on the effect of increasing concentration of T on human prostate stromal cells, obtained from patients with BPH (22). As a function of time, untreated BPH cells progressively increase in number, however they grow faster when exposed to T. Similar results were obtained with DHT (22). Interestingly, we found that blocking growth factor receptors (such as those against type 1 IGF or KGF receptors) with specific antibodies, strongly limited androgen effect (22). The inset of Figure 1 shows the dose-dependency of the androgen-induced prostate growth; the half maximal concentration was obtained at 0.4 nmol/l. Concentrations higher than 1 nmol/l did not further increase BPH cell proliferation, suggesting a saturation model. In other

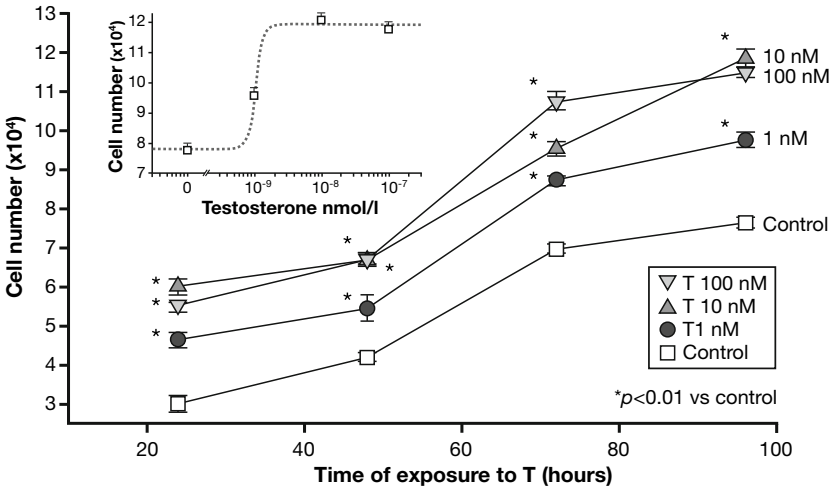

Fig. 1 - *Effect of T on the growth human stromal prostate cell, derived from patients affected by benign prostate hyperplasia (BPH). The figure is derived from ref. 22 and shows a time course (24-96h) analysis of BPH cell proliferation in the absence (control) or presence of increasing concentrations of T (1-100 nM).* Inset: *Sigmoidal dose-response relationship at the latest time point (96h). Note that a "saturation point" is evident above nanomolar concentrations of T. Results are the mean number of cells ±SEM from three separate experiments performed in quadruplicate.*

words, T at sub-physiological concentrations promotes prostate growth, while when in the physiological male range (10-30 nmol/l) it does not further stimulate proliferation.

Morgentaler and Traish (23) first postulated that the human prostate is indeed sensitive to massive androgen ablation (castration levels), but rather insensitive in normal or even in subnormal conditions (such as in mild hypogonadism). According to their hypothesis, the human prostate AR is indeed "saturated" by the circulating androgens and therefore rather insensitive to further T increase, such as those derived from TRT in cases of mild hypogonadism. Hence, they proposed that there is a limit to the ability of androgens to stimulate PC growth (23). The "saturation" hypothesis, if further substantiated, could not only shift the paradigm of T and PC (23) but also establish a satisfactory conceptual framework for clinical management of hypogonadal subjects using TRT.

The "saturation" hypothesis has recently been confirmed in a large series of individuals consulting for erectile dysfunction (ED) (24), one of the more common hypogonadism-associated symptoms, as derived from a recent European survey (25). In particular, it has been reported that PSA is androgen-dependent only in the youngest hypogonadal men, whereas this correlation disappears with higher levels of androgenization (24). Figure 2 (panel A) shows similar results to those previously reported (24), in a larger sample (no.=2757). When the association between PSA and T was evaluated as a function of T deciles, the upper 9 groups had similar PSA values, while the lowest (below 8.2 nmol/l, which roughly corresponds to 231 ng/dl) demonstrated a significantly reduced PSA at ANOVA (p=0.008). A further increase of T levels was not associated with a significant rise in PSA. Figure 2 (panel B) also shows the PSA-T association in a selected sample of subjects apparently free from prostatic diseases (as described in ref. 24), as a scatter plot of PSA *vs* T, along with the best-fitting regression line (calculated R square=0.03, using S-curve regression model; p<0.0001). The PSA regression graph is almost linear at low T values and plateaus at T levels of about 8-10 nmol/l. Hence, the "saturation point" approximately corresponds to the clinical threshold for the diagnosis of hypogonadism.

The concept that reducing T concentration below a critical concentration threshold (the saturation point) leads to an intracellular milieu where prostate tissue grows in an androgen-dependent manner has been recently substantiated also in PC subjects. In a population of 257 patients undergoing radical prostatectomy for PC, we described a significant association between PSA and T only in hypogonadal PC men (T<10.4 nmol/l), whereas this correlation was not evident in men with normal T (26). In a prospective study, involving 72 patients, Takizawa et al. (27) found no correlation of PSA levels with all the androgens studied before ADT (eugonadal state), but a clear correlation after ADT (hypogonadal state).

Permissive effect of circulating androgen on PC

Although in one early study (28), plasma T was found to correlate positively with time of PC progression, in all the later studies there was no evidence for any association between androgen levels and tumor progression or Gleason score (29, 30, see ref. 31 for review). A pooled analysis of worldwide data from 18 prospective studies (more than 3000 cases and 6000 controls) found no association between serum androgen concentrations and PC risk (32). A recent meta-analysis of TRT adverse effect found no significant association between TRT and the incidence of PC, the need for prostate biopsy or a significant PSA increase (33). In a randomized, placebo controlled trial on TRT in 44 hypogonadal men (T<10.4 nmol/l), prostate biopsies performed both at baseline and at 6 months did not show changes in androgen prostate levels nor in several tissue biomarkers related to prostate growth (34). Even variation in AR sensitivity has not been consistently linked to the risk of developing PC (35).

In conclusion, according to the "saturation" model, PC tumor cells are already maximally stimulated at the physiological T concentration. At physiological T concentra-

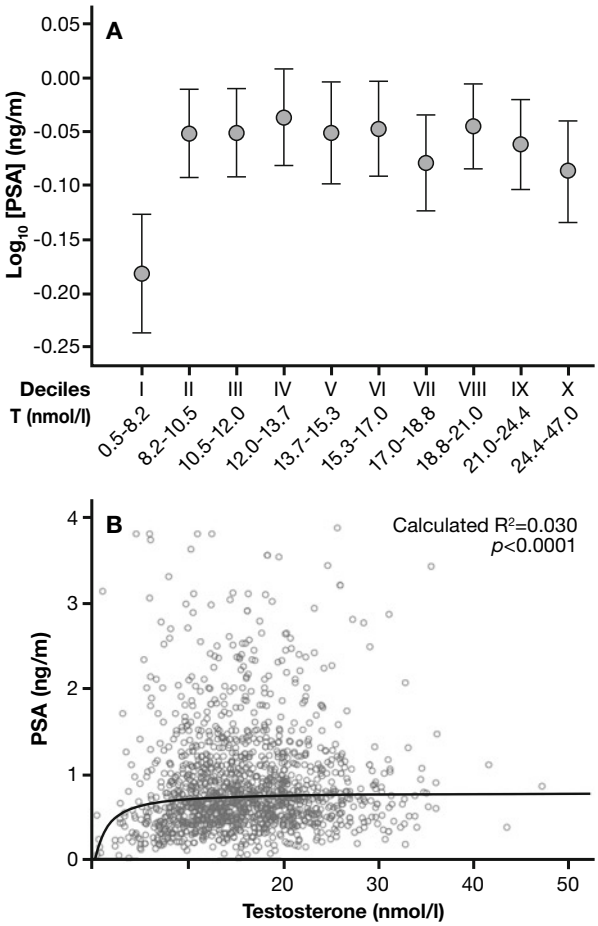

Fig. 2 - Panel A: *PSA levels as a function of total testosterone deciles in a consecutive series of 2757 patients attending our Unit seeking medical care for sexual dysfunction. ANOVA Tukey* post hoc *was p<0.005 for 1st decile vs 2nd or higher.* Panel B: *relationship between testosterone and PSA levels as a scatter plot along with a best-fitting regression curve. Data derived from a selected sample of 1377 of our patients apparently free from prostate diseases: history of prostatitis, benign prostatic hyperplasia, or suspected prostate cancer as assessed by urological examination and/or an abnormal (tender, enlarged, or with suspect nodules) digital rectal examination of the prostate, and/or total PSA above 4 ng/ml, and/or previous prostatic surgery or radiotherapy, and/or using drugs altering prostate growth including testosterone, alpha-blockers, vitamin D, and antiandrogen drugs including GnRH analogues, bicalutamide, 5-alpha-reductase inhibitors, serenoa repens and mepartricin.*

tion, no evidence suggests a link between androgen levels or androgen PC sensitivity and PC risk. Although there is no controversy that lowering the T serum to castrate levels at least temporarily induces PC regression, over time, PC inevitably becomes resistant to ADT and survives in an androgen-independent state (see below).

Androgen deficiency and PC

As reported before, the majority of clinical studies have failed to support the notion that high androgen levels increase the risk of PC. In contrast, some recent data indicates that low serum T is associated with more aggressive, ADT-resistant tumors suggesting that low levels of androgens create a selective pressure for prostate cells which then become less dependent upon androgens (see below). An epidemiological study, including 17,049 men, indicates that high levels of androgens are associated with reduced risk of aggressive PC but not non-aggressive forms of the disease (36, 37) found in 211 patients – who underwent prostatic biopsy due to abnormal digital rectal examination and/or PSA level – and that patients diagnosed with PC have low levels of serum T and high levels of serum FSH, as compared to the patients with BPH. Several studies have also supported the notion that in clinically localized PC a low pretreatment serum T is associated with a more advanced pathological stage, higher recurrence rate following radical prostatectomy and increases the probability of non-organ confined disease and worse survival (38-41, see ref. 42 for review). By using mathematical modeling of the binding kinetics of prostate androgens, their relationship to proliferation and death in the prostate epithelium, and the evolution of AR expression, Eikenberry et al. (43) suggested that low androgen levels can increase selection for phenotypes resistant to hormonal therapy which may also be more aggressive. Whether low T levels are the cause or the consequence of the observed higher PC risk is a matter of speculation. Low T could be a marker of a pre-existing and/or more aggressive PC and not an independent risk factor for PC (44).

Androgen supplementation to hypogonadal PC

Since the traditional belief that PC growth is androgen-dependent in non-castrated men has been challenged (see above), the possibility of offering TRT to symptomatically hypogonadal PC subjects whose cancer has been successfully treated has been addressed by several case report studies. To date, results after radical prostatectomy have been reported in six studies (206 patients, see Table 1; 45-50), after radiotherapy in two studies (11 patients; 49, 51) and after brachytherapy in one study (31 patients; 52). Until the report of Leibowitz, after a quite variable follow-up (from 3.3 months up to 9 yr), clinical recurrence was observed only in one subject (over 158 described) with a Gleason score of 8 (49). In the Leibowitz study, collecting records of 96 patients who received TRT after initial management for PC from 2000 to 2007, nearly 60% of men had increasing PSA levels that triggered discontinuation of TRT, even though biochemical progression was not associated with clinical or symptomatic disease progression. In this series, however, the majority of PC subjects (61%) were treated, as a primary treatment, with ADT and therefore a TRT-associated PSA rise was not surprising. In conclusion, the number of reported cases is still small and heterogeneous; in the absence of randomized controlled trials the concept of using TRT for PC survivors is still debatable. Current recommendations including those of the major Andrology Societies (53) and ISSM (54) suggest to limit TRT to symptomatic hypogonadal men successfully treated for PC, after a prudent interval, the length of which, however, was not specified.

Table 1 - *Studies addressing the effect of testosterone replacement therapy in symptomatic hypogonadal men after radical prostatectomy. TT: total testosterone.*

Study	Age (yr) mean±SD (range)	No. of Subjects	Diagnosis of hypogonadism	Follow up Mean (range)	Recurrence
Kaufman et al, 2004 (45)	62±7 (50-70)	7	TT < 300 ng/dl	12-144 months	None
Agarwal et al, 2005 (46)	64 (59-69)	10	TT < 250 ng/dl	19 (9-29) months	None
Khera et al, 2009 (47)	64 (53-83)	57	TT < 300 ng/dl	13 (1-99) months	none
Leibowitz et al, 2010 (48)	61 (46-85)	96	Not reported	15 (1-83) months	56 discontinued TRT due to increasing PSA levels; 9 stopped TRT for reasons other than progression
Davila et al, 2008 (49)	67.5	14	Not reported	10.5 months	None
Nabulusi et al, 2008 (50)	61±9	22	TT < 300 ng/dl	20 (14-30) months	1/22

METABOLIC COMPLICATIONS OF ANDROGEN DEPRIVATION THERAPY

As previously discussed, ADT is the cornerstone in the treatment of advanced and metastatic prostate cancer. The use of ADT has significantly increased over the last few years from 3.7% in 1991 to 31% in 1999 (55). In particular, it has been reported that about 30% of men diagnosed with PC in the United States have received ADT (600,000 men) (56). Despite widespread use, there is clear evidence that ADT improves disease-free or overall survival only in two conditions: 1) in combination with primary radiation for locally advanced or high-risk diseases and 2) as adjuvant therapy for pN1 diseases after prostatectomy (57-59). Since the 5-yr relative survival for men with all stages of prostate cancer combined is more than 98% (59), it is reasonable to think that a "misuse and/or abuse" of ADT has occurred in the past few years. In addition, because androgens are essential for the regulation of different bodily functions including fat distribution, insulin sensitivity, lipid and bone metabolism, recent publications highlighted the concept that ADT may also be involved with increased overall and cardiovascular morbidity and mortality (57-60). The evidence supporting this hypothesis will be better addressed in the following sections (see also Table 2).

ADT and osteoporosis/bone fractures

The relationship between reduction of bone mineral density (BMD) and ADT is well known. In fact, both sexual hormones, estrogens and androgens, depleted by ADT, are essential in maintaining normal bone mass. Accordingly, it has been reported that BMD in the hip and spine reduces approximately 3% per year during ADT (61). A recent meta-analysis of the available studies has documented that PC patients treated with ADT had a 30% higher risk of osteoporosis and 17% higher risk of fracture when compared to patients not subjected to ADT (61). In addition, the duration of ADT was negatively correlated with lumbar spine and total hip BMD (61). Conversely, patients with PC had similar levels of BMD and lower rates of osteoporosis in comparison to healthy controls confuting the general idea that PC patients have lower BMD and higher risk of osteoporosis even before ADT as a result of age, underlying diseases, or other co-morbidities (62).

ADT and obesity

Both cross-sectional and longitudinal studies have demonstrated that ADT increases fat mass and decreases lean body mass in men with PC (57-60, Table 2). Basaria et al. (63) reported that subjects treated with ADT have increased fat mass in the trunk and extremities, when compared to eugonadal subjects with PC not treated with ADT or age-matched controls. In line with these findings, longitudinal evidence supported the concept that men with PC receiving ADT have significantly higher body weight and percentage of body fat (64-68). Interestingly, these changes appear to be evident even after a short term (3 months) of treatment (64, 65). The specific mechanisms linking low T to fat accumulation have not been completely clarified (69-71). Data derived from the general population demonstrate a bi-directional relationship. In fat, cross-sectional studies have shown a clear stepwise decline of T as a function of body mass index, while low T levels could contribute to the accumulation of excess fat, thus establishing a vicious cycle (69-71). Which molecule plays the major role in this cycle is still unknown; reasonable candidates are estrogens, insulin, leptin, TNFα or other adipokines (69).

The best strategy to prevent the ADT-induced adverse changes in body composition is unknown. Interestingly, Segal et al. (72), in a randomized study involving 155 men with PC treated with ADT, demonstrated that resistance exercise 3 times per week was able to minimize differences in fat distribution.

ADT and insulin resistance/diabetes

Two different meta-analyses of the available prospective studies indicated that low T levels predicted the development of type 2 diabetes mellitus (T2DM) in men (73, 74). In addition, Pitteloud et al. (75), demonstrated that T levels were positively correlated with insulin sensitivity, measured with a hyperinsulinemic-euglycemic clamp, independent of body composition. Similar results were reported by Muller et al. (76), and Yialamas at al. (77) demonstrated that acute withdrawal of TRT was associated with an impairment of insulin sensitivity, without changing body composition. Furthermore, although only few reports have been published, we recently demonstrated, using a meta-analytic method, that TRT is able to

Table 2 - *Descriptive characteristics of the available studies evaluating the impact of androgen deprivation therapy (ADT) levels on cardiovascular morbidity and mortality. PC: prostate cancer; RT: radiotherapy: TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; BMI: body mass index; HOMA: homeostatic model assessment of insulin resistance; CVD: cardiovascular diseases: ↔= non changed; ↑ = increased; ↓ = decreased.*

Study	AGE (yr) mean±SD	No. of subjects under ADT	Duration of ADT	Control group	Fat mass	Lean mass	BMI/ weight	Insulin/ HOMA	Fasting glycemia	Lipid profile	Incident T2DM/CVD
Basaria et al, 2002 (63)	68.4±8.7	20	12-101 months	18 non ADT PC 20 healthy controls	↑	↔	↑	NA	NA	NA	NA
Smith et al, 2001 (64)	67±8	22	3 months	None	↑	↓	↔	↑/NA	↔	↑ TC, HDL	NA
Smith et al, 2002 (65)	66±2	32	12 months	None	↑	↓	↑	NA	NA	↑ TC, LDL, HDL, TG	NA
Smith et al, 2006 (66)	68±2	25	12-101 months	None	↑	↓	↔	↑/NA	NA	↑ TC, HDL, TG	NA
Chen et al, 2002 (67)	74.3	67	12-60 months	47 healthy men	↑	↔	↑	NA	NA	NA	NA
Lee et al, 2008 (68)	66±10	65	12 months	None	↑	↓	NA	NA	NA	NA	NA
Dockery et al, 2003 (78)	70.5±8.0	16	3 months	15 healthy men	NA	NA	NA	↑/NA	↑	NA	NA
Basaria et al, 2006 (79)	70.2±1.8	18	12 months	17 non ADT PC 18 healthy controls	NA	NA	NA	↑/↑	↑	NA	↑/NA
Keating et al, 2006 (80)	66 or older	31,620	at least 6 months	41,548 non ADT PC	NA	NA	NA	NA	NA	NA	↑/↑
Braga-Basaria et al, 2006 (82)	69.6±1.7	16	12-101 months	14 non ADT PC 14 healthy controls	NA	NA	NA	NA	NA	↑ TC, LDL and non-HDL	NA
Saigal et al, 2007 (83)	66 or older	4810	at least 6 months	18,006 non ADT PC	NA	NA	NA	NA	NA	NA	NA/↑
D'Amico et al, 2007 (84)	41-87	Arm 1 102 RT + 6 month ADT	3-8 months	Arm 2 104 RT alone	NA	NA	NA	NA	NA	NA	CV death sooner in those older than 65 yr
Tsai et al, 2007 (85)	65 or older	1015	1-33 months	3877 non ADT PC	NA	NA	NA	NA	NA	NA	NA/↑
Efstathiou et al, 2008 (86)	70	Arm 1 758 long term ADT	Long term, 28 months Short term, 4 months-	Arm 2 763 short term ADT	NA	NA	NA	NA	NA	NA	NA/↔
Roach et al, 2009 (87)	Median 70	Arm 1 221 RT + short term ADT	Arm 1, 4 months Arm 2, NA	Arm 2 232 RT alone	NA	NA	NA	NA	NA	NA	NA/↔
Efstathiou et al, 2009 (88)	70	Arm 1 477 RT + ADT	Arm 1, 0-14.1 yr Arm 2, NA	Arm 2 468 RT + ADT only at relapse	NA	NA	NA	NA	NA	NA	NA/↔
Studer et al, 2006 (89)	65 or older	Arm 1 492 immediate ADT	median follow-up of 7.8 yr	Arm 2 493 deferred ADT	NA	NA	NA	NA	NA	NA	NA/↔
Nanda et al, 2007 (90)	Median 69.5	1521	Median 4 months	3556 non ADT PC	NA	NA	NA	NA	NA	NA	↑ CV only with preexisting comorbidities
Hayes et al, 2007 (91)	65-75	4531	3-5 months	8261 non ADT PC	NA	NA	NA	NA	NA	NA	↑ all cause mortality only with preexisting comorbidities

improve glycometabolic control (reduction of fasting glycemia and glycated haemoglobin) in hypogonadal patients with T2DM. The results observed in subjects treated with ADT for PC are in line with this evidence (Table 2). Short term ADT (3-6 months) is associated with an increase of insulin circulating levels without any significant modification of fasting glucose suggesting the development of insulin resistance (64, 66, 78). Accordingly, Basaria et al. (79) demonstrated, in a small study involving a total of 53 men, 18 of whom had PC treated with ADT for at least 12 months, that 44% of the ADT group developed T2DM compared to 12% and 11% in the non-ADT and control groups, respectively. Similarly, data from Surveillance, Epidemiology, and End Results (SEER) and Medicare, collecting uniform records from 73,196 men diagnosed with local or local regional PC between 1992 and 1999, demonstrated a 44% increased risk of incident diabetes in men treated with ADT (80).

ADT and dyslipidemia

Conflicting results have been reported on this topic (Table 2). In fact, although different studies have documented that ADT in men with PC is associated with an increase of total and LDL-cholesterol and triglycerides (65, 81, 82), one report did not show any change in lipids after three months of ADT. Similarly to that which was observed with glucose modification, the majority of the studies showed that the adverse effects on serum lipids developed within the first three months of ADT. Since HDL-cholesterol also increased in some studies, its contribution to cardiovascular risk in men treated with ADT remains unclear.

ADT and increased cardiovascular risk

The possible relationship between hypogonadism and cardiovascular (CV) risk is under intensive debate. Epidemiological data suggest that low T might be associated with an increased risk of CV death in community-dwelling men, and in men with ED (71). However, the possibility that low T, observed during chronic diseases, provides a type of protective mechanism, which could "turn off" T-dependent functions (such as reproduction and physical labour) which are not desirable when the physical condition is ailing, cannot be excluded at all (71). Data derived from PC patients treated with ADT further complicate the current discussion (Table 2). Some, but not all, studies have reported an association between ADT and forthcoming CV diseases (CVD). Keating et al. (80) from SEER-Medicare dataset, reported a significantly increased risk of incident coronary heart diseases (HR=1.16), myocardial infarction (HR=1.11) and sudden cardiac death (HR=1.16) in men treated with ADT. Similarly, a subsequent retrospective analysis based on 23,000 men from the SEER-Medicare database confirmed that ADT caused a 20% increased risk of CVD after 1 year (83). The pooled data from three randomized PC trials showed that men 65 yr old or older treated with ADT for at least 6 months had shorter times to fatal myocardial infarction (MI) when compared to age-matched men not receiving ADT or younger individuals (84). Furthermore, Tsai et al. (85), evaluating data from the Cancer of Prostate Strategic Urologic Research Endeavour (CaPSURE) database on almost 5000 patients, found that men receiving ADT appear to be associated with an increased risk of death (HR=2.6) from cardiovascular diseases independently of age.

In contrast with the aforementioned reports, three large Radiation Therapy Oncology Group (RTOG) trials involving a total of almost 3000 men with PC have shown comparable CV mortality regardless of ADT assignment (86-88). In addition, the European Organization for Research and Treatment of Cancer (EORTC) trial, enrolling 985 men with PC not suitable for local treatment, did not show any difference in cardiovascular mortality based on timing of ADT (89).

Recent reports have emphasized the concept that ADT might adversely affect CV morbidity and mortality, particularly in men with pre-existing comorbid CV illness. Nanda et al. (90) reported that ADT was significantly associated with an increased risk of all-cause mortality among men with a history of heart failure or MI but not among men with no comorbidity or a single CV risk factor at baseline. Similar data were more recently reported by the Hayes study (91).

MOLECULAR MECHANISMS OF ANDROGEN INDEPENDENCE

The molecular mechanisms leading to the development of androgen-independent (AI, also named castration resistant) prostate cancer (AI-PC) have not yet been fully disclosed and are still being elucidated. The lack of definitive information about this process can be explained by the fact that transition to androgen-independence is complex and involves both selection and outgrowth of pre-existing AI clones, as well as adaptative up-regulation of genes that help the cancer cells to survive and grow after ADT of the patient. The fact that more than one theory has been developed to explain this transition reflects the peculiar characteristics of PC: indeed these tumors are heterogeneous (due to a multifocal origin and/or to a genetic instability of the initial cancer) and comprised of various subpopulations of cells, which respond differently to androgens (92). The presence of multiple genotipically distinct tumours together with prostatic intraepithelial neoplasia (PIN) in untreated patients (93) supports the hypothesis that PC contains cells with different sensitivity to androgens (for instance, it has been calculated that about 1 out of 1 million cells is androgen independent (94). The heterogeneous population of PC also represents a prerequisite for explaining the adaptative theory: indeed cells respond differently to the therapy, and while most of the androgen-dependent cells die, others slowly become AI and start to activate alternative pathways of growth, and to up-regulate anti-apoptotic genes.

Concerning the molecular events that drive transition to AI, they can be divided into those involving the androgen receptor and those that bypass it (Fig. 3).

Mechanisms involving androgen receptor

Several lines of evidence suggest that amplification and mutations of AR may be involved in the AI process. A study performed *in vitro* and *in vivo* in castrated nude

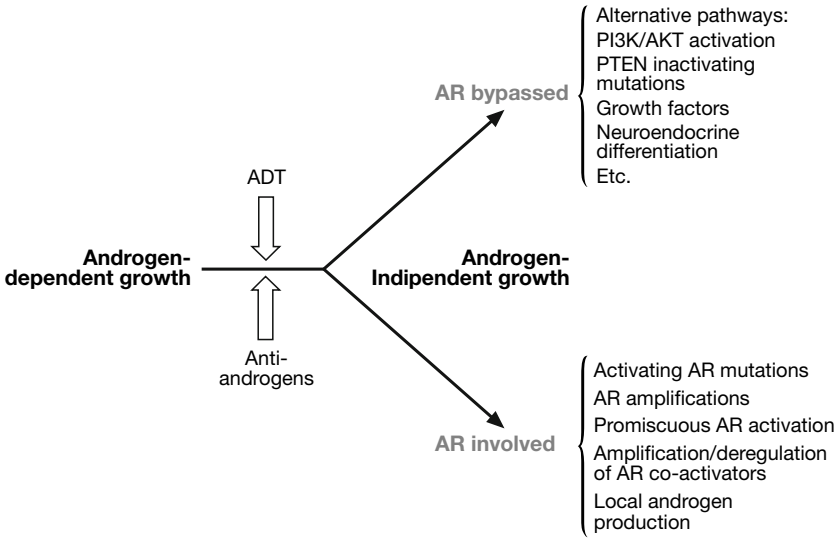

Fig. 3 - *Schematic representation of the main pathways hypothesized to lead to androgen independence after androgen deprivation therapy (ADT). AR: androgen receptor.*

mice employing AI cell lines and xenographs derived from LNCaP (95) demonstrated that an increase in AR expression is the main determinant of the transition to AI. However, whether an increase in AR expression occurs following androgen-ablation *in vitro* in PC cell lines is a matter of substantial controversy (95-97), which appears to depend on experimental conditions. Studies in human tumor specimens demonstrated that amplifications of AR are found in about 30% of AI-PC tumors (98, 99) and appear to develop as a consequence of ADT, as they are not present in the original tumors. Conversely, AR mutations that confer ligand promiscuity may be present also in hormone naïve PC but appear to be more frequent in AI ones (100). Over 90 AR mutations naturally occurring in PC have been described. They regard about 20% of AI-tumors and, interestingly, those naïve tumors bearing AR point mutations seem to be more aggressive and become AI faster. Other mechanisms that may increase AR function leading to AI include alterations of AR co-regulatory molecules, found to be increased in AI tumors (101), ligand-independent activation of AR and increased intraprostatic androgen levels. Concerning the latter, intratumor conversion of adrenal androgens as well as *de novo* synthesis have been hypothesized as possible causes of transition to AI (102, 103). However, a recent study (104) performed in several experimental models of PC cells, as well as in tissue specimens of different origins (see below) has questioned such a theory, concluding that intraprostatic androgen biosynthesis contributes very little to progression.

The demonstration of occurrence of gene fusions between the androgen regulated gene TMPRSS2 and ETS family members in a high percentage of PC (105), has stimulated research on the role of these chromosomal aberrations in progression to AI. So far, however, the role of TMPRSS2 - ETS fusions in PC progression remain unclear and controversial (106, 107).

AR-involving hypotheses do not completely explain why patients receiving ADT tend to have an earlier development of more aggressive types of PC. There is evidence in the literature that AR, besides promoting cancer progression, may also be involved in maintaining a more differentiated phenotype (108; see below) and that the signaling and the function of AR may be different in stromal and epithelial prostate cells and even in cancer cells derived from different tumor foci of the same patient (109).

Androgen receptor bypassing mechanisms

The activation of alternative pathways of growth, such as PI3K/AKT/mTOR, is considered to play a relevant role in the development of AI (see for review, 110). The incidence of activating mutations of PI3K in PC is of about 30% and, more importantly, the occurrence of inactivating mutations/functional loss of the enzyme PTEN (phosphatase and tensin homolog, which is deputed to interrupt AKT activation in the PI3K/AKT pathway) are frequently found in PC metastasis. Abnormal activation of PI3K/AKT/mTOR pathway may also be obtained by activation of growth factor pathways [such as EGF/EGFR pathway (111)] due to abnormal growth factor synthesis or activating mutations/amplifications of their receptors. In these situations, apoptotic pathways are inhibited and proliferation continues in a low/suppressed androgen environment. Activation of PI3K/AKT/mTOR pathway may also lead to activation of AR (112), further supporting AI growth of the cells. Although targeting the PI3K/AKT pathway for the treatment of PC could be of value especially for patients where alterations of the pathway are present (112), the initial enthusiasm for this therapy has been mitigated by a recent report by Alimonti et al. (113). In this study, it has been demonstrated that the PI3K/AKT pathway in tumor cells drives the process of cellular senescence – which is now emerging as an intrinsic tumor suppressive mechanism – if *Pten* is lost or mutated, and that pharmacological inhibition of *Pten* drives senescence in human models of PC. These effects may limit the efficacy of PI3K/AKT targeted therapies.

A relevant event occurring during development of AI is neuroendocrine differentiation (NED) (114). Neuroendocrine cells (NE), which are present both in normal and

cancerous prostate tissue, do not express AR, and produce and secrete serotonin and other peptides able to profoundly influence the surrounding environment. The incidence of NED in prostate tumours is considered a promising prognostic indicator for the development of AI disease (115, 116), as they supply both proliferative and survival factors to cancer cells (117). In addition, NED is related to greater tumour aggressiveness and poor patient prognosis (118). Recently, we have shown that androgen ablation induces NED in androgen-dependent but not in independent PC cell lines, evidencing a possible role of EGF in inducing NED in the latter (119).

The role of androgen receptor in prostate cell differentiation/prostate cancerogenesis

Using ARKO mice, where the receptor has been differentially knocked in epithelial, fibroblast and smooth muscle cells of the prostate, it has been demonstrated that during prostate development, AR has different functions, from proliferative in stromal to growth suppressor in epithelial luminal cells (109). In PC cells, AR seems to have a similar dual function. Mouse PC models lacking the AR only in the prostatic epithelium and/or stroma have been generated (ARKO-TRAMP) (120, 121). These mice paradoxically develop poorly differentiated PC and, more importantly, restoration of AR function only in epithelial basal cells leads to tumor suppression. Conversely, restoration of AR in stromal cells stimulates cancer progression, supporting a differential role of AR in PC depending on its location (differentiating in the epithelium, oncogenic in the stroma). Similarly, *in vitro* studies have shown that enforced expression of AR in AR-negative PC cells may decrease the metastatic/invasive potential of the cells (120, 122-128). Furthermore, enforced expression of AR in transformed prostate epithelial cells induces differentiation to a luminal phenotype similar to that of organ-confined PC (129). In a recent paper evaluating the role of androgen signaling in epithelial-mesenchymal transition, a process involved in progression of PC, Zhu and Kyrianou (130) demonstrated that, in contrast to low AR content, over expression of AR in PC cell lines suppresses androgen-induced epithelial-mesenchymal transition. There are also clinical data supporting a differentiating role of AR in PC and demonstrating promotion of metastatic lesions following ADT (131). Indirect support comes from the clinical evidence that intermittent ADT benefits patients with regards to PC progression (132). In addition, patients with AI-PC displaying amplification of the AR gene in carcinoma cells survive longer than patients without amplification (132).

Intraprostatic androgen synthesis

As mentioned above, although recently questioned, intraprostatic androgen synthesis may contribute to increased proliferation as well as transition to AI and progression of PC. Indeed, intraprostatic androgen levels may remain elevated in PC patients undergoing ADT (11, 12, 103). Using the androgen-dependent LNCaP xenograph model, Locke et al. (102) demonstrated that intratumoral androgen levels increase as the tumor progresses to AI and, more importantly, all enzymes necessary for androgen synthesis are expressed and up-regulated in PC. However, a recent paper by Hofland et al. (104) directly evaluating the expression of steroidogenic enzymes required for *de novo* synthesis of steroids in 88 PC tumors, reported expression of the two essential enzymes for androgen biosynthesis (CYP17A1 and HSD3B1) only in 19 of them, but only 5 of the latter express both enzymes. Conversely, the expression of the enzymes AKR1C3 and SRD5A1, that convert, respectively, androstenedione to T and the latter to DHT are abundantly expressed in all the tumors (104), implying that blocking intraprostatic T conversion may represent a favorable therapeutic option for AI-CP.

CONCLUSIONS

Growth and differentiation of the prostate gland is clearly androgen-dependent, however, clinical data indicate that such dependency is evident only in the hypogonadal condition (the "saturation hypothesis"). At physiological T concentration, the evidence suggests a link between androgen levels or androgen PC sensitivity and PC risk. Androgen receptor has different functions in prostate cells, from proliferative in stromal to growth suppressor in epithelial luminal cells. There is unequivocal evidence that reducing androgen signaling to the hypogonadal range can reduce PC growth and patient symptoms. However, different strategies of ADT for advanced PC are only palliative and rarely cure patients. In fact, in PC cells, AR, besides promoting cancer progression, is also involved in maintaining a more differentiated phenotype. Several studies have also supported the notion that in clinically localized PC, a low pretreatment serum T is associated with a more advanced pathological stage, higher recurrence rate following radical prostatectomy and increases the probability of non-organ confined disease and worse survival.

Transition to AI is complex and involves both selection and outgrowth of pre-existing AI clones, as well as adaptative up-regulation of genes that help the cancer cells to survive and grow after ADT. Intratumoral synthesis of androgens, up-regulation of AR (amplification and/or mutations) or of its co-stimulatory proteins and growth factors, which transactivate the receptors in a ligand-independent manner, might select more aggressive and fast growing clones, which often circumvent ADT. In addition, because androgens are essential for the regulation of different bodily functions including fat distribution, insulin sensitivity, lipid and bone metabolism, recent publications highlighted the concept that ADT may also be involved with increased overall and cardiovascular morbidity and mortality. Hence, while ADT still represents a cornerstone for the palliative therapy of a small fraction of aggressive PC, a "misuse and/or abuse" of ADT should be avoided.

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