Review – Prostate Cancer

Progress in Understanding Androgen-Independent Prostate Cancer (AIPC): A Review of Potential Endocrine-Mediated Mechanisms

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Abstract
This review is triggered by recent developments that offer new explanations for the mechanism of progression of prostate cancer to androgen independence. Established and hypothetical mechanisms, which have been described in the past, are put into perspective with recent progress in the field. A total of seven mechanisms can be identified that relate to progression to androgen independence. Five of those are dependent on the androgen receptor, which is present or over-expressed in androgen-independent prostate cancer tissue. Probably due to selective pressure, AIPC cells have the capability to escape from the effect of castration and antiandrogens; exclusion of the androgen receptor activity by inhibition of dimerisation or inhibition of DNA binding seem to be the logical next steps.

Although androgen levels and androgen synthesis are suppressed in prostatic tissues during the phase of response to endocrine treatment, androgen levels and, specifically, 5-α-dihydrotestosterone (DHT) were elevated in tissues derived from metastases of AIPC. In addition, all enzymes needed to synthesise androgens from the level of pregnenolone on are present or over-expressed in such tissue. This offers new potential for treatment.

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1. Background

The effect on prostate cancer of lowering circulating androgens by castration or by reducing plasma testosterone levels through interference with the estrogen-driven diencephalic pituitary feedback mechanism was first observed and described by Huggins [1]. Eventually, if sufficient time is allowed to elapse, virtually all prostate cancers will become androgen independent (AIPC). The mechanisms leading to androgen independence have been subject to extensive research in the past. Most likely even at this time these mechanisms are only incompletely understood. Recent discoveries and
developments shed new light on different factors and molecular events that may contribute to the development of androgen independence. These recent developments together with more established mechanisms are subjects of this review.

1.1. Epidemiologic aspects

Considering the stage at the time of diagnosis there is strong variability in overall and prostate cancer-specific survival. Of the men initially presenting with metastatic disease 85% will die of prostate cancer. The median survival of such men in older but representative studies is in the range of 2.5–3 yr [2]. These rates are dramatically more favourable if prostate cancer is diagnosed at an early stage. Data from the only available randomised study of radical prostatectomy versus watchful waiting of 695 men with locally confined disease show that with an average follow-up of 8.2 yr 14.4% of men in the watchful waiting arm and 8.6% in the surgery arm died of prostate cancer [3]. Metastatic progression and prostate cancer death in both arms strongly depended on prognostic factors [4].

Despite recent advances in the management of locally confined disease, in most Western countries prostate cancer remains the second or third most frequent cause of death from any type of cancer in men. Table 1 shows numbers of cases and crude rates for incidence and mortality in the world, northern Europe, and northern America for the year 2000. The numbers of prostate cancer deaths illustrate the magnitude of the problem worldwide. Ratios of incidence and mortality are strongly variable among different parts of the world. The high ratio of 7.1 is most likely due to prostate-specific antigen (PSA)-driven early detection that is more common in northern America [5]. The comparison with 1982 data illustrates the effect of recent developments, mainly of screening and the resulting lead time. In the United States between 1995 and 2004 an average annual decrease of prostate cancer mortality of 4.1% has occurred [6]. The reason for this decrease, which is not observed in a similar way in most other countries of the world [7], is not clear. Most likely contributing factors are the increased early detection and early treatment as the result of PSA-driven screening [8], more effective treatment of locally advanced disease [9], and the frequent use of different types of statins in the Western world [10] that will be discussed further in section 3.2.

1.2. Progression to androgen independence

Progression to androgen independence is frequently signalled by a rise of PSA under endocrine treatment and definitively confirmed by progression to metastatic disease. Next to the review of knowledge available prior to the year of 2005, recent developments warrant an update of our understanding of AIPC. Most of the mechanisms of escape from endocrine control are related to the presence of a functional androgen receptor (AR), but its function in AIPC was and is poorly understood. The developments that are described in section 3 are major steps toward an improved understanding of AIPC. Time to progression to AIPC for any tumour stage is not influenced by different types of endocrine treatment. Testicular androgens can be excluded by castration or the use of a luteinising hormone-releasing hormone (LHRH) agonist or antagonist. Androgens derived from the adrenal glands are metabolised peripherally and in the prostate to testosterone and 5-α-dihydrotestosterone (DHT), the most active androgen. Adrenal androgens can be counteracted by the use of antiandrogens. The combination of castration or an LHRH agonist with an antiandrogen was termed maximal androgen blockade. The regimen was established and studied early on by Labrie [11]. A meta-analysis of all subsequently conducted randomised studies [12] and the most relevant single randomised study conducted in the United States [13] did not show statistically significant or clinically relevant differences in overall survival or prostate cancer-specific survival. This is most likely due to a rather small contribution of adrenal androgens to the endocrine control of prostate cancer. As mentioned above, the reason metastatic progression under endocrine treatment does not always lead to death from prostate cancer is due to the high prevalence of

| Table 1 – Incidence and mortality of prostate cancer in different areas of the world [5] |
|----------------|----------------|----------------|----------------|
|                | Incidence      | Mortality      | Ratio          |
|                | Cases, n       | Crude rate     | Deaths, n      | Crude rate |
| World          | 679,023        | 21.7           | 221002         | 7.1        | 3.1 |
| Northern Europe | 46,974         | 100.4          | 16771          | 35.9       | 2.8 |
| Northern America | 257,943       | 163.7          | 36447          | 23.1       | 7.1 |

intercurrent disease at the age of 70–72 yr when prostate cancer mortality cumulates.

2. AIPC: our understanding up to the year of 2005

AIPC recapitulation follows closely the excellent reviews by Feldman and Feldman [14] and Grossman et al [15]. At this time five different potential mechanisms for the development of AIPC were identified. These mechanisms are briefly summarised in Table 2 and are discussed and updated in the following sections. This recapitulation is necessary to provide proper background for understanding section 3 and the conclusions of this review. The puzzle can only be solved if all pieces are available. Also, going back to older findings, it becomes evident that the basic hypotheses-building findings date back many years.

2.1. Preexisting genetic changes in prostate cancer stem cells

On the basis of their experimental observations, Coffey and Isaacs [16,17] postulated that androgen independence of prostate cancer was due to a selective growth advantage of preexisting hormone-independent clonal populations of pre-existing, hormone-independent stem cells. The total population of prostate cancer cells in this setting is considered to be a mixture of hormone-dependent and hormone-independent cells from the time of initiation of the malignancy. Progression to hormone independence would then be explained by proliferation of the hormone-independent cell populations possibly due to a growth advantage originating from suppression by androgen ablation of the growth of hormone-dependent cancer cells. This mechanism, if it would play a major role, is likely to be completely independent of androgen- and AR-dependent growth stimulation.

2.2. Oncogenes, the inhibition of apoptosis

Another androgen- and AR-independent mechanism has been established in experimental systems and is likely to play a role in clinical settings. These mechanisms include the activation of oncogenes, the inactivation of tumour suppressor genes, or the over-expression of Bcl2, which is part of a mechanism capable of inhibiting apoptosis. McDonnell et al found that Bcl2 was undetectable in 13 of 19 cases of androgen-dependent prostate cancer tissues. In contrast, AIPCs showed high levels of Bcl2 staining. In normal human prostate Bcl2 expression was limited to the basal epithelial cells [18]. However, the observation of these coinciding findings does not establish a causal relationship. Others have found that expression of Bcl2 in prostate cancer cells was an independent predictor of treatment failure in localised disease [19]. Bcl2 has been addressed as a therapeutic target in hormone-refractory prostate cancer and antisense therapy was also developed for the MDM2 oncogene. Clinical trials are pending or ongoing [20]. Recent reviews are available [21,22].

Inactivation of the PTEN tumour suppressor gene was found to be associated with locally progressive prostate cancer [23]. Also, biallelic inactivation of PTEN in mouse prostate was shown to induce prostate cancer in an animal that does not usually develop this disease. The resulting proliferation was not accompanied by reduced apoptosis indicating that the proliferative mechanism is independent of the Bcl2-related cascade [24]. Although these mechanisms are primarily androgen and AR independent, the finding of a gene fusion between the androgen-driven TMPRSS2 gene and members of the ERG-ETS oncogene family opens the possibility that other oncogenes may also be dependent on androgens [25].

2.3. Ligand-independent AR activation

Culig at al have shown that the AR can be activated by the insulin-like growth factor 1 (IGF-1), the
keratinocyte growth factor (KGF), and the epidermal growth factor (EGF) [26]. The author also showed that these growth factors are over-expressed in some prostatic cancers and that the antiandrogen bicalutamide blocks the IGF-induced AR activation. This questions the potential of this mechanism to introduce androgen-independent growth. Receptor tyrosine kinase activated pathways have been targeted by several new drugs including Herceptin with limited activity in AIPC [27].

Although the mechanisms described so far seem to have no direct relation to androgen regulation, the intriguing finding that the AR is expressed in most AIPC tissues suggests that different mechanisms may overlap [28]. Other authors have shown that the androgen-binding capacity of the AR in AIPC cells is intact [29]. This leads to the review of those potential mechanisms of progression to AIPC that are dependent on the AR.

### 2.4. AR hypersensitivity

Amplification of the AR leading to increased sensitivity and response to very low levels of androgens was found in about one third of tissues derived from hormone-independent prostate cancer [30,31] and found to be positively related to increased progression measured by the proliferation marker KI-67 [32]. Hypersensitivity of the AR may also explain failure of antiandrogens resulting from an imbalance of the increased number of receptor molecules and the available number of competing molecules of the antiandrogen in the nucleus [33]. Obviously, increased intraprostatic levels of androgens due to increased production of DHT in the prostate as suggested by Labrie [34] or changes of the sensitivity of the AR [35] will have the same result: stimulation of growth due to activation of the AR.

### 2.5. Change of AR specificity (AR mutations)

The AR gene is located on the X chromosome. Germline mutations leading to a loss of AR function and to the clinical androgen insensitivity syndrome are not infrequent. Complete loss of AR function is compatible with life [36]. Veldscholte et al [37] were the first to discover a mutation leading to a functional change of the AR in the LNCaP cell line. This mutation resulted in a substitution of alanine for threonine at position 877 of the AR (the T877A mutation). The presence of this mutation changes the growth inhibitory effect of flutamide to a growth-stimulating effect of the same substance. Numerous other AR mutations were subsequently discovered [38]. Ruijter et al present a summarising review [39].

At present, it remains uncertain how frequently AR mutations lead to androgen insensitivity in a clinical setting. Experience relating to the use of castration or an LHRH agonist in combination with an antiandrogen as initial treatment in prostate cancer (maximal androgen blockade) has shown that flutamide withdrawal may lead to clinical remissions in about 30% of cases with a duration of 3–4 mo [40]. AR mutations seem to be at least in part due to the selective pressure exerted by the use of antiandrogens. Taplin et al identified the T877a mutation in 5 of 16 patients who received maximal androgen blockade using flutamide [41]. Later, in a study of 48 specimens obtained from bone marrow metastases of prostate cancer progressing under endocrine treatment, 5 (10%) had a mutation in the hormone-binding domain [42].

In summary, a number of mechanisms have been identified and related to androgen-independent progression of prostate cancer. Overlapping effects are likely. Androgen insensitivity may at the same time be due to the presence of two or more of the mechanisms described and cited above. Available clinical data do not allow precise determination of the relative frequency with which the events leading to AIPC occur. However, undoubtedly, the mechanisms leading to AIPC even without consideration of the most recent developments during 2006 and 2007 must be considered frequent events that may overlap in their occurrence.

### 3. AIPC developments after 2005

A number of recent discoveries probably will drastically change our understanding of progression of prostate cancer to androgen independence. This review includes information that, so far, is only available in the form of abstracts. This may, with peer-reviewed publications pending, add an element of uncertainty to this review. Still, available data fit the resulting hypothesis quite well. Therefore, their review seems to be justified at this time.

#### 3.1. Gene fusions

Gene fusions between the androgen-dependent TMPRSS2 gene and oncogenes of the ETS family were described by Tomlins et al [25]. Such fusions may play a role in the initiation or promotion of prostate cancer but may also provide a mechanisms for explaining the progression of clinical prostate cancer from the hormone-dependent to the hor-
mone-independent state. However, proof of such causal relationships is largely pending. Expression of the fused gene results from binding of the activated AR to the androgen responsive element of TMPRSS2. In this way, the activated AR actually mediates the function of an oncogene. Presence of the fusion mainly between TMPRSS2 and the ERG and ETV1 members of the ETS oncogene family were observed in hormone-dependent and in hormone-independent prostate cancer xenograft lines [43]. Direct evidence of a more frequent presence and expression of the fusions genes in hormone-unresponsive prostate cancer is, however, not available at this time. Since the original publication [25] at least 14 studies have appeared that confirm the presence of TMPRSS2-ETS family member fusions in a large proportion of clinical cases or prostate cancer lines [42–47]. In the evaluation of clinical cases usually a combination of reverse transcriptase-polymerase chain reaction (RT-PCR) techniques and fluorescence in situ hybridisation (FISH) is used. Several studies report on radical prostatectomy or biopsy specimens and find either one of the fusions in 36%, 42%, 43%, and 50% of cases [43–48]. One recent study of 165 patients treated by radical prostatectomy revealed the presence of one of the gene fusions in 81 (49.1%) of cases. Also, after adjusting for grade, stage, and PSA level the hazard ratio for PSA recurrence of gene-positive cases was 8.6 ($p < 0.0001$; Table 3). This is the only large clinical study suggesting that the TMPRSS2-ERG gene fusion is an independent prognostic parameter in locally confined prostate cancer [49]. It does not relate to AIPC. Only one study [50] so far has shown the TMPRSS2-ERG fusion to be present more frequently in lethal prostate cancer prospectively treated by watchful waiting (cumulative incidence ratio 2.6, $p < 0.01$). The TMPRSS2-ERG gene fusion has also been identified in 21% of 19 evaluated high-grade prostatic intraepithelial neoplasia (PIN) lesions [47]. Attard et al [51] studied 445 tissue specimens from men considered to have minimal prostate cancer and to be candidates for active surveillance; 311 (70%) did not have the fused gene. This is compatible with the unproven possibility that gene fusion varies with the stage of the biologic development of a given cancer. Obviously, markers for potentially indolent disease are urgently needed to help to curb present over-diagnosis and over-treatment of screen-detected prostate cancer.

Although there is theoretical potential for TMPRSS-ETV oncogene family gene fusions to be related to prostate cancer progression to androgen independence, direct causal clinical evidence for the

<table>
<thead>
<tr>
<th>TMPRSS2-ERG gene fusion status</th>
<th>Positive</th>
<th>Negative</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical progression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37 (45.7%)</td>
<td>6 (7.1%)</td>
<td>43 (26.1%)</td>
</tr>
<tr>
<td>Negative</td>
<td>44 (54.3%)</td>
<td>78 (92.9%)</td>
<td>122 (73.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>81 (49.1%)</td>
<td>84 (50.9%)</td>
<td>165</td>
</tr>
</tbody>
</table>

- 165 cases of radical prostatectomy.
- End point: biochemical recurrence.

Fig. 1 – AR expression in BPH and endocrine therapy-unresponsive prostate cancer. Samples obtained via transurethral resection. AR = androgen receptor; BPH = benign prostatic hyperplasia. Reprinted with permission from van der Kwast TH et al. Int J Cancer 1991;48:189–93.
involvement of this mechanism is not available at present.

3.2. **Androgen synthesis in AIPC tissues**

Two findings relating to AIPC obtained in the past have been puzzling and remain unexplained and unconnected to the event of androgen-independent growth of prostate cancer. In 1991, van der Kwast et al showed that almost consistently the AR studied by immunohistochemistry remained over-expressed in primary prostate cancer tissue that was progressive under endocrine treatment [28] (Fig. 1). The study was based on freshly obtained and frozen tissue in 17 cases of local progression under endocrine treatment requiring transurethral prostatic resection. In 13 of the 17 examined specimens at least 80% of the nuclei of tumour cells were AR positive. Only one case seems to be completely AR negative. Shortly thereafter it was confirmed that the AR in AIPC tissue was structurally intact and has a normal binding capacity [29]. The other finding, the fact that PSA still serves as a prognostic marker in AIPC despite castration levels of serum androgens, has always been suggestive of remaining androgen activity that is not counteracted by castration or the use of an LHRH agonist. The promotor region of the PSA gene contains several androgen-responsive elements (AREs); PSA expression, therefore, requires an activated AR.

These findings provide links to recent developments that show that high androgen levels are preserved in tissue derived from metastases of patients with AIPC suggesting a high level of intracrine activity. Stanbrough [52] studied 33 prostate cancer specimens that were obtained from bone marrow metastases and preserved by snap freezing. Findings were compared to a group of 22 prostate cancer specimens obtained from primary prostate cancers. The study showed an increased expression of a number of genes involved in the androgenic part of the steroid metabolism. This included the genes 3βHSD2, AKR1C3, SRD5A1, as well as AKR1C1 and C2 and UGT2B15. These genes all encode for enzymes that are involved in the androgen synthesis from the level of pregnenolone and progesterone on downward. The AR was found to be 5.8-fold over-expressed. Interestingly over-expression was also seen for SRD5A1, the gene that encodes for 5α-reductase type 1 but not type 2, which is predominant in tissue of benign prostatic hyperplasia (BPH).

More recently these data were confirmed in a study of six cases of AIPC with metastatic disease. Tissue from bone metastases was obtained by “warm autopsy” and immediately frozen. The study showed levels of DHT that were equivalent to non-castrate prostate cancer tissue. Testosterone levels, however, were found to be 3.8 times higher than in PC tissue from non–androgen-deprived patients. The group identified transcripts for enzymes of the androgen synthesis including cytochrome P17A, (CyP17A), aldo-keto reductase 1 C3 (AKR1CK), 3β-hydroxysteroid dehydrogenase-2 (3βHSD2) and SRD5A2, and the gene encoding for 5α-reductase type 2 (5-α-R2), which were all shown to be significantly up-regulated in the metastatic samples. The data suggest increased synthesis and degradation of androgens in AIPC tissues [53].

Clearly, probably due to selective pressure of castration and antiandrogenic treatment regimens, prostatic tissue is capable of developing escape mechanisms such as the amplification and over-expression of the AR and the establishment of androgen synthesis in prostatic tissue. Interestingly, the findings above are at variance with the well-documented and frequently described decrease of androgens in serum and in the prostate and prostatic cancer tissue specimens during the phase of response to endocrine treatment. Older observations have recently been confirmed [54,55]. The data referred to above need confirmation but, if confirmed, necessitate a dramatic revision of earlier understanding of the mechanisms of AIPC. At the same time new targets, specifically the enzymes active at different levels of the androgen synthesis, become available. None of the studies so far has shown or targeted therapeutically expression or over-expression in adult prostate or prostate cancer.

![Fig. 2 – Substrate cholesterol. Tissue levels in normal prostate, benign prostatic hyperplasia (BPH), prostatic carcinoma (Ca), and fibromuscular stroma (FM). Reprinted with permission from Goldstein. Ph.D. dissertation, Rutgers University, New Brunswick, NJ, 1975.](image-url)
tissue of the cytochrome P11A, the enzyme that synthesises pregnenolone from cholesterol. CyP11A was, however, shown at high levels by RT-PCR in an fetal prostate [56]. This pathway should be subject to urgent investigation with high priority as studies dating back to 1970s have shown high levels of cholesterol in prostate cancer. Cholesterol in prostate cancer was shown to be considerably higher than in BPH, normal prostate, and liver. Most of the prostatic cholesterol is free (not esterified) contrary to that in the liver [57]. The findings are documented in Fig. 2. In line with this an important study based on the US Male Health Professional Study [10] showed a 51% reduction of advanced prostate cancer in long-term statin users. The possibility that androgens are synthesised in AIPC tissue from cholesterol offers a potential explanation for these findings.

3.3. New targets for treatment of AIPC

Based on recent findings it is evident that enzymes related to androgen metabolism that are over-expressed in AIPC could be targets for endocrine treatment. A recent example is the drug abiraterone acetate (CB7630), which was already been studied in phase 1 and phase 2 investigations [58]. Abiraterone acetate is an oral, irreversible inhibitor of cytochrome 450C17, the 17-α-hydroxylase–C1720-lyase enzyme. Fig. 3 shows that this enzyme blocks subsequent steps of steroid synthesis from the level of pregnenolone and progesterone. The results of a phase 2 study conducted in 38 patients with AIPC were recently reported. All patients had previously been treated by chemotherapy including docetaxel. At the time of analysis 21 of the 38 patients (55%) remained on treatment. For the 38 evaluable patients in the trial, the median time to progression had not been reached at the time of the analysis but is estimated to occur after 8.4 mo, indicating effectiveness at this level [59].

4. Conclusions

It seems that most pieces of the puzzle that are needed to explain the mechanisms of AIPC are now on the table and may be put together. Most of the molecular events causing progression of prostate cancer to androgen independence can probably be explained by AR-related mechanisms. Knowledge obtained from studying metastatic tissues of AIPC dramatically changes our understanding of this disease and opens the possibility to address new potentially effective targets. These targets are mainly the enzymes and related genes that synthesise androgens from the level of progesterone on as well as the AR itself. Clearly prostate cancer is capable of developing escape mechanisms to low serum and tissue androgen levels achieved by castration or the use of LHRH agonists.

The question remains why antiandrogens are not effective in this situation. Clearly, the inhibition of binding of androgens to the AR by antiandrogens is insufficient as escape mechanisms develop and
antiandrogen levels reached in the prostate cell were shown to be too low. Initial data showing biochemical response to a 17–20 lyase inhibitor are encouraging. Hopefully it will be possible to eliminate the functional AR in AIPC all together in the near future.

Conflicts of interest

The author has no direct financial relationship to companies producing or marketing the PSA test. The author is consultant to Ferring Ltd Copenhagen. The ERSPC study Europe wide is co-funded by Beckman Coulter Ltd.

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