Mechanisms of androgen receptor repression in prostate cancer


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Abstract

Anti-androgens used in prostate cancer therapy inhibit AR (androgen receptor) activity via largely unknown mechanisms. Although initially successful in most cases, they eventually fail and the disease progresses. We need to elucidate how anti-androgens work to understand why they fail, and prolong their effects or design further therapies. Using a cellular model, we found different anti-androgens have diverse effects on subcellular localization of AR, revealing that they work via different mechanisms and suggesting that an informed sequential treatment regime may benefit patients. In the presence of the anti-androgens bicalutamide and hydroxyflutamide, a significant proportion of the AR is translocated to the nucleus but remains inactive. Receptor inhibition under these conditions is likely to involve recruitment of co-repressor proteins, which interact with antagonist-occupied receptor but inhibit receptor-dependent transcription. Which co-repressors are required in vivo for AR repression by anti-androgens is not clear, but one candidate is the Notch effector Hey1. This inhibits ligand-dependent activity of the AR but not other steroid receptors. Further, it is excluded from the nucleus in most human prostate cancers, suggesting that abnormal subcellular distribution of co-repressors may contribute to the aberrant hormonal responses observed in prostate cancer. A decrease in co-repressor function is one possible explanation for the development of anti-androgen-resistant prostate cancer, and this suggests that it may not occur at the gross level of protein expression.

Background

The androgen signalling pathway is key in prostate cancer progression and therapy; therefore repressing it is the major aim of therapy. Prostate tumours, like the prostate itself, are dependent on circulating androgens for growth; hence the gold standard for their treatment is orchiectomy or chemical castration, which ablates testicular androgens. However, the adrenal gland secretes weak androgens that can be converted into testosterone and subsequently the potent DHT (dihydrotestosterone), resulting in relatively high intracellular levels of the latter in the prostate (~60% non-castrate levels) [1]. Anti-androgens are thus often used to oppose the actions of residual androgens. This treatment is effective in approx. 80% of cases, but most of the patients relapse after 1–2 years. This advanced, hormone-refractory stage of the disease is aggressive and metastatic and currently there are no effective therapies for it. The mechanisms of progression to hormone-resistant disease are not fully understood, although many hypotheses have been put forward, most of which involve alterations in the AR (androgen receptor) signalling pathway [2].

The AR is a ligand-activated transcription factor and a member of the nuclear receptor superfamily [3]. In the absence of ligand, it is cytoplasmic, existing in a complex containing heat-shock proteins, believed to hold the AR in a ligand binding-competent, inactive conformation. On ligand binding, these proteins were thought to dissociate, although recent evidence indicates that even nuclear steroid receptors are associated with heat-shock proteins [4]. The receptor dimerizes and translocates into the nucleus where it binds to AREs (androgen-response elements) in the promoters of target genes and alters the rate of transcription from these promoters. Increasing transcription requires the recruitment of accessory proteins called co-activators, while inhibiting transcription may require the recruitment of co-repressor proteins [5].

How do anti-androgens inhibit AR activity?

Anti-androgens, which may be steroidal in structure (e.g. cyproterone acetate) or non-steroidal (e.g. hydroxyflutamide and bicalutamide), bind to the ligand-binding domain of the AR and inhibit its activity. The mechanisms by which they do this are not well understood. Since they are a mainstay of prostate cancer therapy, it is imperative that every effort is made to understand how they work and hence why they fail, in a bid to delay or reverse the failure of such therapies. Figure 1 shows the various steps in the AR activation pathway at which anti-androgens could exert their effects. Competition...
with ligand for binding (Figure 1, i) undoubtedly contributes but cannot entirely account for anti-androgen action since the relative binding affinities of the AR for anti-androgens are very low compared with those for androgens. Inhibition of nuclear translocation or increased nuclear export, resulting in cytoplasmic accumulation of the receptor (Figure 1, ii), has been shown for the anti-oestrogen faslodex, used in breast cancer therapy [6]. However, it is known that certain anti-androgens can promote nuclear accumulation of the AR. Recently, inhibition of DNA binding (Figure 1, iii) by bicalutamide and hydroxyflutamide was reported [7]. More downstream events such as the recruitment of cofactor proteins are also likely to be affected (Figure 1, iv). For instance, Masiello et al. [8] showed that bicalutamide-bound AR can bind to DNA but, instead of recruiting co-activator proteins, the co-repressor protein NCoR (nuclear receptor co-repressor) was found at the ARE.

We undertook a cell-based study to compare the three most commonly used anti-androgens and determine their effects on AR localization using in situ cell fractionation followed by immunoblotting [9]. The study used the PC3 cell line, which is negative for AR expression, stably transfected with AR expression plasmid, resulting in AR expression levels comparable with those seen for endogenous AR in the LNCaP prostate cancer cell line. This model was preferred to LNCaP cells since the AR in LNCaP cells has an amino acid substitution that allows activation of the receptor by anti-androgens. The results are summarized in Table 1. Treatment with cyproterone acetate resulted in irreversible AR accumulation in the cytoplasm. Hydroxyflutamide and bicalutamide both promoted nuclear entry, but surprisingly we saw the AR largely in the subnuclear fraction associated with the nuclear matrix, rather than in the nucleoplasm as was the case in the presence of androgen. It is possible that AR in the nuclear matrix fraction is less likely to bind to AREs; thus the anti-androgens are effectively reducing DNA binding. Alternatively or additionally, it may be that the AR in this fraction binds proteins different from those it binds in the presence of its cognate ligand, as suggested by the chromatin immunoprecipitation experiments of Masiello et al. [8]. The isolation and identification of AR-associated proteins in the different cellular fractions after treatment with ligand and anti-androgens will help to elucidate how these anti-androgens are exerting their effects.

Are co-repressors the answer?

Nuclear receptor co-repressors were originally identified as proteins that are recruited to DNA-bound nuclear receptors, such as thyroid hormone receptor, in the absence of ligand and inhibit basal transcription from the target promoters [5]. Steroid receptors generally do not bind DNA in the absence of ligand, but evidence is increasing that they may recruit co-repressors in the presence of antagonists. For instance, direct interaction has been shown between the progesterone receptor and NCoR in the presence of the antiprogestin RU486 [10]; and while a direct anti-androgen-dependent interaction between the AR and NCoR has not been demonstrated, they do co-exist on an ARE in the presence of bicalutamide [8] and interact in the presence of RU486 and DHT [11]. Thus it is likely that nuclear AR-associated proteins in the presence of anti-androgens will include known and/or novel co-repressors. It is not yet clear which co-repressors are important in vivo for AR regulation in the prostate. No naturally occurring anti-androgens have yet been identified, and conceptually it seems unlikely that co-repressor proteins exist that will only interact with the AR

### Table 1 | Androgen receptor targeting by androgen and anti-androgens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle (ethanol)</th>
<th>Androgen (mibolerone)</th>
<th>Hydroxyflutamide</th>
<th>Bicalutamide</th>
<th>Cyproterone acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C &gt;&gt; N</td>
<td>N &gt;&gt; C</td>
<td>N M &gt; C</td>
<td>N M &gt; C</td>
<td>CM = C &gt;&gt; N</td>
</tr>
</tbody>
</table>

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Why do anti-androgens fail?

Resistance to anti-androgen therapy is the major clinical problem in prostate cancer. Understanding and overcoming the progression of tumours to this stage will require the elucidation of how anti-androgens work. A popular hypothesis is that a somatic increase in co-activator proteins, or decrease in co-repressors, may drive higher AR activity, stimulating tumour growth even at low castrate levels of androgens. If co-repressors are indeed required for anti-androgens to inhibit AR activity, a reduction in co-repressor levels could also drive anti-androgen resistance. However, as yet there is little convincing evidence for alterations in cofactor levels in prostate cancer. Several expression studies have shown small changes supportive of the idea, but others show no significant differences in benign versus malignant tissue [13–15]. It may be that the relevant cofactors are not being examined and may not even yet be identified. It may be that differences are not evident at the RNA level, and will only be evident at the protein level. To go further, any changes may not be at the gross level of protein expression, but rather at the level of protein sequence and/or function. In support of this, we found that levels of the AR co-repressor Hey1 were not noticeably altered in prostate cancer versus benign prostate tissue, using immunohistochemistry. However, although immunohistochemistry is not a reliably quantitative technique, it allows visualization of the protein localization within the cell, and we saw that the distribution of Hey1 was altered in the cancer samples [12]. Whereas in normal and benign tissue, Hey1 showed mainly nuclear localization, in eight of ten prostate cancer samples it was exclusively cytoplasmic (Figure 2). This nuclear exclusion would effectively reduce functional co-repressor levels, since the nucleus is where co-repressors act. A similar phenomenon has been observed in NF-κB (nuclear factor κB) signalling, whereby IL-1β (interleukin-1β)-mediated nuclear export of co-repressors caused de-repression of regulated genes [16]. Thus, while cytoplasmic sequestration of the AR itself is one mechanism of action of anti-androgens, cytoplasmic sequestration of co-repressors is a possible mechanism of escape from anti-androgen therapy.

Clinical implications

At present, when a prostate cancer patient relapses on anti-androgen therapy, treatment options are limited. Withdrawal of the anti-androgen, seemingly paradoxically, may result in a short improvement in symptoms, possibly because of an adaptive mutation in the AR causing its activation by the anti-androgen [2]. However, tumour growth will soon recur and often only palliative chemotherapy can be offered. It is not common practice to switch to treatment with a second anti-androgen after one has failed. We found that the mechanisms of action, and perhaps the cofactor proteins involved, are different for different anti-androgens. Such studies could be used as the basis for a rational sequence of anti-androgens in treatment, thus prolonging the relapse-free period and life expectancy. For instance, failure of flutamide therapy may not preclude a response to cyproterone acetate. This has already been established in breast cancer, where treatment of tamoxifen-resistant patients with the second anti-oestrogen faslodex leads to clinical benefit in approx. 40% of cases [17].

Many studies are quantifying levels of co-activator and co-repressor RNA or protein in prostate cancer tissue. However, alterations occurring at the functional level may be visible only if studies are carried out at the cellular level. Our small-scale study revealed that Hey1 is excluded from the nucleus in prostate. If our further studies indicate that this is functionally
significant in prostate cancer, future therapies could be based on reversing such aberrant localization with a view to re-establishing sensitivity to anti-androgens.

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References

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