Molecular aspects of hormone-independent prostate cancer

Jack A. Schalken
Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

INTRODUCTION

In normal prostate epithelial cells, androgens act as differentiation agents, inducing the production of differentiation-associated proteins such as PSA [1]. When the androgen receptor (AR) is inactive, it is bound to heat-shock proteins in the prostate cell cytoplasm. Binding of the androgenic ligand testosterone or dihydrotestosterone (DHT) to the receptor causes it to dissociate from the heat-shock proteins. The androgen-bound receptor then translocates to the nucleus, dimerizes, and binds to androgen-response elements, thereby activating androgen-dependent proteins [2].

It has been known for >50 years that suppression of androgenic activity, either by castration or by suppressing the activity or synthesis of androgens, can inhibit the development and reduce the spread of prostate cancer [3,4]. This implies that androgens and the AR have a role in prostate cancer that is exactly the opposite of that in normal cells, where androgens support differentiation, i.e. in transformed cells, they promote proliferation. How can this happen?

THE AR IN PROSTATE CANCER

Changes that can occur in AR signalling during the development of prostate cancer are illustrated in Fig. 1. Under physiological conditions the action of the AR is typically associated with the induction of terminal differentiation; the induction of transient proliferation is indirect, and it occurs via the AR-positive stromal cells. The remarkable change in AR signalling in prostate cancer is sometimes associated with mutations of the AR, although such mutations are rare [2]. A second possibility is the development of androgen-independent mechanisms that bypass the AR. Possible mechanisms include the development of cells that secrete neuropeptides, or of ways to switch off the apoptosis pathway. Both phenomena might be frequent [2]. A third possibility involves amplification of the gene encoding the AR. Indeed, genetic and histological studies show that levels of the AR are greatly enhanced in prostate cancers that are unresponsive to endocrine therapy (Fig. 2) [5].

LINK BETWEEN THE AR AND ONCOGENES

Tomlins et al. [6] used a novel bioinformatic approach to search for genes that are expressed more frequently in prostate cancer than in normal tissue. Their ‘cancer outlier profile analysis’ was aimed at accentuating the identification of genes encoding two ETS transcription factors, ERG and ETV1, as frequent outliers. The authors argued that enhanced expression of both genes would be redundant. This hypothesis was confirmed by showing that increased expression of the two transcription factors is mutually exclusive.

The next step in this study was to investigate the mechanisms responsible for over-expression of these transcription factors by examining specific cell lines in culture. The authors succeeded in showing recurrent fusion of the ERG or ETV1 gene to the S'-untranslated region of TMPRSS2, a gene encoding an androgen-regulated type II transmembrane protease of unknown function [7].

Tomlins et al. [6] then investigated whether the synthesis of ERG is under androgen control in cells harbouring the fusion hybrid. The authors concluded that this fusion is an early event that precedes chromosome loss. In two patients, fusion transcript was detected in the HGPIN lesion but not in the carcinoma present in the same gland, a finding possibly indicating the multicentric development of cancer, with or without involvement of the ETS pathway.

The nature of the TMPRSS2:ERG fusion product was studied in more detail by Perner et al. [10]; they confirmed the presence of the TMPRSS2:ERG fusion in 49% of 118 primary prostate cancers and in 41% of hormone-naive lymph node metastases. In addition, fluorescence in situ hybridization detected
intronic deletions in 60% (35 of 58) of the primary fusion cases of prostate cancer and in three of seven of the TMPRSS2:ERG hormone-naive lymph-node metastases. These deletions were associated with prostate cancer progression.

In a xenograft study, Hermans et al. [11] transplanted 11 different xenografts of different stages of prostate cancer onto nude mice. All five androgen-dependent samples showed major overexpression of the TMPRSS2:ERG transcript. This fusion gene was also detected (but not expressed) in three of four androgen-independent, AR-negative xenografts. The authors suggested that fusion of the TMPRSS2 and ETS genes is of key importance in most androgen-regulated prostate cancers, but that this fusion is bypassed in late-stage disease.

Iljin et al. [12] reported the presence of the TMPRSS2:ERG fusion in seven of 19 advanced cancer samples and one instance of fusion with the gene encoding the ETV4 factor (another member of the ETS protein family). Expression of ERG was correlated with increased levels of WNT signalling and down-regulation of cell death pathways. Both of these effects have been related to carcinogenesis and are, potentially, under androgen control through the fusion product.

In a study of the diversity of the transcripts arising as a result of fusion between TMPRSS2 and ERG in the human prostate, Clark et al. [13] characterized 14 different hybrid transcripts, each containing different combinations of sequences from the TMPRSS2 and ERG genes. The transcripts included two that were predicted to encode a normal full-length ERG protein, six that encoded N-terminally truncated ERG proteins, and one that produced a chimeric protein. All but one of the protein products contained the DNA-binding domain of ERG. Interestingly, distinct patterns of hybrid transcripts were found in samples taken from separate regions of individual cancerous prostates, a finding.
suggesting that independent fusions arise at different sites.

Laxman et al. [14] undertook the first preliminary study on the possible diagnostic relevance of the TMPRSS2:ERG fusion. They used PCR analysis to assess the expression of ERG and TMPRSS2:ERG in urine after prostate massage. Both transcripts were detectable in eight (42%) of 19 patients. These encouraging results support a larger research study, although further development work would be needed to ensure the detection of alternative isoforms of the transcript.

Taken together, these results suggest that androgen-dependent expression of ERG (and related transcription factors) is of key importance in explaining the proliferation and epigenetic programme of many prostate cancers, at least those with ARs. Further work is needed to establish the prognostic and diagnostic significance of the different isoforms of the fused product. These results also have enormous implications for research on other forms of carcinoma.

What are the clinical implications? It seems clear that reduction of androgen concentrations to negligible levels would be an effective treatment of many cases of prostate cancer. To what extent do we achieve this reduction now?

ANDROGEN LEVELS IN ADVANCED PROSTATE CANCER

Nishiyama et al. [15] used highly specific HPLC mass spectrometry to measure concentrations of testosterone, DHT, dihydroepiandrosterone (DHEA), DHEA sulphate, and androsteredione in serum and in prostate tissue. Of particular interest were the prostate levels of DHT, the natural ligand of the AR [2]. The measurements were first obtained from 103 patients who had or were suspected of having prostate cancer, and then repeated in 30 patients with confirmed prostate cancer treated by castration and flutamide for 6 months.

Before treatment, the correlation between prostatic and serum DHT concentrations was highly significant ($P = 0.025$). There were also significant correlations between prostatic DHT and serum concentrations of DHEA ($P = 0.014$) and DHEA sulphate ($P = 0.015$). There was no correlation between prostatic DHT and serum testosterone concentrations ($P = 0.923$). These results indicated that prostatic DHT is synthesized outside the prostate from DHEA or related metabolites. Reduction of externally supplied testosterone to DHT within the prostate does not appear to be an important pathway.

After treatment, the results were strikingly different. There were no longer significant correlations between prostatic and serum DHT concentrations ($P = 0.869$) or between prostatic DHT and serum DHEA concentrations ($P = 0.708$). The amount of prostatic DHT was no longer significantly correlated with serum DHEA sulphate concentration ($P = 0.065$), but it was significantly correlated with serum testosterone concentration ($P = 0.033$). These results indicated that after treatment, prostatic DHT is synthesized within the prostate from externally supplied testosterone or, possibly, DHEA or related metabolites.

As a result of treatment, there were significant decreases in the amount of prostatic DHT and in serum testosterone, DHEA and DHEA sulphate concentrations (all $P < 0.001$). However, the mean (±SD) amounts of prostatic DHT remained at 25% of the control values, i.e. before treatment, 5.44 (2.84), after treatment, 1.35 (1.32) ng/g tissue (both 30 samples). The authors concluded that as a result of androgen deprivation therapy, the mode of formation of DHT switches from prostatic to intracrine production and that relatively high levels of this androgen remain. It also follows that advanced prostate cancer is often incorrectly thought to be hormone-independent, but the cancer is in fact still dependent on the hormone remaining in the prostate.

CONCLUSIONS

The available results suggest that activation of the AR provides an important proliferative signal in many cases of prostate cancer, often including advanced disease. The results also allow the conclusion that current approaches to androgen-deprivation therapy are inadequate, because substantial levels of active androgen remain in the prostate. It therefore appears that new methods to achieve radical androgen deprivation offer a promising therapeutic approach. One possibility might be to combine androgen-deprivation therapy with inhibition of the enzyme 5α-reductase, which converts testosterone to DHT. Dutasteride might be better than finasteride in this respect, because it inhibits both forms of the enzyme present in the prostate [16,17]. No combination trial of this kind has been completed. Furthermore, inhibition of AR expression by antisense AR oligonucleotides might be a promising new approach for treating hormone-independent prostate cancer.

However, attempts must also be made to individualize therapy, because prostate cancer is not one disease [18]. Total DHT suppression would be most promising for the subset of patients with TMPRSS2:ERG-positive cancers (≈ 30%). Patients who are negative for the AR (≈ 50%) could be given chemotherapy alone. It might be appropriate to give the remaining patients a combination of chemotherapy and endocrine therapy, or simply active surveillance, depending on the presence or absence of stem cell markers. Further studies are needed to clarify these issues.

CONFLICTS OF INTERESTS

JAS has been a paid consultant for GlaxoSmithKline and has received payment for speaking.

REFERENCES

6 Tomlins SA, Rhodes DR, Perner S et al. Recurrent fusion of TMPRSS2 and ETS


Correspondence: Jack Schalken, University Medical Centre Nijmegen, 267 Experimental Urology, PO Box 9101, 6500 HB Nijmegen, Netherlands.

e-mail: J.Schalken@uro.umcn.nl.

Abbreviations: AR, androgen receptor; DHT, dihydrotestosterone; DHEA, dihydroepiandrosterone; HGPIN, high-grade prostatic intraepithelial neoplasia.