20
Castration-Recurrent Prostate Cancer Is Not Androgen-Independent

James L. Mohler

Significance

In the USA in 2006, an estimated 234,460 new cases of prostate cancer (PC) will be diagnosed and 27,350 men will die from PC (1). Despite the increased use of digital rectal examination and serum prostate-specific antigen (PSA) measurement for early detection, ~30% of men treated with curative intent suffer PC recurrence. These men and those who present with locally advanced or metastatic PC can be palliated by androgen deprivation therapy (ADT), a treatment that remains unimproved since its discovery more than 60 years ago (2). Over 80% of men with disseminated PC demonstrate clinical or biochemical response that is associated with a mean life expectancy of ~3.5 years in contrast to nonresponders or untreated patients who live an average of 9 months. Regardless of the androgen responsiveness of incurable PC, almost all patients succumb to castration-recurrent PC because it responds poorly to all known therapies.

AR Protein Expression in Castration-Recurrent CaP

Androgen receptor (AR) immunostaining of 19 specimens of castration-recurrent PC [% positive nuclei, 83.7 ± 11.6, and mean optical density (MOD), 0.284 ± 0.115] was similar to 16 specimens of benign prostate (% positive nuclei, 77.3 ± 13.0, and MOD, 0.315 ± 0.044) (p = 0.25 for % positive nuclei and 0.48 for MOD) (Fig. 1) (3). These findings, measured using an AR monoclonal antibody and automated image analysis, were similar to earlier reports that used qualitative methods (4, 5). High levels of AR expression in castration-recurrent PC in the absence of testicular androgens provides the potential for enhanced AR sensitivity to available androgens or alternate mechanisms of activation that would allow AR to remain central to growth regulation of castration-recurrent PC (6–9). The central question becomes *"How is AR activated after medical or surgical castration?"*

J.J. Li et al. (eds.), *Hormonal Carcinogenesis V.*

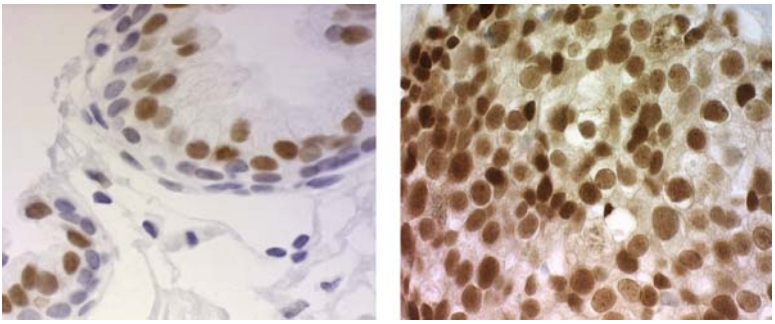

Fig. 1 AR protein expression is similar in androgen-stimulated benign prostate (*left*) and castration-recurrent PC (*right*)

AR Mutations in Castration-Recurrent Prostate Cancer

At the molecular level, AR mutations have been reported with frequencies ranging from 0 (10) to 44% (11) in androgen-stimulated PC, and 0 (12) to 50% (13) in castration-recurrent PC. Most investigators use a single method to search for mutations and since evaluation of exon A is technically difficult, investigators evaluate only exons B-H, although exon A may harbor many mutations (11). Specimens of castration-recurrent PC from 25 men were used to test whether the AR mutation frequency variation in castration-recurrent PC resulted from methodological differences. Mutation analysis used denaturing gradient gel electrophoresis (DGGE) of all exons except the first fragment of exon A [4 PCR products span exon A (A1–4)], single-strand conformational polymorphism (SSCP), and direct sequencing of all exons. The three mutational analysis methods were similar in sensitivity; i.e., the frequency of LNCaP mutation was 10% by DGGE and direct sequencing, and 20% by SSCP (14). A silent mutation was found in exon F (800C→T). A second patient had two changes: the LNCaP mutation (877T→A) in exon H and a CAG repeat deletion from 25 in genomic DNA to 10. The mutation was confirmed by cloning. Neither mutation was present in peripheral blood mononuclear cells nor in the original androgen-stimulated PC. A third patient had CAG repeat expansion from 21 to 26 and GGN repeat deletion from 23 to 10 in castration-recurrent PC compared with his original PC. SSCP suggested mutations (1) in fragment A3 and 4 in exon E) not confirmed by direct sequencing. A consensus has developed that AR mutations are infrequent (6, 7), although in bone metastases, they may occur with a frequency as high as 30% when ADT includes antiandrogens (15), mutagenesis rates are high in general. When characterized functionally, most of the mutant ARs retain transcriptional activity in response to androgens and some have altered steroid-binding specificity that changes the spectrum of ligands capable of activating AR (13, 16–19).

AR Amplification in Castration-Recurrent Prostate Cancer

Chen et al. used in vitro and in vivo models to suggest that castration-recurrent PC results from increased expression of AR protein through AR gene amplification that allows expression of androgen-regulated genes despite castrate levels of serum androgens (20). This hypothesis was tested clinically using a tissue microarray constructed from 24 samples of castration-recurrent PC and six samples of benign prostate (21). Eight (33%) castration-recurrent PCs exhibited AR gene amplification, a frequency similar to that reported by others. AR was immunostained more intensely in PC with amplified AR (MOD 0.36 ± 0.07) than nonamplified AR (MOD 0.24 ± 0.09) ($p < 0.01$) but AR immunostaining intensity was unrelated to the degree of AR amplification. A single laboratory has reported on AR gene amplification and survival; a survival advantage was found for amplified patients in their first two reports but not in their most recent publication (22). We found that AR gene amplification was unrelated to duration of survival after ADT.

Ligand-Independent AR Activation

AR is activated by IL-6 (23). Phosphorylation of the coactivator SRC-1 is regulated by IL-6 causing protein interaction between the N-terminal domain of AR and SRC-1 (24). Growth factor kinase signaling pathways may activate AR directly or sensitize AR by regulation of coactivator interaction with AR (25–29). Evidence is strong for HER-2, but HER-2 receptor levels are low in castration-recurrent PC at the protein and mRNA levels; however, HER-2 amplification was not detected in any of 39 androgen-stimulated or castration-recurrent PC specimens tested (44). "Ligand-independent" AR activation may occur when AR is sensitized to low androgen levels by growth factors, change in AR coregulator profiles, or microenvironmental hypoxia. For example, the neuropeptide growth factor bombesin synergizes with 10 pmol dihydrotestosterone (DHT) to activate AR in PC-3 cells that overexpress transfected AR (30).

AR Activating Levels of Androgens in Castration-Recurrent Prostate Cancer

A group of 21 patients aged 57–86 years demonstrated clinical evidence of castration-recurrent PC (3) (Fig. 2 and Table 1). All underwent transurethral prostatectomy for urinary retention from local recurrence that occurred from 7–92 months after medical (10 men) or surgical (11 men) ADT. Histologic examination revealed poorly differentiated carcinoma (Gleason sum 8–10) that represented an

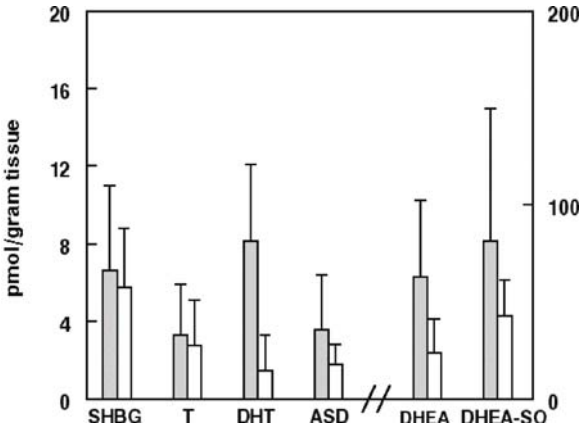

Fig. 2 Tissue androgen levels in castration-recurrent PC (*open bars*) vs. androgen-stimulated benign prostate

Table 1 Androgen levels in castration-recurrent PC

LC/MS/MS			**RIA**		
Titus 2005			**Page 2006**		
	T	**DHT**		**T**	**DHT**
AS-BP (n = 18)	2.75	13.7	AS-BP (n = 4)	1.84	9.26
RCaP (n = 18, 37 m)	3.75	1.25	LHRH+T (n = 4, 1 m)	1.38	6.8
Nishiyama 2004			LHRH (n=4, 1 m)	0.56	1.94
	T	**DHT**	**Mohler 2004**		
AS-BP (n = 30)	-	18.7		**T**	**DHT**
CaP (n = 30, 3–6 m)	-	4.65	AS-BP (n = 30)	3.26	8.13
Mizokami 2004			RCaP (n = 15, 37 m)	2.78	1.45
	T	**DHT**	**Labrie 1989**		
AS-BP (n = 15)	-	8.53		**T**	**DHT**
CaP (n = 15, 3–6 m)	-	2.13	Human CaP (n = ?)	-	18.6
			Orch (n = 5, 2–12 m)	-	9.29
			Orch + flu (n = 4, 2 m)	-	ND
			Rat prostate	-	14.6
			Orch, orch ± flu	-	ND
			Guinea pig prostate	-	32.4
			Orch, orch ± flu	-	ND
			Geller 1979		
				T	**DHT**
			AS-BP (n = 17)	-	17.6
			CaP orch ± DES (n=9)	-	4.47
			CaP DES 1 mg (n = 6)	-	12.4

average of 92% (range 72–99%) of the cross-sectional area of the tissue sections. To compare these tissues to androgen-stimulated prostate tissue, frozen specimens of benign prostate tissue were obtained from radical prostatectomy specimens. The frozen tissues were assayed for total levels of T, DHT, androstenedione (ASD),

dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-SO_4), estradiol, sex hormone-binding globulin (SHBG), and PSA. Although tissue levels of DHT, DHEA, and ASD were lower in castration-recurrent PC from men undergoing ADT than in benign prostate from untreated men ($p<0.01$), DHT tissue levels averaged 1.45 nM in castration-recurrent PC and 8.14 nM in benign prostate. Tissue levels of testosterone (T) were similar in castration-recurrent PC (2.78 nM) and benign prostate (3.27 nM) ($p=0.21$). Tissue levels of PSA in castration-recurrent PC were approximately 1/10 the level measured in benign prostate ($p<0.000001$). Castration-recurrent PC tissue levels of androgens, estradiol, SHBG, and PSA did not differ between three patients who received flutamide and 12 patients who did not. In particular, tissue levels of DHT were similar ($p=0.29$) in both groups (flutamide, 3.75 ± 3.58 pmol/g tissue, range 0.40–7.53 pmol/g tissue; no flutamide, 0.87 ± 0.53 pmol /g tissue, range 0.37–2.17 pmol /g tissue).

These results obtained using radioimmunoassay (RIA) were surprising. Therefore, the data were confirmed using mass spectrometry (MS) (31). A prostate tissue homogenization and androgen extraction protocol and a liquid chromatography (LC)/electrospray ionization (ESI)/MS analytic method were developed in collaboration with Dr. K. Tomer, Director, NIEHS MS Facility. T levels were similar in castration-recurrent PC (3.75 pmol/g tissue) and benign prostate (2.75 pmol/g tissue, $p=0.30$). DHT levels in castration-recurrent PC (1.25 pmol/g tissue) were less than in benign prostate (13.7 pmol /g, tissue $p<0.0001$), although, in most specimens of castration-recurrent PC, DHT levels were sufficient for AR activation. DHT levels in castration-recurrent PC compared with benign prostate decreased 91% by MS and 82% by RIA.

Are Tissue Androgen Levels Really Elevated During ADT?

Dr. Labrie et al. gained widespread recognition for their work on tissue androgen levels during ADT measured by RIA. PC tissue DHT levels decreased from 5.24 ng / g tissue in noncastrated men 55–68 years of age to 2.7 ng /g tissue in five men who castrated 2–12 months before radical prostatectomy (32). Among four castrated men receiving flutamide, 250 mg three times daily for 2 months prior to prostatectomy, tissue DHT was undetectable. It was postulated that flutamide, competing for high affinity DHT binding to AR, decreased prostate DHT levels by increasing its degradation. These data led to the use of "total androgen blockade" where tissue DHT was eliminated using antiandrogens (33). However, a metaanalysis of clinical trials comparing LH-RH agonists and anti-androgens vs. LH-RH agonists alone (34) and a study comparing orchiectomy and anti-androgens vs. orchiectomy alone (35) demonstrated no survival benefit to combination therapy. Careful review of older literature and recent findings cast further doubt on Labrie's hypothesis.

In 1979, Geller et al. published an analysis of tissue androgen levels in prostate and non-androgen target tissues with a specific emphasis upon tissue steroid levels as markers of tumor differentiation and adequacy of anti-androgen therapy (36).

Tissues procured by transurethral resection of the prostate were assayed by RIA. They reported that 1 mg of DES did not adequately suppress tissue DHT levels since the levels remained intermediate between androgen-stimulated benign prostate and prostate tissue procured from castrated men. They concluded that their findings "support the long suspected theoretical role of andrenal cortical androgens as biologically important sources of DHT in relapse of PC." The clinical importance of their findings were obscured by Labrie's assertion that coadministration of anti-androgens cured PC by reducing tissue DHT levels to 0, a finding that was based upon experimental data in a total of four men. Interest and attention on Geller's original hypothesis has been rekindled by our RIA and MS data from men with castration-recurrent PC. MS findings are supported by two recent reports. Mizokami et al. (37) showed that average tissue DHT levels measured by LC/MS/MS decreased 75% in prostatectomy specimens obtained after 3–6 months of ADT, and Nishiyama et al. (38) found tissue DHT concentration decreased 75% in prostate tissue from 30 men receiving ADT for 6 months. Recently, Page et al. (39) reported that tissue levels of RIA testicular androgens in benign prostate may be sufficient for AR activation as early as 1 month after castration! Twelve men underwent prostate biopsies on day 28, four men received placebos, a long acting LHRH antagonist, acyline, or acycline and T. In four men who received acycline, T, and DHT, prostate tissue levels decreased by 70 and 80%, respectively. Despite this decrease in prostate tissue levels of androgens, IHC revealed no detectable differences among the three groups in cellular proliferation, apoptosis, and PSA or AR expression. This report is especially important for two reasons. First, it suggests that benign prostate recovers the ability to produce testicular androgens as soon as 1 month after institution of ADT. Second, normal tissue homeostasis was recovered 1 month after ADT. Yet, in benign prostate, prostate volume remains reduced forever; ADT cures benign prostate enlargement. In contrast, PC cells must develop the ability to use these tissue androgens to recur as castration recurrent PC to kill the patient.

Clinical Relevance of AR Activating Levels of Tissue Androgens

Are levels of ~3 nM T and DHT measured in castration-recurrent PC tissues sufficient to activate AR? Simard et al. (40) were the first to suggest that residual DHT in prostatic tissue after castration was androgenic. On the basis of traditional transient transfection experiments in PC cell lines, 1 nM T efficiently activates most androgen-regulated reporter genes. We (41) and others (42) have shown that the "supersensitive" AR is activated in castration-recurrent PC cell lines by pM DHT. The presence of PSA in these specimens of castration-recurrent PC and in serum of patients is consistent with the presence of an activated AR, although PSA levels in castration-recurrent CaP tissue were only 7.6% of levels in benign tissue. Stege et al. (43) reported a PSA level of 4,973 µg/g tissue (assuming 1 mg DNA per gram tissue) in aspirated benign prostate, which was similar to the level we found in benign prostate (3,198 µg per gram tissue). They reported a tissue PSA level of 458 µg per gram tissue in PC from noncastrated

patients that were similar to the level we measured for castration-recurrent PC (297 µg per gram tissue). In transurethral resection specimens, Yang et al. (44) reported tissue PSA levels of 1952.27 µg/g protein in benign prostate, and 583.75 µg/g protein in PC from noncastrated patients. Since we and others obtained similar PSA levels in androgen-stimulated benign prostate, the similar PSA levels measured by us in castration-recurrent PC and those of others in androgen-stimulated PC and benign prostate suggest that the AR is activated in all tissues despite the castrate serum levels of androgens.

Adrenal Androgens may be the Source of Prostate Tissue DHT

Belanger et al. (32) suggested that persistent levels of prostatic DHT after castration alone resulted from metabolism of adrenal-derived DHEA, DHEA-SO_4, and ASD in prostate tissue. Serum DHEA-SO_4 levels can be 300–500 times the concentration of DHEA, and the sulfatase present in human prostate converts DHEA-SO_4 to DHEA (45). In the only report of tissue levels of DHEA, nonhyperplastic tissue specimens obtained by open prostatectomy contained 90 pmol mg^{-1} DNA (equivalent to 90 nM DHEA assuming 1 mg DNA per gram tissue) (45). These levels of DHEA cause detectable activation of AR in cotransfection assays (46). Moreover, small amounts of DHT have been reported to be formed from DHEA and DHEA-SO_4 in benign prostate (47).

Some preliminary data support the possibility that conversion of DHEA and DHEA-SO_4 to DHT in castration-recurrent PC contributes to AR activation. Thirty-six tissue homogenates were made from 12 samples each of frozen operative specimens of benign prostate, androgen-stimulated PC, and castration-recurrent PC. [^{3}H]-ASD appeared as [^{3}H]-DHT in all three tissue types (thin layer chromotography, data not shown) suggesting that the androgen metabolic enzymes present in androgen-stimulated benign prostate and PC remain in castration-recurrent PC. The androgen metabolism pathway from adrenal androgens to DHT appears present in the CWR-R1 cell line that was generated from a castration-recurrent CWR22 human xenograft tumor. An average of 5% of [^{14}C]-DHEA appeared as [^{14}C]-DHT in three experiments (Fig. 3).

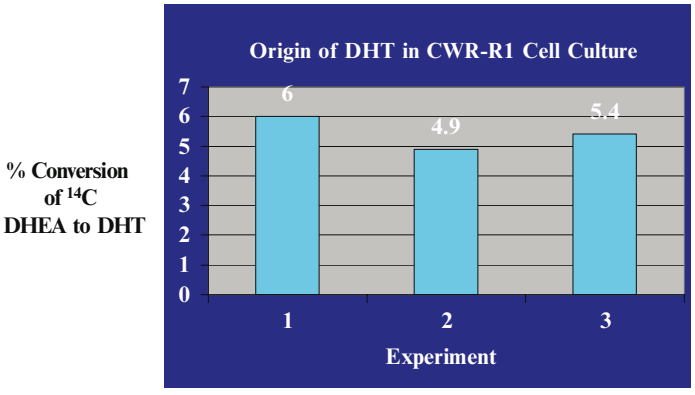

Fig. 3 % Conversion of [^{14}C]-DHEA to DHT in three individual experiments

These observations are consistent with recent reports of upregulation of androgen metabolism enzymes (45, 47) during ADT that allow T formation from adrenal androgens or androgen metabolites.

Model of Castration-Recurrent Prostate Cancer

The benign and malignant prostate is stimulated by circulating and tissue levels of androgens from puberty through adulthood. Both benign and malignant prostate epithelium grows slowly; rates of apoptosis and cellular proliferation are similar. Circulating T is reduced to DHT, the preferred AR ligand. Castration reduces circulating T and DHT to castrate levels that remain so indefinitely. Both benign and malignant prostate respond to this insult with a massive wave of apoptotic cell death, which peaks on day two (29) after castration (48). Both castration-recurrent PC (3, 31) and benign prostate (39) demonstrate tissue androgen levels sufficient for AR activation soon after castration. When PC recurs clinically after castration, PSA begins to rise in patients and xenograft models (48) and, for unknown reasons, castration-recurrent PC begins to grow again whereas benign prostate hyperplasia remains permanently dormant (Fig. 4).

Conclusion

AR remains active in growth signaling despite castrate levels of circulating androgens (49). AR protein and AR-regulated proteins are expressed in PC that recurs during ADT in both primary (3, 4, 50, 51) and bone metastases (52, 53). The substrates and metabolic pathways (54) responsible for maintenance of functional tissue levels of T and DHT in castration-recurrent PC remain to be clarified. New therapies that target AR directly (8) and prevent the formation of androgens within PC tissue (55) may offer novel approaches to prolong remission or induce reremission of castration-recurrent PC.

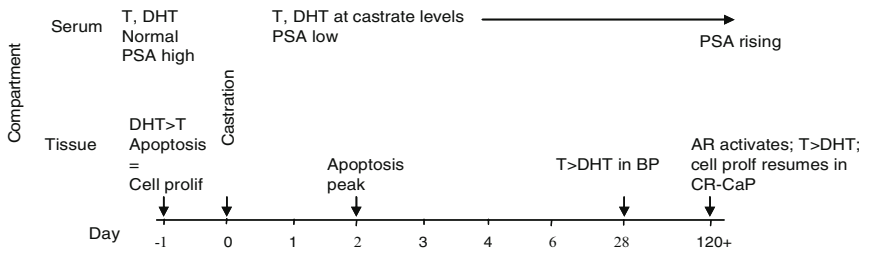

Fig. 4 Model of castration-recurrent prostate cancer

Summary

An American man is diagnosed with prostate cancer (PC) every 3 min and dies from the disease every 17 min. Although androgen receptor (AR) expression is diminished following androgen deprivation therapy (ADT) that induces clinical remission in most patients, castration-recurrent PC expresses levels of AR protein similar to those found in androgen-stimulated PC and benign prostate. This observation suggests that the AR may be as important for growth regulation in castration-recurrent PC, as it is in androgen-stimulated PC and benign hyperplasia. Neither ligand-independence, point mutations, glutamine and/or glycine repeat expansion nor amplification have explained AR activation in most cases of castration-recurrent PC. Castration-recurrent PC tissue has levels of testosterone (T) similar to androgen-stimulated benign prostate and levels of dihydrotestosterone (DHT), the most active androgen for AR activation that are approximately 10% of androgen-stimulated benign prostate. These levels of tissue androgens appear capable of activating the AR since prostate-specific antigen (PSA), the classic androgen-regulated gene, is expressed at similar tissue levels in castration-recurrent and androgen-stimulated PC. These startling findings suggest a paradigm shift; *PC that recurs during ADT is not androgen-independent*.

References

1. Jemal A, Siegel R, Ward E, et al. (2006) Cancer statistics, 2006. CA Cancer J Clin 56: 106–130.
2. Huggins C, Hodges CV (2002) Studies on prostatic cancer: I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941. J Urol 168: 9–12.
3. Mohler JL, Gregory CW, Ford OH III, et al. (2004) The androgen axis in recurrent prostate cancer. Clin Cancer Res 10: 440–448.
4. van der Kwast TH, Schalken J, Ruizeveld de Winter JA, et al. (1991) Androgen receptors in endocrine-therapy-resistant human prostate cancer. Int J Cancer 48: 189–193.
5. Visakorpi T, Hyytinen E, Koivisto P, et al. (1995) In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9: 401–406.
6. Feldman BJ, Feldman D (2001) The development of androgen-independent prostate cancer. Nat Rev Cancer 1: 34–45.
7. Gelmann EP (2002) Molecular biology of the androgen receptor. J Clin Oncol 20: 3001–3015.
8. Grossmann ME, Huang H, Tindall DJ (2001) Androgen receptor signaling in androgen-refractory prostate cancer. J Natl Cancer Inst 93: 1687–1697.
9. Sadar MD, Hussain M, Bruchovsky N (1999) Prostate cancer: molecular biology of early progression to androgen independence. Endocr Relat Cancer 6: 487–502.
10. Takahashi H, Furusato M, Allsbrook WC, Jr, et al. (1995) Prevalence of androgen receptor gene mutations in latent prostatic carcinomas from Japanese men. Cancer Res 55: 1621–1624.
11. Tilley WD, Buchanan G, Hickey TE, et al. (1996) Mutations in the androgen receptor gene are associated with progression of human prostate cancer to androgen independence. Clin Cancer Res 2: 277–285.

12. Ruizeveld de Winter JA, Janssen PJ, Sleddens HM, et al. (1994) Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer. Am J Pathol 144: 735–746.
13. Taplin ME, Bubley GJ, Shuster TD, et al. (1995) Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 332: 1393–1398.
14. Greene SL, Stockton P, Kozyreva OG, et al. (2003) Mutational analysis of the androgen receptor using laser capture microdissection and direct sequencing. In: Terrain D (ed.) Cancer Cell Signalling: Methods and Protocols. Humana Press, Totowa, NJ, pp. 287–302.
15. Taplin ME, Bubley GJ, Ko YJ, et al. (1999) Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 59: 2511–2515.
16. Shi XB, Ma AH, Xia L, et al. (2002) Functional analysis of 44 mutant androgen receptors from human prostate cancer. Cancer Res 62: 1496–1502.
17. Culig Z, Hobisch A, Cronauer MV, et al. (1993) Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. Mol Endocrinol 7: 1541–1550.
18. Peterziel H, Culig Z, Stober J, et al. (1995) Mutant androgen receptors in prostatic tumors distinguish between amino-acid-sequence requirements for transactivation and ligand binding. Int J Cancer 63: 544–550.
19. Tan J, Sharief Y, Hamil KG, et al. (1997) Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol Endocrinol 11: 450–459.
20. Chen CD, Welsbie DS, Tran C, et al. (2004) Molecular determinants of resistance to antiandrogen therapy. Nat Med 10: 33–39.
21. Ford OH, 3rd, Gregory CW, Kim D, et al. (2003) Androgen receptor gene amplification and protein expression in recurrent prostate cancer. J Urol 170: 1817–1821.
22. Palmberg C, Koivisto P, Kakkola L, et al. (2000) Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. J Urol 164: 1992–1995.
23. Ueda T, Bruchovsky N, Sadar MD (2002) Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. J Biol Chem 277: 7076–7085.
24. Ueda T, Mawji NR, Bruchovsky N, et al. (2002) Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem 277: 38087–38094.
25. Yeh S, Lin HK, Kang HY, et al. (1999) From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci USA 96: 5458–5463.
26. Liu Y, Majumder S, McCall W, et al. (2005) Inhibition of HER-2/neu kinase impairs androgen receptor recruitment to the androgen responsive enhancer. Cancer Res 65: 3404–3409.
27. Mahajan NP, Whang YE, Mohler JL, et al. (2005) Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: role of Ack1 in polyubiquitination of tumor suppressor Wwox. Cancer Res 65: 10514–10523.
28. Park SY, Kim YJ, Gao AC, et al. (2006) Hypoxia increases androgen receptor activity in prostate cancer cells. Cancer Res 66: 5121–5129.
29. Smitherman AB, Gregory CW, Mohler JL (2003) Apoptosis levels increase after castration in the CWR22 human prostate cancer xenograft. Prostate 57: 24–31.
30. Dai J, Shen R, Sumitomo M, et al. (2002) Synergistic activation of the androgen receptor by bombesin and low-dose androgen. Clin Cancer Res 8: 2399–2405.
31. Titus MA, Schell MJ, Lih FB, et al. (2005) Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. Clin Cancer Res 11: 4653–4657.
32. Belanger B, Belanger A, Labrie F, et al. (1989) Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. J Steroid Biochem 32: 695–698.

33. Labrie F, Dupont A, Belanger A, et al. (1982) New hormonal therapy in prostatic carcinoma: combined treatment with an LHRH agonist and an antiandrogen. Clin Invest Med 5: 267–275.
34. Prostate Cancer Trialists' Collaborative Group (1995) Maximum androgen blockade in advanced prostate cancer: an overview of 22 randomized trials with 3283 deaths in 5710 patients. Lancet 265–269.
35. Eisenberger MA, Blumenstein BA, Crawford ED, et al. (1998) Bilateral orchiectomy with or without flutamide for metastatic prostate cancer. N Engl J Med 339: 1036–1042.
36. Geller J, Albert J, Loza D (1979) Steroid levels in cancer of the prostate –markers of tumour differentiation and adequacy of anti-androgen therapy. J Steroid Biochem 11: 631–636.
37. Mizokami A, Koh E, Fujita H, et al. (2004) The adrenal androgen androstenediol is present in prostate cancer tissue after androgen deprivation therapy and activates mutated androgen receptor. Cancer Res 64: 765–771.
38. Nishiyama T, Hashimoto Y, Takahashi K (2004) The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. Clin Cancer Res 10: 7121–7126.
39. Page ST, Lin DW, Mostaghel EA, et al. (2006) Persistent Intraprostatic Androgen Concentrations after Medical Castration in Healthy Men. J Clin Endocrinol Metab 91: 3850–3856.
40. Simard J, Luthy I, Guay J, et al. (1986) Characteristics of interaction of the antiandrogen flutamide with the androgen receptor in various target tissues. Mol Cell Endocrinol 44: 261–270.
41. Gregory CW, Johnson RT, Jr, Mohler JL, et al. (2001) Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 61: 2892–2898.
42. Culig Z, Hoffmann J, Erdel M, et al. (1999) Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. Br J Cancer 81: 242–251.
43. Stege R, Tribukait B, Lundh B, et al. (1992) Quantitative estimation of tissue prostate specific antigen, deoxyribonucleic acid ploidy and cytological grade in fine needle aspiration biopsies for prognosis of hormonally treated prostatic carcinoma. J Urol 148: 833–837.
44. Yang Y, Chisholm GD, Habib FK (1992) The distribution of PSA, cathepsin-D, and pS2 in BPH and cancer of the prostate. Prostate 21: 201–208.
45. Bartsch W, Klein H, Schiemann U, et al. (1990) Enzymes of androgen formation and degradation in the human prostate. Ann N Y Acad Sci 595: 53–66.
46. Gregory CW, He B, Johnson RT, et al. (2001) A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 61: 4315–4319.
47. Harper ME, Pike A, Peeling WB, et al. (1974) Steroids of adrenal origin metabolized by human prostatic tissue both in vivo and in vitro. J Endocrinol 60: 117–125.
48. Kim D, Gregory CW, French FS, et al. (2002) Androgen receptor expression and cellular proliferation during transition from androgen-dependent to recurrent growth after castration in the CWR22 prostate cancer xenograft. Am J Pathol 160: 219–226.
49. Taplin ME, Balk SP (2004) Androgen receptor: a key molecule in the progression of prostate cancer to hormone independence. J Cell Biochem 91: 483–490.
50. Edwards J, Krishna NS, Grigor KM, et al. (2003) Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. Br J Cancer 89: 552–556.
51. Linja MJ, Savinainen KJ, Saramaki OR, et al. (2001) Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 61: 3550–3555.
52. Brown RS, Edwards J, Dogan A, et al. (2002) Amplification of the androgen receptor gene in bone metastases from hormone-refractory prostate cancer. J Pathol 198: 237–244.
53. Hobisch A, Culig Z, Radmayr C, et al. (1995) Distant metastases from prostatic carcinoma express androgen receptor protein. Cancer Res 55: 3068–3072.

54. Hsing AW, Reichardt JK, Stanczyk FZ (2002) Hormones and prostate cancer: current perspectives and future directions. Prostate 52: 213–235.
55. Stanbrough M, Bubley GJ, Ross K, et al. (2006) Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 66: 2815–2825.