**Pten Inactivation and the Emergence of Androgen-Independent Prostate Cancer**

Michael M. Shen¹ and Cory Abate-Shen²

Abstract

Hormone refractory disease represents a late-stage and generally lethal event in prostate tumorigenesis. Analyses of mouse models have recently shown that the onset of hormone independence can be uncoupled from disease progression and is associated with activation of the phosphoinositide-3 kinase/Akt as well as Erk mitogen-activated protein kinase signaling pathways in the prostate epithelium, which act in part to counterbalance the inhibitory effects of androgen receptor signaling in the prostate stroma. These observations have potential implications for the treatment of patients with hormone refractory cancer and highlight the role of epithelial-stromal interactions for androgen independence. [Cancer Res 2007;67(14):6535–8]

Introduction

Similar to other male secondary sexual tissues, the development and homeostasis of the prostate gland requires androgens. When the fully mature prostate is deprived of testicular androgens, either through castration or chemical androgen ablation, it undergoes rapid regression, in which ~90% of prostate epithelial cells undergo apoptosis. Prostate epithelial cells remain androgen dependent even in the transformed state, and thus androgen deprivation represents an effective therapeutic intervention for prostate carcinoma, as first shown by Huggins and Hodges (1). Nonetheless, androgen ablation is generally followed by the eventual emergence of hormone refractory prostate cancer, which represents a major clinical challenge.

Despite the greatly reduced levels of androgens in the hormone-refractory state, it is now well established that maintenance of prostate cancer cells requires androgen receptor (AR), a member of the nuclear hormone receptor family (reviewed in refs. 2–6). In particular, the androgen-independent state still requires a functional AR signaling pathway, AR is usually wild type during initial emergence of androgen independence but may acquire mutations during androgen ablation therapy (2–4, 6). Moreover, the expression level of AR is frequently increased in androgen-independent prostate cancer (7), perhaps consistent with selection for adaptation to reduced androgen levels. Furthermore, hormone refractory tumors may produce sufficient levels of androgens to activate AR after androgen ablation therapy (8).

These observations indicate that activity of the AR signaling pathway is maintained, and perhaps even amplified, after the reduction or absence of ligand in the androgen-independent state. Several possible molecular mechanisms have been proposed for this outcome, including (a) the development of hypersensitive AR responses due to increased AR levels or production of endogenous androgens within the prostate, (b) altered transcriptional activity of AR due to changes in expression of co-repressors and/or co-activators, or (c) ligand-independent activation of AR (reviewed in refs. 2, 5). Notably, these categories of models imply that androgen independence arises from molecular alterations that occur during carcinogenesis and that androgen-independent cells undergo molecular adaptation during this process (9). Alternatively, other models presume that there are preexisting androgen-independent cells that occur infrequently within tumors and that these undergo clonal selection during androgen ablation therapy to result in androgen-independent tumors (9, 10). In particular, the existence of androgen-independent prostate cancer stem cells might be consistent with such a clonal selection mechanism.

Early Emergence of Androgen-Independent Prostate Cancer in Nkx3.1; Pten Mice

The analysis of the molecular mechanisms underlying androgen-independent prostate cancer has been greatly facilitated by the generation of mouse models that can recapitulate key features of hormone refractory disease. Work in our laboratories has used mouse models that contain null mutant alleles of two key regulatory genes known to be inactivated in human prostate cancer progression, Nkx3.1 and Pten (11, 12). The Nkx3.1 homeobox gene encodes a transcriptional regulator that is expressed from the earliest stages of prostate organogenesis and is essential for proper branching morphogenesis of the prostate and expression of secretory proteins (13). Homozygous Nkx3.1 mutant mice are healthy and viable but develop a prostate epithelial hyperplasia that leads to the development of low-grade prostatic intraepithelial neoplasia (PIN) with increasing age (13). The Pten tumor suppressor encodes a lipid phosphatase that negatively regulates the phosphoinositide-3 kinase/Akt pathway and, thus is intimately involved with the regulation of cellular proliferation and survival (14). Although homozygous Pten null mutants display homozygous embryonic lethality, compound heterozygotes for Nkx3.1 and Pten display high-grade PIN and invasive carcinoma with increasing age (12). Notably, older Nkx3.1; Pten mutant mice can develop metastases as well as androgen independence (11, 15–17).

Although hormone refractory disease arises at late stages of prostate cancer progression, our recent studies have shown that androgen independence can be largely uncoupled from disease progression (17). To assess the onset of androgen-independent
phenotypes, Nkx3.1; Pten mutant or wild-type control mice were castrated at ages from 3 weeks to 6 months. At 2 days after castration, large-scale apoptosis was observed in the control prostate but not in the Nkx3.1; Pten mutant prostates, which continued to display a highly proliferative phenotype. When analyzed at age of 8 or 10 months, the castrated mice displayed androgen-independent lesions with high-grade PIN or carcinoma phenotypes similar to those found in intact (noncastrated) mice of the same genotypes and ages. Interestingly, even mice castrated at age of 3 weeks, before sexual maturation, displayed a high frequency of androgen-independent PIN lesions. Such lesions were still observed in animals that had undergone both surgical castration as well as adrenalectomy, suggesting that androgen independence was not simply a consequence of adaptation to reduced androgen levels.

Notably, in the androgen-independent lesions arising in Nkx3.1; Pten mutant mice, the AR remains wild type in sequence and in expression levels (16). Furthermore, the growth of these lesions is blocked by treatment with the AR inhibitor flutamide, either in castrated Nkx3.1; Pten mice or in prostate tissue grafts derived from these mice and grown under the renal capsule in immunodeficient nude mice, indicating their dependence on AR. Because these tissue grafts are generated by recombination of Nkx3.1; Pten mutant epithelium with wild-type urogenital mesenchyme, their androgen-independent growth in castrated mice is strictly dependent upon the loss of function of Nkx3.1 and Pten in the epithelium rather than in the stroma/mesenchyme.

Furthermore, our observations suggest that inactivation of Pten plays a primary role in emergence of androgen independence. In particular, androgen-independent lesions can also be found in Pten heterozygous mice that are wild type for Nkx3.1, whereas androgen-independent lesions were never observed in Nkx3.1 mutant mice (17). However, inactivation of Pten leads to rapid down-regulation of Nkx3.1 expression (12, 18, 19); and consequently, the role of Nkx3.1 in this process remains unclear. It is important to note that these mouse models are predisposed to Pten inactivation because they contain germline deletion of one Pten allele and undergo stochastic mutational inactivation of the wild-type allele. In contrast, the initial absence of a mutated Pten allele in human patients may partially account for the long latency of prostate cancer and the observed association between late-stage cancer and androgen independence. Although this is indeed the case, analyses of human tissue samples have shown that inactivation of Pten frequently occurs by the onset of prostate carcinoma (e.g., refs. 20, 21) before clinical treatment; and thus, it is likely that androgen-independent epithelial cells are present long before the actual detection of hormone refractory disease.

The absence of a strict sequential relationship between disease progression and acquisition of androgen independence may help explain the paradoxical outcome of a major clinical study of the efficacy of finasteride in chemoprevention of prostate cancer (22). Finasteride is an inhibitor of 5α-reductase, which converts testosterone into the active metabolite dihydrotestosterone, and therefore reduces androgen levels. Although this Prostate Cancer Prevention Trial showed that finasteride successfully reduces the overall incidence of prostate cancer, those patients who did develop cancer displayed a higher-grade disease. Therefore, it is conceivable that reduction of androgen levels may accelerate disease progression by selection for prostate epithelial cells that are predisposed to survive in the absence of androgens.

Synergistic Activities of Akt and Erk Signaling in Androgen Independence

We noted that carcinoma in both intact and castrated Nkx3.1; Pten mice displayed significant activation of the Akt pathway, as expected for inactivation of Pten, but also showed strong activation of the Erk mitogen-activated protein kinase (MAPK) pathway (16). These pathways are also activated in human prostate cancer, particularly in androgen-independent specimens (23–25), and cooperate in androgen-independent growth in human prostate cancer cells (26). In addition, Akt and AR have been shown to cooperate in cancer progression in a tissue recombinant model in vivo (27). Notably, several lines of evidence have suggested that Akt may directly phosphorylate AR, thus providing a potential mechanism by which it may contribute to androgen independence (reviewed in ref. 28). However, the physiologic significance of these findings has not been resolved.

For further analysis of molecular mechanisms of androgen independence, we have taken advantage of androgen-dependent and androgen-independent prostate epithelial cell lines, which we have termed CASP cell lines, that were derived from primary tumors of the mutant mice in the absence of selection for androgen independence (15). Importantly, androgen dependence strikingly differs between existing prostate epithelial cell lines and prostate epithelium in vivo. In the absence of androgens, androgen-dependent cell lines in culture do not undergo apoptosis but instead fail to proliferate (e.g., ref. 29). This distinction is observed for androgen-dependent CASP cell lines (16); similar observations have been made for other mouse lines (30). The mechanistic basis for this key distinction between the behavior of prostate epithelial cells in vivo versus in cell culture has remained unclear.

To investigate the functional significance of the activated Akt and Erk signaling pathways in androgen-independent lesions, we took advantage of matched pairs of androgen-dependent and androgen-independent CASP cell lines (15). Retrovirally mediated overexpression of either activated Akt (Akt) or activated B-raf (B-raf), an upstream regulator of the Erk pathway, or both resulted in androgen-independent proliferation of the androgen-dependent CASP 2.1 cell line in culture (16). Consistent with its dependence on AR signaling activity together with Akt or Erk MAPK pathway stimulation, this proliferation in culture could be blocked by flutamide or by inhibitors of the Akt and B-raf pathways.

In contrast with the additive effects of Akt and B-raf on androgen-independent growth of CASP 2.1 cells in culture, the identical cells behaved differently when grown in renal grafts in vivo. Cells infected with control retroviruses failed to proliferate in the absence of androgens in culture but underwent apoptosis when grown in renal grafts with wild-type urogenital mesenchyme followed by castration. Forced overexpression of either Akt or B-raf failed to result in growth of renal grafts in castrated nude mice, whereas coexpression of Akt and B-raf resulted in a strong synergistic androgen-independent growth response. Thus, the combined activation of two distinct proliferative pathways is required for the ability of prostate epithelial cells to overcome the apoptotic consequences of androgen deprivation in vivo.

An Epithelial-Stromal Competition Model for Androgen Independence

Taken together, these studies provide support for a new model for the emergence of androgen independence (Fig. 1). The
difference in behavior of the CASP 2.1 cells in culture versus in vivo indicates that interaction with the wild-type tumor environment is likely to be responsible for the apoptosis of the epithelial cells as typically observed for androgen-dependent prostate tumors. Consistent with this interpretation, other studies have shown that stromal AR, but not epithelial AR, is necessary for the apoptotic response after castration (31), indicating that the androgen-deprived stroma produces paracrine apoptosis–inducing factor(s) that act upon the epithelium. Moreover, AR expression has been shown to be down-regulated in the stroma of hormone-refractory prostate tumors (32). At present, the nature of the stromal proapoptotic signal(s) is unknown but identity of these signals should be of considerable interest.

In this view, the emergence of androgen independence reflects the outcome of a contest between the proliferative ability of the epithelial cells and the nonautonomous proapoptotic activity produced by the stroma. Notably, both of these competing activities require wild-type AR function, consistent with the wild-type sequence of AR in most hormone refractory cancers. In the simplest case, inactivation of Pten would strongly influence the outcome of this epithelial-stromal competition by promoting the survival and proliferation of epithelial cells. Furthermore, the combined activation of the Akt and Erk MAPK pathways can overcome the stromal proapoptotic factor(s), resulting in androgen independence.

Finally, our epithelial-stromal competition model does not necessarily presume that all cells within an androgen-independent lesion are truly androgen independent at the single-cell level, as the hormone refractory state could be partially promoted by nonautonomous survival/proliferation signals. This would potentially be consistent with the heterogeneous nature of androgen-independent cancer (33) and would be compatible with a clonal selection model of androgen independence. Further analyses of androgen independence in mouse model systems will undoubtedly provide tests of the validity of these ideas.

Acknowledgments


Grant support: National Cancer Institute (NCI); National Institutes of Diabetes, Digestive and Kidney Diseases; and Department of Defense Prostate Cancer Research Program.

We apologize to numerous colleagues whose work could not be cited due to length constraints.
Cancer Research

References


Announcements

MEETING OF THE RADIATION RESEARCH SOCIETY

The annual meeting of the Radiation Research Society will be held at the State University of Iowa, Iowa City, on June 22-24, 1953. The Society will be the guest of the University, and all meetings will be held on the campus. The program will consist of: (1) Two symposia, one on "The Effects of Radiation on Aqueous Solutions," which includes the following speakers: E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen. The second is "Physical Measurements for Radiobiology" and companion talks by Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne S. Harris. (2) On Monday night, June 22, a lecture by Dr. L. W. Alvarez on meson physics has been tentatively scheduled. On Tuesday night, June 23, Dr. L. H. Gray of the Hammersmith Hospital, London, will speak on a topic to be announced. Dr. Gray's lecture is sponsored by the Iowa Branch of the American Cancer Society. Those desiring to report original research in radiation effects, or interested in attending or desiring additional information, please contact the Secretary of the Society, Dr. A. Edelmann, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

ERRATUM

The following correction should be made in the article by Beck and Valentine, "The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia. I. Glycolysis and Respiration," November, 1952, page 891: substitute for the last paragraph:

The data in Table 8 permit several interesting calculations. If one compares the amount of glucose actually disappearing with the sum of the amount equivalent to lactic acid produced plus that equivalent to O₂ consumption, it is seen that the amount of glucose "cleavage products" exceeds the amount of glucose utilized by 12 per cent in N and 27 per cent in CML and is exceeded by the glucose utilized by 16 per cent in CLL. If the assumption is made that, in this respect, the myeloid and lymphoid cells of leukemia are similar to those of normal blood, it may be that the computed normal figure represents a summation of the myeloid (M) and lymphoid (L) cells that make up the normal leukocyte population. Thus, if M = +0.27 and L = −0.16 and the normal differential is 65 per cent M and 35 per cent L, then

\[ 0.65 (+0.27) + 0.35 (-0.16) = +0.12 \]

a figure identical to the observed +0.12 for normal leukocytes.
Pten Inactivation and the Emergence of Androgen-Independent Prostate Cancer

Michael M. Shen and Cory Abate-Shen


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/14/6535

Cited articles
This article cites 33 articles, 20 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/14/6535.full#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/67/14/6535.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/67/14/6535.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.