Urol Res (1987) 15:133–138

Urological Research

Development of Androgen-Independent Tumor Cells and Their Implication for the Treatment of Prostatic Cancer

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Accepted: January 12, 1987

Summary. Development of androgen-independent prostatic cancer cells from androgen-responsive cells can occur by a variety of mechanisms (e.g., environmental adaptation, multifocal origin, or genetic instability). Regardless of the mechanism of development, however, once androgen-independent cancer cells become present within prostatic cancer, the tumor is no longer homogeneous but is now heterogeneous. Once a prostatic cancer is heterogeneously composed of both androgen-dependent and -independent cancer cells, androgen withdrawal therapy, no matter how complete, cannot be curative. In order to produce cures of such heterogeneous prostatic cancers, hormonal therapy must be combined simultaneously with chemotherapy early in the course of the disease so that all the cancer populations (i.e., androgen-dependent and -independent) can be simultaneously affected within an individual patient.

Key words: Androgen independent tumor cells, Prostatic cancer heterogeneity.

Introduction

Nearly all men with metastatic prostatic cancer treated by androgen ablation do respond, indicating that at least a portion of their cancer cells are androgen responsive. Unfortunately, however, essentially all of these patients eventually relapse to a state unresponsive to further antiandrogen therapy, no matter how aggressive their secondary treatment [23, 31, 32]. Because of this nearly universal relapse phenomenon, the annual death rate from prostatic cancer has not decreased at all over the subsequent 40 years since androgen withdrawal has become standard therapy [2]. Over the last 40 years, the superficially benign nature of androgen withdrawal therapy has tended to disguise the fact that metastatic prostatic cancer is still a fatal disease for which no therapy is available which effectively increases survival [21].

What is the mechanism for this relapse phenomenon wherein an initially androgen-responsive prostatic cancer progresses following androgen ablation to an androgen-resistant state? The answer to this question is fundamental since, depending on the mechanism, it may or may not be possible to prevent the development of such androgen resistance. The significance of such a possibility is that once resistance to androgen withdrawal therapy has occurred, any possibility of curing the patient solely with hormonal therapy is lost [30].

One way in which the relapse to androgen withdrawal can occur is that prostatic cancers initially could be composed of tumor cells that are homogeneous, at least in regard to their dependence on androgenic stimulation for their maintenance and continuous growth (i.e., androgen-dependent cancer cells). Following castration, most of these dependent cells stop proliferating and die, thus producing an initial positive response to withdrawal therapy. Some of these androgen-dependent cells, however, under environmental pressure, randomly adapt to become androgen-independent. These androgen-independent cells, once formed, proliferate without the requirement for androgenic stimulation, and thus repopulate the tumor producing a relapse after castration. In such an explanation, the changing host environmental conditions following castration are assumed to be critically involved in inducing the adaptive transformation of an initially androgen-dependent to an androgen-independent tumor cell. This process is therefore called the environmental adaptation model. In contrast to this environmental adaptation model where the changing androgen environment following castration is assumed to play a direct inductive role, an alternative explanation is possible in which the role by the changing androgen environ-

Part of this paper was presented at the 5th Congress of the European Society for Urological Oncology and Endocrinology, 18–20 August, 1986, Edinburgh, UK

ment following castration is only indirect. It is possible that initially prostatic cancers are heterogeneous, being composed of preexisting clones of androgen-dependent and -independent tumor cells. The androgen-independent cancer cells can be of two types: cells which are neither dependent on nor sensitive to androgenic stimulation for their growth (i.e., androgen-independent insensitive cells) or cells which grow faster in the presence of adequate androgen levels but which can still grow continuously even when no androgen is present (i.e., androgen-independent sensitive cancer cells; for example, of both of these types of androgen-independent cells see ref. [7]). Regardless of what type of androgen-independent cells (i.e., insensitive or sensitive) are present, castration, in such a context, would result in the death of only the androgen-dependent cells without stopping the continuous growth of the androgen-independent ones. While this would produce an initial positive response, these independent cells would continue to proliferate following castration. Even if these androgen-independent cells initially represented only a small fraction of the starting tumor, their continuous growth would eventually not only replace completely any tumor loss due to the death of the androgen-dependent cells, but progressively re-expand the tumor producing a relapse.

Regardless of whether environmental adaptation or selection is the mechanism for relapse to androgen withdrawal therapy, eventually clones of androgen-independent prostatic cancer cells grow to kill the patient. Therefore, of what clinical importance is the resolution as to which of the two possible mechanisms is actually responsible for relapse? The importance of resolving whether adaptation *versus* clonal selection is the mechanism responsible for relapse is that the optimal therapy for prostatic cancer is very different depending upon the answer.

The Environmental Adaptation Model – The case for a More Complete Androgen Withdrawal

If the environmental adaptation model is the mechanism for the relapse to standard androgen ablation therapy, then it is possible that the presently utilized forms of androgen ablation may not be ideal for the treatment of prostatic cancer. This is because while the standard forms of surgical or chemically induced castration do lower the serum testosterone level by over 90%, they do not completely eliminate all potential serum androgens. Since low levels of non-gonadal (e.g., adrenal) androgens are left following castration, this treatment induces a partial, not a complete, androgen withdrawal. This has led some investigators to suggest that a more complete form of androgen withdrawal in which the very low levels of non-testicular serum androgen remaining following castration are neutralized by simultaneous treatment with a direct acting antiandrogen might be more effective than simply castration alone [5, 16]. In particular, Labrie et al. [16] have used a combination of a luteinizing hormone-releasing hormone (LHRH) analog plus an antiandrogen to produce what they term "a complete androgen withdrawal". The rationale for such a combination approach is based upon Dr. Labrie's premise that "all or nearly all tumoral prostatic cells are androgen dependent at the beginning of the disease and that loss of androgen dependence occurs by genetic loss of this property during cell divisions ... it appears logical to propose the initiation of this (complete androgen withdrawal treatment) as early as possible after diagnosis to minimize the appearance of androgen-insensitive cancer cell clones" [16]. In addition, Labrie asserts that "99% of prostatic tumors, even at the stage of metastases are still androgen-sensitive. Instead of being present at the beginning of treatment, most androgen-insensitive cells develop when tumor cells are exposed to the low androgen milieu provided by the adrenal androgens" [17]. Since once these androgen-independent prostatic cancer cells develop, the patients become incurable by any type of hormonal therapy, Labrie has postulated that by combining antiandrogen with castration (either surgical or chemical), it might be possible to kill all of the androgen-dependent prostatic cancer cells, before they can progress to become androgen-independent cancer cells.

Labrie's group termed such a combination of surgical or medical orchiectomy with the additional administration of the antiandrogen flutamide "complete androgen blockade", and have reported preliminary studies using this form of hormonal therapy [19, 20, 21]. While these investigators interpret their preliminary data as suggesting improvement in response and survival, the short follow-up time for their study and their patient selection and evaluation criteria appear to influence their conclusions. Although the number of patients has continued to increase since their initial reports, the average follow-up time (i.e., 1.4 years) has remained constant in their later studies. Nevertheless, the projected response and survival rate have decreased continuously over the last years. For example, the projected 2-year response rate decreased from 81 percent in their 1985 report (calculated on 87 patients) [20] to 60 percent in their 1986 report (calculated on 119 patients) [21]. Therefore the question remains as to how much further the response and survival rates will decrease when the patients in the study of Labrie et al. have been followed for a longer period of time, so that actual rates are obtained than projected.

In addition, there are other major limitations to the Labrie study. First, the study is a non-randomized clinical trial; no partial androgen withdrawal group was randomized and followed prespectively in parallel over the same time interval with the complete androgen withdrawal group. Second, the trial was not a double blind study (i.e., the evaluating physician knew the treatment the patient received). These last two points are critical since in other clinical trials, more complete androgen withdrawal therapy has not been shown to be any more effective than castration alone. For example, Beland et al. [1] performed a randomized double blind clinical trial comparing the effectiveness of surgical castration with or without simultaneous treatment with the antiandrogen, Anadron, on previously untreated men with

metastatic prostatic cancer. In this study, the evaluating physician did not know which patients were or were not receiving the antiandrogen or placebo. This randomized double blind study could not demonstrate any statistically significant increase in survival between castrated patients treated with or without antiandrogen.

Likewise, Schroeder et al. [31], in a randomized clinical trial of previously untreated D2 prostatic cancer patients, demonstrated that androgen withdrawal consisting of daily LHRH analog plus cyproterone acetate was no more effective in preventing progression of the cancer than was LHRH analog alone (i.e., 39% of patients progressed within 12 months on the combinational treatment group vs. 38% progression in the LHRH alone group). In addition, similar negative results have been reported by Zadra et al. [40] using the non-steroidal antiandrogen, Anandron. In this clinical trial, previously untreated D2 prostatic cancer patients were randomized to receive either: 1) castration alone, 2) castration plus simultaneous Anandron, or 3) LHRH plus simultaneous Anandron. All patients were followed for 14–19 months. The progression rate for the castration alone group was 30%; for the castration + Anandron group, it was 50%; for the LHRH + Anandron group, it was 36%. In addition to these negative clinical trials in humans, animal studies, using well characterized serially transplantable rat prostatic cancers, also have failed to demonstrate any additional effect of combining an antiandrogen, be it either cyproterone acetate or flutamide, with surgical or medical castration [5, 30].

The Environment Selection Model – The Case for Combining Androgen Withdrawal and Chemotherapy and/or Radiation

There are several major clinical observations which do support the critical assumption upon which the complete androgen withdrawal theory is based. *First,* while the adrenals do supply steroids to the blood which theoretically could act as androgen, no one has actually demonstrated that in humans these adrenal androgen precursors in fact have major effects upon prostatic cell growth. Indeed, a recent study by Oesterling et al. [28] demonstrates that the adrenal glands do not have a significant stimulatory effect on the human prostate and are not capable by themselves of supporting prostatic growth. This conclusion is based upon the following findings. In patients with panhypopituitarism, in whom there is no testicular nor adrenal function, the prostates are completely atrophic by both histological and morphological criteria, thus demonstrating a complete lack of androgenic stimulation. When these atrophic prostates were compared to those of patients with normal adrenal function but no testicular function (hypogonadotropic hypogonadism or prepubertal castration), no difference was found in their degree of atrophic morphology or histology demonstrating that adrenal function alone does not have a stimulatory effect on the human prostate and is not capable of supporting prostatic growth.

Second, prostatic cancers, when they become clinically manifest, are rarely phenotypically homogeneous with regard to the clones of cancer cells comprising individual tumors. For example, Kastendieck [15] demonstrated that of 180 clinically manifest prostatic cancers removed surgically from previously hormonally untreated patients, 60% of these cancers were already histologically heterogeneous being composed of a mixture of several different cancer cell types of widely varying differentiation (admixture of glandular, cribiform, and anaplastic morphology within the same cancer). These results clearly demonstrate that prostatic cancer cell heterogeneity can occur early in the clinical course of the disease and there is no requirement for any reduction in serum androgen levels (i.e., no requirement for hormonal therapy) in order to induce this morphological heterogeneity. This last point is also demonstrated by the study of Viola et al. [38] in which immunoperoxidase staining methods were used to examine the cellular distribution of prostate-specific antigen, carcinoembryonic antigen and p21 Harvey-ras oncogene protein within individual prostatic cancers from patients with metastatic disease and who had received no prior hormonal therapy. This study again demonstrates that each of these phenotypic parameters was heterogeneously distributed with multiple foci of both nonreactive and reactive cancer cells present within individual prostatic cancers. Similar cellular heterogeneity within individual prostatic cancers, even before hormonal therapy was initiated, was also demonstrated by Mostofi et al. [26] using immunocytochemical localization of prostatic specific acid phosphatase as a phenotypic marker.

Based upon these morphological and immunocytochemical studies, it is clear that individual prostatic cancers are heterogeneously composed of clones of phenotypically distinct prostatic cancer cells even before hormonal therapy is begun. This has led a series of investigators [7, 29, 35, 36] to suggest that the major reason androgen withdrawal therapy is not curative, is not due to an inadequate decrease in the systemic level of androgen following therapy, but is instead due to the fact that prostatic cancers are heterogeneously composed of clones of both androgen-dependent and -independent cancer cells even before hormonal therapy is begun. If this is correct, then treatment of such a heterogeneous prostatic cancer with androgen withdrawal alone would kill only the androgen-dependent clones of cancer cells present without stopping the continuous clonal growth of the preexisting androgen-independent prostatic cancer cells, no matter how complete this androgen withdrawal therapy might be.

Animal models have clearly demonstrated not only the fact that prostatic cancer can be heterogeneously composed of androgen-dependent and -independent prostatic cancer cells before hormonal therapy is begun [7, 36], but also the fact that an increase in survival above that produced by castration alone cannot be produced no matter how complete

the androgen withdrawal therapy utilized [5]. Using these animal models, it can be shown that an increase in survival, above that produced by castration alone, can only be produced, if nonhormonal chemotherapy is given simultaneously in combination with castration and as early as possible in the course of the disease [10]. For example, in studies using Cytoxan as a model chemotherapeutic agent, it has been found that if rats bearing androgen responsive prostatic cancers are given Cytoxan starting simultaneously at the time of castration early in the course of the disease, i. e., tumor smaller than 1 cm^3, that it is possible to increase survival by over 200% in these combinationally treated animals as opposed to tumor bearing animals simply castrated. Such early combinational treatment has resulted in >50% of these tumor bearing animals being cured. In order to cure animals, Cytoxan has to be given simultaneously with, not sequential to, androgen ablation and it has to be given for at least four cycles [12]. These studies demonstrate that if an effective chemotherapy is available to eliminate the androgen-independent prostatic cancer cells, cures can be obtained if such effective chemotherapy is simultaneously combined with androgen ablation early in the course of the disease.

Mechanism for the Development of Cellular Heterogeneity Within Individual Prostatic Cancers

Once a prostatic cancer is heterogeneously composed of clones of both androgen-dependent and -independent cancer cells, androgen withdrawal therapy, no matter how complete, cannot be curative. Therefore, it is critically important to resolve whether it is possible to prevent the development of such heterogeneity. To answer this question, some ideas as to the mechanism(s) for such tumor cell heterogeneity must be known.

1. Multifocal Origin of Prostatic Cancer

One way in which individual prostatic cancers could be heterogeneous with regard to their androgen responsiveness is that instead of a monoclonal origin for the original prostatic cancers, the tumor could initially arise as a polyclonal mixture of androgen-dependent and -independent cancer cells. This suggestion is strengthened by the observation that when Byar and Mostofi [2] performed careful histological examination of serial step-section on a series of 208 consecutive human prostates removed by radical prostatectomy for early prostatic cancer (i.e., B disease), 85% of prostates had anatomically distinct multifocal cancer areas. If some of these distinct cancer areas are androgen-dependent and others independent, then a heterogeneously responsive tumor would exist even before hormonal therapy is begun. Such a heterogeneous situation could occur rather simply since the normal prostate is heterogeneously composed of both androgen-dependent and -independent prostatic cells even before malignant transformation occurs [13]. The fact that within any individual malignant prostate these multiple cancer foci can have different phenotypic characteristics has been demonstrated by Kirchheim et al. [16]. These authors demonstrate, for example, using histochemical staining techniques, that within the same prostate there can be both cancer foci which were positive and negative for the presence of the enzyme leucine-β-aminopeptidase.

2. Genetic Instability of Prostatic Cancer Cells

Another possibility for the development of heterogeneity in androgen responsiveness within an individual prostatic cancer is that while a prostatic cancer may initially develop monoclonally from a single androgen-dependent malignant cell, as this parental cell continues to proliferate, eventually an occasional progeny cell becomes genetically unstable. The concept of genetic instability in cancer cells has been discussed in detail by Nowell [27] and therefore the general thesis will not be developed except to say that genetic instability can eventually lead to changes in the genome of an occasional cancer cell such that a genetically altered clone of cells which is no langer androgen-responsive but is now androgen-independent, can be added to the original homogeneous tumor. When such an addition occurs, the tumor is no longer homogeneous, but is instead now heterogeneous. Is there any evidence to support the concept of genetic instability as a mechanism for the development of heterogeneity in androgen responsiveness in prostatic cancer? Using the serial transplantable Dunning R3327 system of rat prostatic cancers, it has been possible consistently to demonstrate that androgen-dependent prostatic cancer cells can randomly give rise to completely androgen-independent cancer cells even when the original androgen-dependent prostatic cancer cells are grown in an intact (i.e., noncastrated) male host [9]. This progression to the androgen-independent state has been documented to involve genetic instability since definitive changes in the genetic make-up of the prostatic cancer cells have been demonstrated [39]. In addition, Thompson et al. [37] have demonstrated that when an androgen-dependent subline of Dunning 3327 series is grown in culture, rapid heterogeneity develops such that multiple clones of phenotypically distinct prostatic cancer cells can be isolated. These clonal variations do have in common, however, that they are all androgen-independent even though the original parental cancer cells from which each was derived are initially androgen-dependent. Similar loss of androgen dependency of cancer cells during in vitro culture has also been confirmed by Labrie and Veilleux [22]. Using the Shionogi mouse mammary cancer as a model, this group has confirmed that a wide range of androgen sensitivity develops when clonal variations of the original androgen-dependent Shionogi mammary cancer are grown continuously in culture. Such a heterogeneous distribution of androgen sensitivity develops even if the clonal cells are grown in the continuous presence of physiological levels of androgen. This again confirms that genetic insta-

bility of androgen-dependent cancer cells leads to the development of androgen-independent cancer cells and that such development does not require any decrease in the androgenic stimulation to these cells. Such genetic instability thus results in the production of a heterogeneous prostatic cancer even if the tumor is growing in the presence of normal non-ablated levels of androgen. Once a prostatic cancer is heterogeneously composed of clones of both androgen-dependent and -independent cancer cells, the ability to cure the patient with androgen ablation alone is lost. This is because even if the clones of androgen-independent prostatic cancer cells are of the androgen-independent sensitive type and are "hypersensitive" to the low levels of adrenal androgen as proposed by Labrie and Veilleux [22], these clones would not be eliminated even if total androgen ablation is given since their growth rate is only sensitive, not absolutely dependent upon, androgenic stimulation.

Conclusions

Androgen-dependent cells within an individual prostatic cancer can be effectively eliminated by any of a series of presently available forms of androgen ablation (e.g., castration, DES, LHRH analogs/antagonists plus/minus antiandrogen). A substantial number of prostatic cancer patients prefer a medical, as opposed to a surgical form of castration. Since DES produces cardiovascular toxicity, the use of LHRH analogs as a means of medical castration, will undoubtedly increase in the future. Due to the flare reaction, an antiandrogen will probably be begun simultaneously with the LHRH analog, and thus the androgen withdrawal therapy combining the two agents likely become a standard form of therapy for prostatic cancer. This combinational hormonal therapy will probably be as effective as surgical increase in survival compared to simple surgical castration alone. In order to increase survival, what is desperately needed is a modality which can effectively eliminate the clones of androgen-independent cancer cells already in the prostatic cancer even before therapy is begun. By combining such an effective modality with any of the various types of androgen ablation presently available, it should be possible to affect all of the populations of tumor cells within individual heterogeneous prostatic cancers and thus optimize the possibility for cure. Indeed, this theroretical possibility has been experimentally demonstrated. For example, Cytoxan has sufficient effectiveness upon the androgen-independent cancer cells in rat prostatic cancers that if Cytoxan is simultaneously combined with androgen ablation therapy when the tumor is small, cures can be produced [12]. Unfortunately, Cytoxan may not have sufficient effectiveness against the androgen-independent cells within human prostatic cancers to allow cures, even if simultaneously combined with androgen ablation [4]. While combining Cytoxan with androgen ablation has not produced any substantial increase in cures, these previous attempts have usually been begun late in the course of the disease when tumor burden is large and rately has Cytoxan been begun simultaneously with androgen ablation. In most studies, Cytoxan has been given to patients relapsing to androgen ablation [4]. Servadio et al. [34] has reported, however, on a small group of non-randomized stage D patients that such combinational treatment increases survival above that obtained with androgen ablation alone. Thus, while there is hope that Cytoxan might be helpful when combined with androgen ablation, especially if given earlier in the disease, there are substantial reasons to believe that Cytoxan may not be the optimal agent to control the growth of androgen-independent human prostatic cancer cells [11]. Thus the search for effective agents targeted at the androgen-independent human prostatic cancer cells must continue. New approaches to such development have recently been summarized [11].

Acknowledgements. This work was supported by the DHHS (USA) Grant CA 15416.

References

1. Beland J, Elhilali M, Fradet Y, Llaroche B, Ramsey EW, Venner PM (1986) Total androgen blockade vs. orchiectomy in stage D2 prostatic cancer. In: Murphy G (ed) Second international symposium on prostatic cancer. Alan R Liss, New York (in press)
2. Byar DP, Mostofi FK (1972) Carcinoma of the prostate: Prognostic evaluation of certain pathological features in 208 radical prostatectomies, examined by the step section technique. Cancer 30:5–13
3. Devesa SS, Silverman DT (1978) Cancer incidence and morbidity trends in the United States: 1935–1974. Natl Cancer Inst 60:545–571
4. Elder JS, Gibbons SRP (1985) Results of trends of the USA National Prostatic Cancer Project. Prog Clin Biol Res 185(A): 221–242
5. Ellis WJ, Isaacs JT (1985) Effectiveness of complete versus partial androgen withdrawal therapy for the treatment of prostatic cancer as studied in the Dunning R-3327 system of rat prostatic adenocarcinoma. Cancer Res 45:6041–6050
6. Giuliani L, Pescatore D, Giberti C, Martorana G, Natta G (1980) Treatment of advanced prostatic carcinoma with Cyproterone acetate and orchiectomy. 5 year follow-up. Eur Urol 6:145–148
7. Isaacs JT, Coffey DS (1981) Adaptation vs. selection as the mechanism responsible for the relapse of prostatic cancer to androgen ablation as studied in the Dunning R-3327-H adenocarcinoma. Cancer Res 41:5070–5075
8. Isaacs JT (1982a) Hormonally responsive versus unresponsive progression of prostatic cancer to antiandrogen therapy as studied with the Dunning R-3327-AT and -G rat adenocarcinomas. Cancer Res 42:5010–5014
9. Isaacs JT, Wake N, Coffey DS, Sandberg AA (1982b) Genetic instability coupled to clonal selection as a mechanism for tumor progression in the Dunning R-3327 rat prostatic adenocarcinoma system. Cancer Res 42:2353–2361
10. Isaacs JT (1984) The timing of androgen ablation therapy and/or chemotherapy in the treatment of prostatic cancer. Prostate 5:1–18
11. Isaacs JT (1985) New principles in the management of metastatic prostatic cancer. Prog Clin Biol Res 185(A):383–406
12. Isaacs JT (1986a) The effects of increasing the rate of cell death on the curability of prostatic cancer by chemotherapy as stud-

ied with the Dunning R-3327-G rat prostatic adenocarcinoma (submitted for publication)
13. Issacs JT (1986b) Control of cell proliferation and cell death in the normal and neoplastic prostate: A stem cell model. NIADDK Monograph BPH (in press)
14. Karr JP, Murphy GP (1985) Treatment of prostatic carcinoma with combinations of drugs and hormones. In: Bruchovsky N, Chapdelaine A, Newman F (eds) Regulation of androgen action: the proceedings of an international symposia in Montreal. Congressdruck R. Bruchner, West-Berlin, pp 81–86
15. Kastendieck H (1980) Correlation between atypical primary hyperplasia and carcinoma of the prostate. Histologic studies on 180 total prostatectomies due to manifest carcinoma. Pathol Res Pract 169:366–387
16. Kirchhiem D, Niles NR, Frankus E, Hodges CV (1966) Correlative histochemical and histological studies on thirty radical prostatectomy specimens. Cancer 19:1683–1696
17. Labrie F, Dupont A, Belanger A, Lacourciere Y, Raynaud JP, Hassan JM, Gareau J, Fazekos AT, Sandow J, Monfette G, Girard JH, Emond J, Houle J (1983) New approach in the treatment of prostate cancer: Complete instead of partial withdrawal of androgens. Prostate 4:579–594
18. Labrie F, Dupont A, Belanger A (1985) Complete androgen blockade for the treatment of prostate cancer. In: Devita VT, Hellman S, Rosenberg S (eds) Important advances in oncology 1985. JB Lippincott, Philadelphia, pp 193–217
19. Labrie F, Belanger A, Dupont A, Emond J, Lacourciere Y, Monfette G (1984) Combined treatment with LHRH agonist and pure antiandrogen in advanced carcinoma of the prostate. Lancet II:1090
20. Labrie F, Dupont A, Belanger A, Giguere M, Lacourciere Y, Emond Jj, Monfette G, Bergeron V (1985) Combination therapy with Flutamide and castration (LHRH agonist or orchiectomy) in advanced prostatic cancer: a marked improvement in response and survival. J Steroid Biochem 23:833–841
21. Labrie F, Dupont A, Lacourciere Y, Giguere M, Belanger A, Monfette G, Emond J (1986a) Combined treatment with Flutamide in association with medical or surgical castration. J Urol 135:203A
22. Labrie F, Veilleux R (1986b) A wide range of sensitivities to androgen develop in clonal Shionogi mouse mammary tumor cells. Prostate 8:293–300
23. Lepor H, Ross A, Walsh PC (1982) The influence of hormonal therapy on survival of men with advanced prostatic cancer. J Urol 128:335–340
24. The Leuprolide Study Group (1984) Leuprolide versus diethylstilbestrol for metastatic prostatic cancer. N Engl J Med 311:1281–1286
25. Menon M, Walsh PC (1979) Hormonal therapy for prostatic cancer. In: Murphy GP (ed) Prostatic cancer. PSG Publishing, Littleton, Mass, pp 175–200
26. Mostofi FK, Sesterhenn J (1981) The role of prostatic acid phosphatase in histological diagnosis of carcinoma of the prostate. Proceeding of the Seventy-Sixth Annual Meeting of the American Urological Association, Abstract #42
27. Nowell PC (1976) The clonal evolution of tumor cell populations. Science 194:23–28
28. Oesterling JE, Epstein JI, Walsh PC (1986) The inability of adrenal androgens to stimulate the adult human prostate – An autopsy evaluation of men with hypogonadotropic hypogonadism and panhypopituitarism. J Urol (in press)
29. Prout GR, Leiman B, Daly JJ, MacLoughlin RA, Griffin PP, Young HH (1976) Endocrine changes after diethylstilbestrol therapy. Urology 7:148–155
30. Redding TW, Schally AV (1985) Investigation of the combination of the agonist D-Trp-6-LHRH and the antiandrogen Flutamide in the treatment of Dunning R-3327-H prostatic cancer model. Prostate 6:219–232
31. Schroeder FH, Klijn JG, deJorg FA (1986) Metastatic cancer of the prostate managed by Buserelin acetate versus Buserelin acetate plus Cyproterone acetate. J Urol 135:202A
32. Schulze H, Isaacs JT, Coffey DS (1986a) A critical review of the concept of total androgen ablation in the treatment of prostatic cancer. In: Murphy G (ed) Second international symposium on prostatic cancer. Alan R Liss, New York (in press)
33. Scott WW, Menon M, Walsh PC (1980) Hormonal therapy of prostatic cancer. Cancer 45:1929–1936
34. Servadio C, Mukamel E, Kahan E (1984) Carcinoma of the prostate in Israel: Some epidemiological and therapeutic considerations. Prostate 5:375–382
35. Sinha AA, Blackard CE, Seal US (1977) A critical analysis of tumor morphology and hormone treatments in the untreated and estrogentreated responsive and refractory human prostatic carcinoma. Cancer 40:2836–2850
36. Smolev JK, Heston WD, Scott WW, Coffey DS (1977) Characterization of the Dunning R-3327-H prostatic adenocarcinoma. An appropriate animal model for prostatic cancer. Cancer Treat Rep 61:273–287
37. Thompson SA, Johnson MP, Heidger PM, Lubaroff DM (1985) Characterization of the heterogeneity of R-3327 rat prostate tumors derived from single-cell clones. Prostate 6:369–387
38. Viola MV, Fromowitz F, Oravez MS, Deb S, Finket G, Lundy J, Harel P, Thor A, Schlom J (1986) Expression of ras oncogene p21 in prostatic cancer. N Engl J Med 314:133–137
39. Wake N, Isaacs JT, Sandberg AA (1982) Chromosomal changes associated with progression of the Dunning R-3327 rat prostatic adenocarcinoma system. Cancer Res 42:4131–4142
40. Zadra J, Bruce AW, Trachtenberg J (1986) Total androgen ablation therapy in the treatment of advanced prostatic cancer. J Urol 135:201

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