Evidence for Clonal Outgrowth of Androgen-independent Prostate Cancer Cells from Androgen-dependent Tumors through a Two-Step Process

Noah Craft, Chloé Chhor, Chris Tran, Arie Beldegrun, Jean DeKernion, Owen N. Witte, Jonathan Said, Robert E. Reiter, and Charles L. Sawyers

ABSTRACT

Prostate cancers require androgen for growth but progress to an androgen-independent stage under the selective pressure of androgen ablation therapy. Here we describe a novel human prostate cancer xenograft (LAPC-9) propagated by serial passage in male severe combined immunodeficient mice that expresses prostate-specific antigen and wild-type androgen receptor. In response to castration, LAPC-9 cells undergo growth arrest and persist in a dormant, androgen-responsive state for at least 6 months. After prolonged periods of androgen deprivation, spontaneous androgen-independent outgrowths develop. Thus, prostate cancers progress to androgen independence through two distinct stages, initially escaping dependence on androgen for survival and, subsequently, for growth. Through the use of serial dilution and fluctuation analysis, we provide evidence that the latter stage of androgen independence results from clonal expansion of androgen-independent cells that are present at a frequency of about 1 per 10^6 androgen-dependent cells. We conclude that prostate cancers contain heterogeneous mixtures of cells that vary in their dependence on androgen for growth and survival and that treatment with antiandrogen therapy provides selective pressure and alters the relative frequency of these cells, thereby leading to outgrowths of androgen-independent cancers.

INTRODUCTION

Androgen withdrawal leads to apoptosis of the secretory epithelium and growth arrest of the basal epithelium in the normal prostate (1). Androgen replacement stimulates the repopulation of secretory epithelium through induction of a differentiation program in a subset of basal cells (2, 3). Thus, basal cells are androgen responsive but are not dependent on androgen for survival, whereas secretory cells require androgen to avoid apoptotic cell death. Most prostate cancers are considered androgen dependent, based on the high response rate of these tumors to antiandrogen therapy. The mechanism for the clinical response to androgen withdrawal therapy is not clearly defined but is likely to result from a combination of tumor cell death through induction of apoptosis as well as growth arrest (4–6). Eventually, prostate cancers will resume growth despite antiandrogen therapy, at which point the tumors are termed androgen independent or hormone refractory.

At a molecular level, androgen-independent progression has been associated with mutations or amplification of the androgen receptor gene (7–12) and activation of intracellular signal transduction pathways that stimulate the androgen receptor (13–15). These observations have led to the concept that androgen-independent prostate cancers have reactivated the androgen receptor pathway by a ligand-independent mechanism (16, 17). Interestingly, comparative gene expression studies indicate that some androgen-independent cancers may express genes normally restricted to the basal epithelium of normal prostate (18, 19). In contrast, their androgen-dependent counterparts have a gene expression profile more typical of differentiated, secretory epithelial cells. These findings raise questions about the cell of origin in prostate cancer and are consistent with the notion that androgen-independent cancers represent the outgrowth of a rare, pre-existing subclone of tumor cells with basal cell characteristics. This concept has been supported previously by fluctuation analysis in the rat Dunning system (20–23). Additionally, recent evidence in the transgenic mouse TRAMP model suggests that androgen-independent prostate cancer cells occur very early in the progression of disease (24). Alternative possibilities are that androgen-dependent prostate cancer cells develop secondary genetic mutations that allow androgen-independent growth or that populations of androgen-dependent cells adapt to the altered hormonal environment caused by androgen deprivation, as reported in the Shionogi mouse mammary carcinoma model (25–27). Although these questions have been well studied in rodents, it has been difficult to distinguish between these concepts in human prostate cancer cells, largely because appropriate models to study this question have been lacking.

Our laboratory previously reported a human prostate cancer xenograft called LAPC-1-4, which progresses from androgen dependence to androgen independence in SCID mice in response to castration (28). Here we describe a new prostate cancer xenograft, LAPC-9, that also requires androgen for growth, synthesizes PSA, and expresses a nonmutant androgen receptor. Through kinetic analysis of in vivo proliferation and cell death, we show that a small fraction of the cells in LAPC-9 tumors die by apoptosis in response to castration, whereas the majority withdraw from the cell cycle. These cells remain in a dormant yet viable state and respond rapidly when reexposed to androgen by reentering the cell cycle and resuming tumor growth, even after 6 months of androgen deprivation. After longer intervals, some LAPC-9 tumors resume growth as androgen-independent cancers.

The availability of two androgen-dependent xenografts that develop androgen independence after castration provides an opportunity to investigate the cellular basis of this progression in an experimental model. Through the use of serial dilution studies and fluctuation analysis, we show that injection of as few as 10 cells will consistently lead to tumor formation in intact male mice, but only a fraction of such injections will produce tumors when implanted in castrated mice. Fluctuation analysis was used originally to provide evidence that the emergence of bacterial strains resistant to bacteriophage lysis is a consequence of preexisting genetic mutations in the bacteria rather than an adaptive response to altered nutrients (29). Our results are consistent with the hypothesis that hormone-refractory cancer evolves through clonal outgrowth of a small number of androgen-independent
tumor cells that are preexisting or develop at a low frequency due to secondary genetic mutations. We propose that prostate cancers contain a mixture of cells that differ in their need for androgen as a growth or survival factor and that antiandrogen therapy givesrogen-independent cells a selective growth advantage that results in outgrowths of hormone-refractory tumors.

MATERIALS AND METHODS

Development of the LAPC-9 Xenograft. LAPC-9 was derived as described previously for LAPC-4 (28). After obtaining informed consent, clinical material was obtained at the time of surgery from the femoral metastasis of a patient who had disease progression while receiving hormone ablation therapy. The tissue was minced and implanted with 200 µl of Matrigel (Collaborative Research, Bedford, MA) s.c. into several male SCID mice under methoxyflurane anesthesia. After initial tumor formation, tumors were harvested, minced, and reimplanted with Matrigel into male SCID mice. Androgen ablation was achieved by surgical castration under anesthesia. Androgen replacement after castration was achieved using implantable sustained release DHT pellets (12.5 mg/90-day release; Innovative Research of America, Sarasota, FL).

Androgen Receptor Sequencing. Individual pairs of oligonucleotides were used to amplify segments of the androgen receptor gene as described previously (30). The PCR product was cloned into pZero Blunt (Invitrogen, San Diego, CA) according to the manufacturer’s protocol and sequenced using automated methods through the UCLA DNA sequencing facility.

Preparation of Single-Cell Xenograft Suspensions. Tumors were dissociated into single-cell suspensions by enzymatic digestion with modifications to a protocol described previously (31). Briefly, LAPC xenografts were harvested using sterile technique from the flanks of SCID mice. Tumors were minced to 1-mm³ pieces in serum-free Iscove’s medium on ice. This tissue was washed twice with Iscove’s medium, and then incubated in a 1% solution of Pronase E (EM Science, Gibbstown, NJ) in Iscove’s for 18 min at room temperature using 10 times the original tumor volume. Tissue was washed twice in Iscove’s medium, filtered through sterile 200-µm nylon mesh (Biodesign, Inc. of New York, Carmel, NY), and plated overnight at 37°C in serum-free PrEGM (Clonetics, San Diego, CA) containing Fungizone. The next day, tissue was disaggregated again by pipetting, refiltered through nylon mesh, and replated in PrEGM overnight to obtain a homogenous single-cell preparation. On the third day, the cells were washed once in PrEGM, counted, serially diluted in PrEGM, then injected s.c. into both flanks using a 25-gauge needle with 100 µl of Matrigel extracellular matrix. For tissue chunks, 2-mm³ chunks of tissue were implanted using a trocar as described previously (28). Tumors were monitored by palpation every 1–2 weeks and measured in three orientations using calipers.

Protein Expression Studies. PSA levels in mouse serum were determined by ELISA (American Qualex, San Clemente, CA) according to product literature and calibrated to the PSA controls provided. Tissues were fixed for 4 h in 10% neutral buffered formalin and then embedded in paraffin for histological sectioning. Antigen retrieval was performed using a commercial steamer and incubation in a 0.01M citrate buffer (pH 6). Serial sections were incubated with monoclonal antibody to PSA diluted 1:3000 in PBS (DAKO Corp., Carpinteria, CA) or MIB-1 antibody (against Ki-67) diluted 1:60 (Immuno-tech, Westbrook, ME). Slides were then incubated sequentially with peroxidase-conjugated rabbit anti-mouse antibodies, peroxidase-conjugated swine anti-rabbit antibodies, and peroxidase-conjugated rabbit anti-swine (DAKO Corp.). Antibody localization was performed using the diaminobenzidene reaction, and slides were counterstained with hematoxylin. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining was performed using the ApopTag Plus kit (Intergen, Purchase, NY) according to the manufacturer’s instructions.

RESULTS

Characterization of a New PSA-positive, Androgen-dependent Human Prostate Cancer Xenograft. To expand the spectrum of prostate cancer phenotypes represented by cell lines and xenografts, we have continued our efforts to develop new human prostate cancer xenografts in SCID mice (28). Similar to the previously reported xenografts LAPC-3 and LAPC-4, LAPC-9 was developed by implanting prostate cancer cells obtained at the time of surgery directly into SCID mice. LAPC-9 has been maintained for >20 passages in male SCID mice over a 2-year period without administering supplemental testosterone. ELISA analysis of serum from mice bearing LAPC-9 xenografts (Fig. 1) demonstrated expression of the androgen-dependent PSA gene, indicating that LAPC-9 tumors are of human prostatic origin and have an intact androgen receptor signaling pathway. As expected, this conclusion was confirmed by immunoblot analysis showing expression of androgen receptor protein in LAPC-9 cells (data not shown).

Although mutations in the androgen receptor gene occur in some prostate cancers (7–11), most clinical samples appear to express wild-type androgen receptor. To determine the status of the androgen receptor in LAPC-9, we sequenced the coding regions of the gene using genomic DNA. No mutations were found based on sequence analysis of PCR products obtained by amplification of exons 1–8 using intron-based primers and genomic DNA as template.
To examine the role of androgen in LAPC-9 tumor growth, we set up a series of experiments in which tumors were established s.c., subjected to androgen deprivation, and in some cases, reexposed to androgen 3–6 months later. We implanted LAPC-9 cells into nine intact male mice, allowed tumors to develop, then castrated four animals. In uncastrated control mice, tumor size (Fig. 1A) and serum PSA (Fig. 1B) continued to rise for 60 days until the mice were euthanized because of tumor burden. In castrated animals, the serum PSA fell by 70% after 7 days and remained at a low but detectable level for the duration of the experiment (120 days). Tumor size showed little decrease after castration and remained unchanged throughout the experiment. These data indicate that LAPC-9 cells require androgen for continued growth.

Secretory epithelial cells in the normal prostate gland as well as some prostate cancer xenografts undergo apoptosis in response to androgen withdrawal (32–34). We used standard immunohistochemical markers of cell growth and cell death at various time points after castration to determine the effect of androgen withdrawal on these parameters in LAPC-9 tumors. In the presence of androgen, a high fraction of LAPC-9 cells expressed the proliferation marker protein Ki-67, indicating a high growth fraction (Fig. 2, upper left panel, day 0). After castration, the number of Ki-67-positive cells fell more than 10-fold over 7 days and remained low throughout the period of androgen deprivation (Fig. 2, 110 days). A very small fraction (~1%) of cells were terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling-positive at all time points before and after castration, indicating a low rate of apoptosis (data not shown). Therefore, the primary consequence of androgen deprivation in the LAPC-9 tumor model is a decrease in cell proliferation, a finding consistent with the lack of significant regression in tumor size (Fig. 1A).

LAPC-9 Cells Remain Dormant but Are Androgen Responsive after Prolonged Periods of Androgen Ablation. The fact that a significant fraction of LAPC-9 tumor cells fail to die after castration raises the possibility that these residual cells are androgen independent. Alternatively, they may no longer require androgen for survival but remain dependent on androgen for growth. To examine this possibility, we asked whether LAPC-9 cells remain androgen responsive after prolonged periods of androgen deprivation. LAPC-9 tumors were established in intact male mice, and then the mice were castrated. PSA levels fell by 70% and reached a plateau phase, as in Fig. 1B. One hundred ten days after castration, DHT pellets were implanted s.c. in a cohort of animals to reexpose the residual tumor cells to androgen. Within 14 days, serum PSA levels rose 30-fold in the DHT-treated group (Fig. 1B) but not in untreated animals. Histological analysis showed a 15- to 20-fold increase in the number of Ki-67-positive cells within 7 days (Fig. 2). This was followed by a rapid increase in tumor size with kinetics comparable with tumors implanted into intact males (data not shown). These results indicate that LAPC-9 tumors remain androgen responsive after prolonged periods of androgen deprivation.

One explanation for the androgen-independent survival of LAPC-9 tumors is the presence of an established vasculature that might provide essential survival factors in the absence of androgen. Alternatively, LAPC-9 cells may be completely independent of androgen for survival, yet require androgen for growth. To distinguish between these possibilities, we injected LAPC-9 cells that had been disaggregated into a single-cell suspension into the flanks of female mice at doses that fail to give rise to tumors after 1 year, reasoning that any tumors that formed after reexposure to androgen could not be explained by tumor vasculature. Six months after injecting 10^3 cells s.c., no tumors were present, and serum PSA levels were undetectable. We then implanted DHT pellets and monitored serum PSA levels and tumor formation. Within 7 days, serum PSA levels rose from undetectable levels to a mean of 13.2 ng/ml (Fig. 1C). After 14 days, PSA levels reached 51.4 ng/ml, and tumors were palpable at the site where cells had been injected s.c. 6 months earlier. By 28 days, PSA levels were >200 ng/ml, and the mice were sacrificed shortly thereafter because of tumor burden. These results indicate that single-cell suspensions of LAPC-9 cells implanted directly into an androgen-deprived environment remain viable but do not proliferate. We conclude that androgen confers a potent growth signal in the LAPC-9 prostate cancer model but is not required for survival.

Isolation of Androgen-independent LAPC-9 Sublines. The previously developed androgen-dependent LAPC-4 xenograft, which grows reproducibly in intact male mice within 4 weeks after trocar
implantation of minced tumor tissue, will form tumors spontaneously in castrated male mice after 13–16 weeks without the readdition of androgen. We have demonstrated previously that these LAPC-4 tumors derived from castrated animals (called LAPC-4 AI) are androgen independent because they grow at comparable rates when passed into intact or castrated male mice (28). We performed a similar experiment with LAPC-9 and found that trocar implants in intact male mice formed tumors in 5 weeks, whereas similar implants in castrated males formed tumors after 26 weeks (Table 1). Of note, these tumors (called LAPC-9 AI) regrew within 4 weeks when passed into female mice, confirming that these sublines are androgen independent for growth. Therefore, sublines of LAPC-4 and LAPC-9 can be derived that are hormone refractory in that they do not require androgen for growth or survival.

Evidence for Clonally Derived, Androgen-independent Cells in Androgen-dependent Xenografts. On the basis of studies of androgen-responsive Shionogi mouse mammary carcinoma cells, it has been argued that conversion to androgen independence is an adaptive process that occurs in response to androgen ablation therapy (25–27). This argument is based on the observation that the frequency of androgen-independent cells in Shionogi tumors that regress after castration is much lower than in recurrent hormone refractory Shionogi tumors, implying that androgen-dependent cells adapt to an altered hormonal environment. Alternatively, it has been proposed from studies of the Dunning rat prostate cancer model that these tumors are heterogeneous and that androgen-independent cells undergo clonal expansion in the setting of antiandrogen therapy (20, 22, 23). These cells could preexist at low frequency in the original androgen-dependent tumor, as suggested by studies in the TRAMP model (24), or develop as a consequence of secondary mutations or epigenetic changes arising during androgen ablation therapy. Because the Shionogi, Dunning, and TRAMP models use rodent carcinoma cells rather than human prostate cancer cells, we examined these concepts in LAPC-4 and LAPC-9 cells. If the clonal expansion hypothesis is true, it should be possible to subdivide a population of androgen-dependent tumor cells into pools, some of which will contain androgen-independent cells and some of which will not, analogous to the fluctuation analysis strategies used to demonstrate preexisting genetic resistance of bacteria to bacteriophage lysis (29) and to show clonal selection in the Dunning system (20). If the adaptive model is correct, then all pools should give rise to androgen-independent tumors. The size of the pools required to dilute out androgen-independent cells will depend on the frequency of these cells in the original androgen-dependent population. Because LAPC-4 and LAPC-9 give rise to androgen-independent sublines with different kinetics (Table 1), we reasoned that the frequency of androgen-independent cells may be different in these two lines.

We performed a serial dilution analysis of androgen-dependent LAPC-4 and LAPC-9 cells in intact male mice. The purpose was to define the smallest number of cells capable of forming a tumor in the presence of androgen to establish the limits of the model. For both xenografts, the latency for tumor formation was strictly related to the dose of cells injected, and as few as 10 cells was sufficient to form a tumor (Fig. 3A). These results establish a high cloning efficiency for LAPC-4 and LAPC-9 cells when isolated from androgen-dependent xenografts and implanted in intact males.

To determine whether androgen-independent cells are present in these tumors and to define their frequency, androgen-dependent LAPC-4 and LAPC-9 tumors were divided into pools ranging in size from 10 to 1 × 10^6 cells and injected into female mice (Fig. 3B). For LAPC-4, all of the animals injected with 10^6 cells developed tumors, indicating that the frequency of androgen-independent cells is at least 1 per million. However, at a pool size of 5 × 10^5 cells, 67 of 104 (64%) female animals developed tumors. Only one of 14 animals (7%) developed a tumor at a pool size of 1 × 10^4, and no tumors were observed at lower doses. When the androgen-independent LAPC-4 tumors that did develop were subjected to the same serial dilution analysis, as few as 100 cells (versus 5 × 10^3 cells) was sufficient to form tumors in female mice (data not shown), demonstrating a 500-fold enrichment for androgen-independent cells by passage in an androgen-depleted environment. For LAPC-9, 13 of 88 (15%) female mice developed tumors at a pool size of 5 × 10^5, and no tumors were observed at lower cell doses. The fact that some but not all female animals develop tumors at defined cell doses argues against the hypothesis that androgen independence results from adaptation of a population of androgen-dependent cells to an androgen-depleted environment. Rather, the data support the presence of a small number of cells that are androgen independent or have the capacity to become androgen independent in some, but not all, pools of androgen-dependent cells. If these cells preexist, we estimate their frequency to be

| LAPC tumor latency in male and castrate male mice |  
|--------------------------------------------------|---|---|
| **LAPC4** | **LAPC9** |  
| AD in males | 28 days (6/6) | 35 days (6/6) |
| AD in castrates | 98 days (5/6) | 182 days (4/6) |
| AI in castrates | 28 days (6/6) | 28 days (6/6) |

Table 1 LAPC tumor latency in male and castrate male mice

LAPC-4 or LAPC-9 tumors were harvested and prepared as in "Materials and Methods." Minced tissue was mixed with Matrigel and implanted by trocar s.c. into intact or castrated male mice. The average number of weeks to tumor formation (≥0.5 cm) is shown for each condition. The number of tumors and sample sizes are shown in parentheses.

Fig. 3. Limiting dilution and fluctuation analysis of LAPC-4 and LAPC-9 xenografts. LAPC-9 and LAPC-4 cells were harvested from intact male mice and prepared as described in "Materials and Methods." A, cells were implanted s.c. into intact male mice at the cell dose specified (n = 6–14 mice at each cell dose), and tumors were measured by calipers weekly. Data points represent the time point at which 50% of implantations formed tumors >0.5 cm in all dimensions. B, LAPC-4 or LAPC-9 cells were implanted s.c. into female mice and monitored weekly. Tumor formation was positive if a tumor was >0.5 cm in all dimensions. Data points represent the percentage of implantations that resulted in tumor formation at 1 year. Absolute number of tumors formed and sample sizes are shown next to each column.

5033

Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 1999 American Association for Cancer Research.
about 1 per $10^5$ for LAPC-4 and 1 per $10^6$ for LAPC-9, which is consistent with the different latencies for LAPC-4 and LAPC-9 to develop androgen-independent tumor outgrowth after trocar implantation (Table 1). Alternatively, if these cells develop as a consequence of secondary genetic events that allow androgen-independent growth, the frequencies of 1 per $10^5–10^6$ could reflect different mutation rates in LAPC-4 versus LAPC-9 because of genomic instability.

**Histological Evidence for Outgrowth of Androgen-independent Subclones.** One prediction of the hypothesis that androgen-independent cells are clonally derived from androgen-dependent cancers is that these cells should proliferate despite androgen ablation therapy. Because these cells represent such a small fraction of the androgen-dependent tumor, it would be difficult to visualize these cells using an immunohistochemical marker for cell proliferation unless the androgen-independent clone was allowed to expand to a detectable size. We tested this possibility by establishing androgen-dependent LAPC-9 tumors in intact males, castrating the mice, and examining serial histological sections of LAPC-9 tumors at time points before (day 110) and during (day 180) the outgrowth of androgen-independent tumors for evidence of proliferation using Ki-67. In this experiment, no animals were given supplemental testosterone after castration. At early time points (day 7), no foci of Ki-67-positive cells were observed, as seen previously in Fig. 2. However, at day 110, we observed small clusters of Ki-67-positive cells in a background of low-level, sporadic Ki-67-positive staining (Fig. 4, compare panels 2 and 3 at day 110), indicating the presence of a focus of cell proliferation at a time prior to the outgrowth of an androgen-independent tumor. At 180 days, tumor size began to increase, indicating that an androgen-independent tumor had developed, and the majority of cells stained positive for Ki-67. These results provide histological evidence that androgen independence occurs by clonal outgrowth of a small number of androgen-independent cells.

**DISCUSSION**

Androgen plays a pivotal role in regulating the growth and differentiation of normal and malignant prostate epithelial cells. Although androgen ablation therapy produces dramatic clinical responses in prostate cancers, this treatment is palliative because androgen-independent or hormone-refractory tumors eventually regrow (35). The mechanism for this progression to androgen independence is unclear. In this report, we have taken advantage of two human prostate cancer xenografts developed recently by our group, each of which expresses PSA and wild-type androgen receptor, to characterize this process in more detail at the cellular level. Our results suggest that androgen-independent progression occurs in two distinct stages (Fig. 5). At the time of initial diagnosis, a fraction of cells in a prostate cancer tumor are dependent on androgen for survival (Fig. 5, lightly stippled cells) and undergo apoptosis in response to androgen ablation therapy, similar to the secretory epithelial cells in normal prostate tissue. Clinical evidence for this conclusion has been well documented in studies of prostate cancer tissue from patients who receive neoadjuvant hormone ablation therapy prior to radical prostatectomy surgery (5, 36, 37). The first step in androgen-independent progression is a transition stage in which tumor cells remain androgen responsive yet no longer require androgen for survival (Fig. 5, striped cells). The second stage involves the outgrowth of a tumor that is independent of androgen for both growth and survival, as observed clinically with hormone-refractory cancers that progress despite androgen ablation therapy (Fig. 5, dark gray cells). Through serial dilution studies and fluctuation analysis of LAPC-4 and LAPC-9, we provide evidence that this second stage results from clonal expansion of a small number of androgen-independent cells.

The strongest evidence for the first stage of androgen independence is the demonstration that a small number of LAPC-9 cells injected into castrated animals can survive for 6 months or more and remain...
The first step in progression to androgen independence (stage I) is enrichment for cells present in the androgen-dependent xenografts under the selective pressure of androgen ablation therapy: (a) we can identify focal areas of cell proliferation that develop in LAPC-9 tumors that have undergone prolonged growth arrest in response to castration. We postulate that these foci undergo further clonal expansion and become hormone-refractory tumors; and (b) serial dilution studies demonstrate that androgen-independent cells account for 1 in $10^5$–$10^6$ cells in our androgen-dependent xenografts.

The second stage of androgen-independent progression, in which tumors grow despite antiandrogen therapy, has been recognized for decades, but the cellular details have been unclear. Two findings from the xenograft studies presented here argue that this occurs through preferential expansion of a small number of androgen-independent cells present in the androgen-dependent xenografts under the selective pressure of androgen ablation therapy: (a) we can identify focal areas of cell proliferation that develop in LAPC-9 tumors that have undergone prolonged growth arrest in response to castration. We postulate that these foci undergo further clonal expansion and become hormone-refractory tumors; and (b) serial dilution studies demonstrate that androgen-independent cells account for 1 in $10^5$–$10^6$ cells in our androgen-dependent xenografts. This frequency could reflect the relative abundance of preexisting androgen-independent cells or the mutation rate for acquiring a genetic or epigenetic event that allows androgen-independent growth. A mutational frequency of $10^{-7}$ is consistent with the background of genomic instability known to exist in human prostate cancers (47–51) and shown previously to occur in the Dunning R-3327 rat prostatic adenocarcinoma system (22).

Our proposal that human prostate cancers progress to androgen independence through clonal evolution is similar in concept to conclusions about the process of cancer metastasis. The work of Fidler and colleagues (52, 53) has established that metastasis occurs by selection of a rare subpopulation of cells with metastatic potential from a heterogeneous starting population of tumor cells. Molecular evidence in support of this concept as applied to metastasis is now available from clinical studies comparing the frequency of cells harboring p53 mutations in metastatic prostate cancer lesions versus the primary tumor (54). Similar evidence for clonal expansion of hormone-refractory prostate cancer cells will require further progress in identifying the molecular lesions responsible for late-stage, androgen-independent disease.


Evidence for Clonal Outgrowth of Androgen-independent Prostate Cancer Cells from Androgen-dependent Tumors through a Two-Step Process

Noah Craft, Chloe Chhor, Chris Tran, et al.