CWR22: The First Human Prostate Cancer Xenograft with Strongly Androgen-dependent and Relapsed Strains Both in Vivo and in Soft Agar

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ABSTRACT

Most patients' prostate cancers respond to androgen deprivation but relapse after periods of several months to years. Only two prostate cancer xenografts, LNCaP and PC-346, have been reported to be responsive to androgen deprivation and to relapse subsequently. Both of these tumors exhibit deceleration of their rates of growth after androgen withdrawal, and, in a proportion of animals, evolves to a stage that has been used subsequently in many laboratories. After androgen withdrawal, the human primary prostate cancer xenograft CWR22 regresses markedly, and prostate-specific antigen (PSA) falls up to 3000-fold in the blood of mice. PSA usually returns to normal. In some animals, the tumor relapses and is then designated CWR22R. In these animals, PSA starts to rise approximately 2-7 months, and tumor begins to grow 3-10 months after castration. Animals with CWR22 need to be euthanized because of large tumors 6-12 weeks after the transplantation of CWR22. Androgen withdrawal prolongs life approximately 3-4-fold.

INTRODUCTION

In men, most prostate cancers respond to androgen withdrawal (1) but relapse after the initial response. Few, if any, prostate cancers in humans have been cured by hormonal manipulation (2). Models for the development of experimental therapy are limited. To our knowledge, only two xenografts, LNCaP (3, 4) and PC-346 (5), have been reported to show initial responses to androgen withdrawal followed by resumption of growth. Both of these tumors exhibit deceleration of their rates of growth after androgen withdrawal by castration; occasionally, these tumors regress slightly after castration, usually with less than a 10% reduction in volume. Unlike most hormonally responsive prostate cancers in men, both of these tumors may undergo a more rapid rate of growth less than 5 weeks after castration (3, 5). PSA in the sera of animals bearing LNCaP tumors may decrease up to 8-fold after castration (3).

In 1994, we (6) described CWR22, an androgen-dependent, serially transplantable xenograft derived from a primary human prostate cancer that has been used subsequently in many laboratories. After castration, CWR22 shows marked regression, not just deceleration or stabilization of growth, and may regress completely. We now describe serially transplanted CWR22R tumors, xenografts that have relapsed several months to more than 1 year after the castration of the hosts. To our knowledge, this is the first report of a human prostate cancer xenograft that both regresses markedly for long periods of time after androgen withdrawal, and, in a proportion of animals, evolves to a tumor that resumes growth in the castrated animal. All previously reported prostate cancer xenografts either (a) regress and disappear never to recur or (b) do not regress significantly after castration. After castration of animals that bear CWR22, serum PSA has fallen 15-3080-fold; in all but one animal, >50-fold. Physically measurable recurrence is preceded by an elevation of serum PSA. The general availability of this model will offer a unique opportunity to compare a human prostate cancer that regresses after castration with tumor that relapses several months to more than 1 year later in the castrated animals.

MATERIALS AND METHODS

The origin (7) and detailed characterization (6) of the serially transplantable human primary prostate cancer xenograft CWR22 have been described. CWR22 causes elevations of PSA in mouse peripheral blood that correlate with growth (size and weight) of the tumors in nude mice. PSA in 50 µl tail vein blood was assayed at intervals ≤5 weeks, as shown in the data from individual experiments, with a slight modification of the method used by us previously (8). Suspensions of single cells for transplantation and for culture in soft agar were obtained by digestion of xenografts with 0.1% Pronase (VWR Scientific, Bridgeport, NJ) as before (6). Nude mice were housed and cared for as described previously (6, 7). Mice with CWR22 were given 12.5-mg sustained-release testosterone pellets (Innovative Research of America, Sarasota, FL) s.c. before receiving tumors and at intervals of 3 months until death.

Cells from freshly dissociated, serially transplanted CWR22 and CWR22R that were serially transplanted from CWR22 tumors that relapsed after androgen deprivation were used in soft agar assays as described by Hamburger and Salmon (9) with the following modifications: MEM+ culture medium with supplements as described in Ref. 10) with 20% normal (Life Technologies, Inc., Gaithersburg, MD) or charcoal-stripped (Calico Biologicals, Inc., Reams-town, PA) calf serum was used as the solvent for the noble agar layers (Difco, Detroit, MI). Cell suspensions were filtered through a single layer of Nitex (Tetko, Inc., Briarcliff Manor, NY) with a 48-µm porosity immediately before use for culture. After preliminary experiments to determine the concentrations of cells that would allow for the most satisfactory growth for counting, cells were plated at final concentrations of 0.05 and 0.025 million cells/ml. Cell suspensions were plated in a volume of 0.4 ml at the tip of a horizontal 5-ml tube. Dilutions of testosterone in 0.04 ml were plated near the opening of this horizontal tube, and 3.6 ml 0.33% soft agar at 44°C were added rapidly to the tube. The tube was mixed three times by inversion, and 1 ml soft agar (final concentration 0.3%) was plated on top of a previously plated 1-ml layer 0.5% agar. The cells in the several dilutions of testosterone (Sigma Chemical Co., St. Louis, MO) shown below and the control plates were cultured and counted in triplicate. The plates were incubated in a humidified chamber with 5% CO2 in air at 37°C. After 14 days in culture, the number of clusters per plate was counted and plotted as the mean ± SD. Groups of cells greater than eight cells per group were scored (11) and termed "clusters."

In preliminary experiments, variables that might affect the soft agar assay were explored. After filtration through 48-µm mesh Nitex as described above, cells were cultured in soft agar over a wide range of concentrations. Concentrations of 0.05 and 0.025 million cells/ml gave approximately 50-100 clusters/plate in the absence of testosterone. Cultures observed at intervals of 1 week for 4 weeks gave similar numbers of clusters (more than eight cells per
RESULTS

PSA in the sera of mice declined much more rapidly after the resection of tumors than after the castration of mice bearing tumors (Fig. 1). The half-life of PSA after resection (Fig. 1A) appears to be less than 1 day, a value that is consistent with the values reported for a study of the LNCaP xenograft (3). Based on only five animals for which PSA values were obtained at intervals after castration and removal of sustained release testosterone pellets without resection of tumors, the half-life of the serum PSA appears to be variable over a range of approximately 3–8 days. The wide variation observed among different animals in this experiment may be related in part to the fact that PSA was elevated 1 day after castration as compared with the value obtained just before castration in some animals. In the most extreme example of this, seen in animal 2276 (Fig. 1B), the PSA was 522 ng/ml immediately before castration, 1504 ng/ml at 1 day, 558 ng/ml at 2 days, 417 ng/ml at 5 days, and 289 ng/ml at 7 days after castration. We speculate that the elevation of PSA in some animals on the day following androgen deprivation may have been related to injury of the neoplastic cells. The regression of tumors proceeded much more slowly than the decrease in PSA, i.e., the PSA usually declined by 50% in a maximum of 1 week after castration while the tumor volume usually took more than 1 month to decline by 50%; the tumor volume often continued to decline for more than 3 months before becoming stable.

The experiment in which mice have been followed for the longest period after castration was a part of a separate ongoing study. Historically, of the 151 animals that have received injections of >1000 CWR22 cells in Matrigel and observed for at least 3 months over the past 3 years, all but 5 have developed tumors. In our longest (>600 days) experiment, CWR22 cells were transplanted by the injection of 8.3 million cells in 0.05 ml Matrigel into single subscapular sites bilaterally in nine animals. By design, when the largest tumor reached 7 mm in its smallest dimension, all nine animals were castrated, and testosterone pellets were removed. The larger of the two tumors was resected from each of five of the animals 1 day after castration; from each of the remaining four animals, 4 days after castration. At the time of castration, PSA values in the blood of these mice ranged between 41 and 282 ng/ml. Both of these mice died without tumors and 0.27 ml, respectively. After resection of the larger of the two tumors that could be measured with calipers and 0.95 and 0.72 ml. After resection of the larger of the two tumors and 220-230-fold after resection of the larger of their two tumors and castration.

After castration, five of the nine animals failed to develop recurrent tumors that could be measured with calipers; the remaining four animals developed relapsed tumors with the first evidence of elevation of blood PSA seen 92, 151 (two animals), and 221 days after castration (Fig. 2). As detailed below, some of the five mice in which relapsed tumors were not detected grossly showed evidence of the persistence of small amounts of residual tumor. To date, these nine animals have been followed for >600 days after castration or until death. Two of the nine animals, (mice 2144 and 2148) are currently alive and without recurrence of growing tumors or abnormal serum PSA. Mice 2146 and 2162 had the lowest PSA values at the time of resection and had tumor burdens estimated from external measurements of 0.95 and 0.72 ml. After resection of the larger of the two tumors in these two animals, the estimated tumor burdens were 0.29 and 0.27 ml, respectively. Both of these mice died without tumors detectable by caliper measurements or abnormal circulating PSA at autopsy, mouse 2146 showed no tumor at death 362 days after castration; mouse 2162 died 443 days after castration and was found to have minute amounts of histologically detectable tumor (estimated to be <1 mm³) in the organized Matrigel at the site of injection, despite the normal serum PSA. The fifth animal (mouse 2160) that died without recurrent growth of tumor that could be detected with calipers had a slight elevation (0.8 ng/ml) of PSA that developed 359 days after castration. It died 373 days after castration; despite the abnormal PSA, no tumor could be detected at autopsy including a thorough histopathological analysis of the site of injection. All other
of two sites, located in the subscapular areas bilaterally, with 8.3 million cells in Matrigel.

Developed progressive elevation of their PSAs and growth of their tumors that were days), and last in mouse 2154 (PSA. 1.2 ng/ml at 221 days). All four of these mice tumors with the elevation of PSA in the tail vein blood being observed first in mouse 2152 (PSA, 4.7 ng/ml at 92 days), next in mice 2142 and 2150 (PSA, 2.0 and 0.6 ng/ml at 151 days), and last in mouse 2154 (PSA, 1.2 ng/ml at 221 days). All four of these mice developed progressive elevation of their PSAs and growth of their tumors that were documented histopathologically at the time when they were euthanized 144, 193, 227, and 344 days after castration. In mouse 2142; •¿ mouse 2144; µ, mouse 2146; △, mouse 2148; ■, mouse 2150; ○, mouse 2152; ●, mouse 2154; ▲, mouse 2160; ▼, mouse 2162.

Animals that have developed blood PSA levels of 0.8 ng/ml after months of normal blood PSA levels have developed recurrent tumors.

In seven of the nine animals, PSA values dropped to 0.1 or 0.2 ng/ml. The remaining two animals (mice 2152 and 2154) never developed normal PSA values after castration. In mouse 2152, PSA dropped from 114 ng/ml just prior to castration to 0.6 ng/ml at 64 days, 4.7 ng/ml at 92 days, 8.8 ng/ml at 123 days, and 73.1 ng/ml at 144 days after castration; weekly measurements with calipers first suggested relapse 119 days after castration. In mouse 2154, PSA dropped from 201 ng/ml just prior to castration to 0.4 ng/ml at 92 days and again at 123 days after castration. In the blood of this mouse, PSA values were still 0.5 ng/ml at 186 days, 1.2 ng/ml at 221 days, and 13.7 at 254 days before rising progressively to 515.3 ng/ml on the day when the mouse was euthanized, 344 days after castration; measurement with calipers showed relapse 279 days after castration.

Four of the nine animals developed recurrent tumors that could be measured with calipers and resulted in their being euthanized 144, 193, 227, and 344 days after castration. These results are particularly interesting in light of the fact that all injection sites received 8.3 million cells, i.e., 8300 times as many cells as are tumorigenic in >95% of the animals maintained with s.c. sustained release testosterone. Most animals given 1000 cells and sustained release testosterone s.c. are euthanized with large tumors <3 months after the 1000 cells are injected.

Fig. 3 shows representative examples of the relationships between PSA in the sera of mice and the growth of tumors as measured with calipers. Mouse 2150 (Fig. 3A) was castrated on day 0; one of its two tumors was resected on day 4. The tumor that was not resected began growth as a relapsed tumor, as measured by calipers, approximately 180 days after castration; PSA indicated recurrent tumor 151 days after castration. Mouse 2274 (Fig. 3B) was allowed to develop large tumors bilaterally before castration in an attempt to develop relapsed tumor after castration. The change in PSA as a function of time after castration reversed its course 79 days after castration, indicating relapsed tumor; however, the two tumors did not appear to relapse as measured by calipers for >6 months after castration. Although CWR22R tumors derived from relapsed tumors (a) cause less elevation of PSA/g tumor in blood and (b) are more heterogeneous than CWR22 tumors with respect to the amount of PSA/g tumor in blood, the amount of PSA released into the blood by CWR22R has always been sufficient to predict the relapse of tumors at least 1 month before the relapsed growth is detectable with caliper measurements.

In other experiments, small numbers of animals with large tumor burdens and serum PSA between 369 and 1142 ng/ml have been castrated, and their sustained release testosterone pellets have been resected. Animals whose tumors have recurred have never shown progressive elevations of the PSA in their sera <2 months after castration. Enlargement of tumors that can be measured with calipers occurs >1 month and usually many months after the elevation of PSA is first detected.
In experiments designed to test the hormonal dependence of CWR22, three female mice and three male mice were given injections of the same suspension of tumor in 0.5 ml Matrigel. All three male mice were euthanized 6 weeks later because of large tumors. All three female mice were euthanized 4 months after injection without any evidence of tumor. In another experiment, two female mice and two male mice were given injections of 1.1 million CWR22 cells in Matrigel. The male mice were euthanized 6 and 9 weeks after transplantation with large tumors. Both female mice were euthanized 6 months after they received these cells but failed to develop tumors. In contrast to CWR22, CWR22R grows in female mice.

We have passaged relapsed tumors serially from five different mice. The relapsed tumors, CWR22R, have varied widely in their rates of growth; however, in general, CWR22R tumors have grown more slowly than CWR22. CWR22R tumors generally reach sizes that require that the animal be euthanized 6–12 weeks after transplantation. Some of the five strains of relapsed tumors reach similar sizes as early as 9 weeks; most require 3–7 months after transplantation. The basis for their slower growth and the marked differences among different relapsed tumors and their progeny is not yet known.

CWR22 and CWR22R differed in their responses to stimulation with testosterone in soft agar (Fig. 4). Cluster formation by CWR22 was increased in the presence of testosterone in a dose-related fashion up to an optimal concentration in the range of 25–35 nM testosterone. The dose-response curves were parallel in normal calf serum and in charcoal-stripped calf serum with the formation of more clusters in the normal serum than in charcoal-stripped serum. In both kinds of sera, cluster formation was increased 2–3-fold in the presence of testosterone. The response of CWR22R to testosterone was less consistent than that of CWR22. The experiments with CWR22R (Fig. 4) were carried out with different CWR22R tumors that arose as separate events in different animals over different intervals of time. In some experiments, small increases in cluster formation were observed in the presence of doses of testosterone that were optimal for CWR22. In other instances, cluster formation of CWR22R appeared to decrease slightly in the presence of testosterone. The only generalization about cells from all CWR22R tumors that seems very important in the light of the available data is that CWR22R cells responded much less vigorously than did CWR22 cells to testosterone in soft agar.

DISCUSSION

CWR22R enhances the value of CWR22 as a relatively unique model of human prostate cancer. The availability of CWR22 and CWR22R as serially transplanted xenografts provides prostate cancer researchers, for the first time, a xenograft derived from a primary prostate cancer that regresses markedly after androgen deprivation and relapses as new tumor growth in approximately one quarter to one half of the mice usually 3–10 months after castration, and 1–5 months after serial measurements of PSA in the tail vein blood has heralded the impending relapse. Even very large CWR22 tumors shrink by >50% after tumor-bearing mice are castrated, and tumors of <1 g at the time of castration usually regress to <3 mm in diameter even when they are fated to recur. Both LNCaP (3, 4) and PC-346 (5) are reported to be androgen responsive; however, they regress only slightly, if at all, and only for 3 to 5 weeks after tumor-bearing mice are castrated (3, 5). The decrease in PSA in the blood of castrated mice bearing CWR22 is much greater than has been reported for other prostate cancer xenografts that relapse following regression. PSA provides a very sensitive indication of the growth of CWR22 (6) that is independent of other indicators of tumor growth such as size as measured with calipers.

CWR22 and the relapsed CWR22R provide investigators with an approach to the investigation of prostate cancer that is relatively unique. Differences between CWR22 and the two other prostate cancer xenografts for which relapsed and hormonally responsive variants are available include the marked physical regression of CWR22 that is measurable with calipers for months after castration and the marked drop in PSA in the peripheral blood of animals bearing CWR22 after castration.

Note Added in Proof

The two animals still alive and without evidence of tumor >600 days after castration (Fig. 2) were given sustained release testosterone s.c. 603 and 609 days after castration. One died without tumor 3 weeks later; the other was euthanized with tumor 7 weeks later.
REFERENCES

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