Abstract. The generation of suitable in vitro models is critical for understanding the process associated with the development and progression of prostate cancer in high-risk African-American men. However, the generation of long-term human prostate epithelial cell lines derived from primary human prostate epithelium have been unsuccessful due to the absence of in vitro immortalization. We have successfully established an immortal human prostate epithelial cell line from primary benign tissues of African-American prostate cancer patients by using telomerase. The actively proliferating secondary African-American prostate epithelial RC-165N cells, derived from benign prostate tissue of a radical prostatectomy specimen, were transduced through infection with a retrovirus vector expressing the human telomerase catalytic subunit (hTERT). A high level of telomerase activity was detected in RC-165N/hTERT cells but not in RC-165N cells. RC-165N/hTERT cells are currently growing well at passage 50 whereas RC-165N cells senesced within passage 3. RC-165N/hTERT cells exhibit epithelial morphology. These immortalized cells showed no cell growth in soft agar, and no tumor formation in SCID mice. The RC-165N/hTERT cells express androgen-regulated prostate-specific homobox gene. NKX 3.1 and epithelial cell specific cytokeratin 8, androgen receptor (AR), prostate stem cell antigen and p16, but not PSA. AR protein was detected by Western blot analysis. AR gene sequencing analysis indicated that this cell line expresses wild-type AR. The cell growth is stimulated by dihydrotestosterone (1.0nM DHT) and the stimulatory effect is blocked by flutamide (50 nM). Chromosome analysis revealed that this cell line is aneuploid human male (XO), with most chromosome counts in the 80’s-90’s range. There were losses of chromosomes Y, 1q, 4q, 10q, 12p and 22 and gains of chromosomes 15 and 20. This is the first documented case of the establishment of an androgen and androgen receptor antagonist responsive African-American epithelial cell line derived from benign prostate tissue with telomerase. This unique in vitro model may be useful for the study of prostate cancer biology and the molecular basis of prostate carcinogenesis, especially for high-risk African-American men.

African-American men have a significantly higher incidence and mortality rates from prostate cancer compared to Caucasian men in North America, indicating that prostate cancer is a major public health problem in this population (1). The etiology of these racial differences in the clinical manifestation of prostate cancer is unclear; hormonal, genetic, behavioral and environmental factors have all been implicated (2). To understand the many factors suspected of contributing to the development of this malignancy, there is a need for an in vitro culture system. However, no suitable in vitro models for the study of African-American prostate cancer are available.

Androgen regulation of prostate growth, as well as the widely-used androgen deprivation therapy for prostate cancer treatment, necessitates a better understanding of the role of androgen in prostate cancer biology. The well-characterized LNCaP prostate cancer cell line is known to express AR, but it is a mutant receptor (3). Therefore, generation of primary prostate cell lines expressing AR will have significant impact in evaluating the role of the androgen signaling pathway.

Telomerase is an enzyme responsible for replicating telomeres and is composed of an RNA subunit containing
an integral catalytic subunit, hTERT (human telomerase reverse transcriptase) (4). Telomerase is expressed low in most normal tissues in vivo, but is known to become activated during tumorigenesis, including prostate carcinogenesis (5). Recent findings have directly implicated telomerase in the escape from senescence (4). Senescence can be prevented by introducing the hTERT into human cells (6-8). Thus, an alternative approach to immortalizing various human cells has been developed recently by transfecting human cells with a vector expressing the hTERT gene. To our knowledge, no successful establishment of immortal primary African-American prostate epithelial cells with viral agents or telomerase has been reported. Our goal is to generate new, continuously proliferating human prostate epithelial cell lines from primary prostate benign tissues of African-American prostate cancer patients by using telomerase.

Materials and Methods

Cell culture. Epithelial cells (RC-165N) were derived from benign prostate tissue of an African-American patient who underwent radical prostatectomy. Benign prostate tissue was confirmed under light microscopy by an experienced pathologist. Primary cultures were established by the explant-outgrowth method as described (9). For serial passages, routine trypsinization was used. Keratinocyte serum-free medium (Cat. 10724-011, Gibco, Gaithersburg, MD, USA) supplemented with bovine pituitary extract (BPE), recombinant epidermal growth factor (rEGF), 1% PSN antibiotic mixtures (Cat. 15640-055, Gibco), and 1% amphotericin B (Cat. 15290-018, Gibco) was used for growing and maintaining the cells and designated as KGM medium (9).

Generation of the RC165N/hTERT cell line. The actively proliferating secondary RC165N cells were infected with a recombinant retrovirus construct, LXS-hTERT (generously provided by Vimla Band, Ph.D., New England Medical Center, Boston, MA, USA) containing the hTERT (7).

Telomerase assay. Cellular extracts were assayed for telomerase activity with TRAP assay (10). This assay amplifies telomeric repeats; the repeat length is longest in cells exhibiting telomerase activity with TRAP assay (10). This assay amplifies telomeric repeats; the repeat length is longest in cells exhibiting telomerase activity.

RT-PCR assay. The RT-PCR assay was performed as described previously (9). Briefly, total RNAs from culture cells were extracted with RNAzol B (TEL-TEST Inc., Friendswood, TX, USA). One μg of total RNA was reverse transcribed into cDNA with RNA PCR-kit (Perkin-Elmer, Foster, CA, USA) and 1/10 of the reverse-transcribed product from each sample was used for PCR to amplify NKX 3.1, AR, PSA, PSCA, CK 8, GAPDH and p16, respectively. The primer sequences and the expected size of the PCR products were the same as described previously (9).

Western blot analysis. Cell lysates were prepared from confluent cells by T-PER tissue protein extraction reagent (PIERCE, Rockford, IL, USA) containing protease inhibitor cocktail (Calbiochem, San Diego, CA, USA). The Protein concentration was determined with BCA protein assay kit (PIERCE). Twenty mg of proteins from each lysate were electrophoresed in 4 – 12% SDS- polyacrylamide gels and then electro-transferred to PVDF membrane (Invitrogen, Carlsbad, CA, USA). The PVDF membrane was blocked in 5% nonfat milk in 1xPBS and 0.05% Tween-20 and then exposed to AR specific monoclonal antibody (SC-7305, H-280, Santa Cruz, CA, USA) at a dilution of 1:100 for 1 hour at room temperature. The antimouse secondary polyclonal antibody (SC-2005, Santa Cruz) was then applied at a dilution of 1:500 for 1 hour at room temperature. Antigen antibody complexes were detected with an enhanced chemilluminescence detection kit (ECL. PIERCE).

Androgen and AR antagonist sensitivity assay. Both stock solution of DHT (10 mM) and flutamide (10 mM) were prepared in absolute ethanol and subsequently diluted by corresponding medium. The same passage of the cells was used for the DHT and flutamide sensitivity assays. The RC-165N/hTERT cells (1 x 10^5) in the KGM medium were plated into individual wells of sterile six-well plates. After 24 hours, the BPE and rEGF in the KGM were replaced by 0.1% BSA solution (designated as KGM/BSA medium) and androgen was added at 0, 0.01, 0.1, 1.0 and 10.0 nM DHT (Sigma, St. Louis, MO, USA) in a final concentration of ethanol 0.01% (v/v), respectively. The plates were placed into a 37°C incubator with 5% CO2 atmosphere for 4 days with a change of medium including DHT doses after 2 days. The cells were counted by a particle counter (Coulter Counter Z1, Beckman) after trypsinization. For AR blocking study in the presence of exogenous DHT doses, cells (3 x 10^4) in the KGM/BSA medium were plated into individual wells of sterile 24-well plates. After 24 hours settlement, a single dose of flutamide was then spiked into 24-well plate at a final concentration of 50 nM. For the dose-dependent antagonist sensitivity assay, cells (3 x 10^4) in the KGM medium were plated into individual wells of sterile 24-well plates. After settlement, flutamide (0, 0.1, 1, 10, 100 nM) was added in sterile 24-well plates without addition of DHT. After the cells had been allowed to grow 4 days, a colorimetric assay kit (Cell Titer 96 One Solution Cell Proliferation Assay, G388/1, Promega, Madison, WI, USA) was used to determine the number of viable cells in proliferation assay following the manufacturer’s instruction. In brief, 120 μL of reagent solution was added into individual wells (600 μL medium) and the plates were incubated for 2 hours at 37°C in a humidified 5% CO2 atmosphere. A 96-well plate reader recorded the absorbance at 490 nm after incubation and transfer of 200 μl of resulting reaction solution from 24-well plates. The dynamic linear range between absorbance at 490 nm and up to 2 x 10^5 cells/well (RC-165N/hTERT) was examined and used to keep the measured absorbance within linear range.

Statistical analysis. The differences in mean values of the relative intact cell ratio between untreated and treated groups were analyzed by one-way analysis of variance with Dennett’s pair-wise multiple comparison test (n=3). p<0.05 was considered statistically significant.

AR sequencing. Total RNA was extracted from RC165N/hTERT cells and mRNA was reverse transcribed into cDNA with the RNA PCR-kit (Perkin-Elmer). Six pairs of primers were used to amplify 6 fragments which cover the whole encoding sequence of AR; the detailed primer sequence information is:
PfuTurbo DNA Polymerase (Stratagene, La Jolla, CA, USA) was used to increase the fidelity of PCR (11). Gel purification was performed to isolate the PCR products. Direct sequencing was carried out by 6 purified fragments. To confirm the repetition number of CAG and GGC, the PCR products containing CAG and GGC repeats were amplified by the following two pairs of primers:

<table>
<thead>
<tr>
<th>Sense</th>
<th>Anti-sense</th>
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<tbody>
<tr>
<td>(Forward)</td>
<td>(Reverse)</td>
</tr>
<tr>
<td>5'-GAAGTAGGGTGAAGATTCAGCCA-3'</td>
<td>5'-GTGTGCA GAAATGGTGAAGTG-3'</td>
</tr>
<tr>
<td>5'-ATGGGAAGTGCAGTTAGGGCTGGGA-3'</td>
<td>5'-ACTTCG CGACGCTCTGGA-3'</td>
</tr>
<tr>
<td>5'-AGGAAAGCATTCAGGGAAGCACA-3'</td>
<td>5'-GCCAGG GTACCACACATCAGG-3'</td>
</tr>
<tr>
<td>5'-ACCTGATGTGTTGATCCCTGGA-3'</td>
<td>5'-ATCAGG GGGGAAGTAGGCAC-3'</td>
</tr>
<tr>
<td>5'-AGCCCATCTGAGGAGACAACC-3'</td>
<td>5'-TCACTG GGTGATGGAATAGATGGG-3'</td>
</tr>
<tr>
<td>5'-GAAGCTGACAGTGTCACACAT-3'</td>
<td>5'-TCACGG GGGTGAGTGAATAGATGGG-3'</td>
</tr>
</tbody>
</table>

Figure 1. Telomerase activity in extracts from cultured RC-165N/hTERT cells. Cells were analyzed for telomerase activity by TRAP assay as described (10). (A) LNCaP cells, (B) RC-165N/hTERT (passage 2), (C) RC-165N/hTERT (passage 10).

Table I. Properties of RC-165N cells and the telomerase-transduced RC-165N/hTERT cell line.

<table>
<thead>
<tr>
<th></th>
<th>RC-165N</th>
<th>RC-165N/hTERT</th>
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<tbody>
<tr>
<td>Life-span</td>
<td>&gt;Passage 3</td>
<td>&lt;Passage 50</td>
</tr>
<tr>
<td>Growth (PD time)</td>
<td>ND</td>
<td>35 hours</td>
</tr>
<tr>
<td>Telomerase activity</td>
<td>Not present</td>
<td>Present</td>
</tr>
<tr>
<td>Colony formation in soft agar</td>
<td>ND</td>
<td>Negative</td>
</tr>
<tr>
<td>Gene expression by RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKX3.1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSCA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P16</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GAPDH</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tumorigenicity in SCID mice</td>
<td>ND</td>
<td>Negative</td>
</tr>
</tbody>
</table>

ND=not done
PD=population doubling
These two amplified fragments were integrated into Zero Blunt TOPO PCR Cloning plasmid (Invitrogen), and transformed into E. coli. Plasmids extracted from positive colonies were analyzed with EcoRI. Plasmids containing the correct size of fragments were sequenced with M13 forward and reverse primers. The 3100 ABI PRISM Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) was used following the manufacturer’s instruction.

**Colony formation in soft agar.** A cell suspension (5x10⁵ cells/ml) in 5 ml of 0.35% Noble agar with KGM was overlaid into a 60-mm dish containing 0.5% agar base. Colonies of > 0.2 mm in diameter were counted on day 21.

**Tumorigenicity in SCID mice.** Tumorigenicity assay was carried out in SCID mice. Briefly, viable cells (1x10⁷) in 0.2 ml of PBS were injected subcutaneously into the mid-dorsal infrascapular region of adult SCID mice to determine tumorigenicity. The SCID mice were observed for 6 months for the appearance of tumor developments.

**Cytogenetic analysis.** Chromosome counts, ploid distribution and Giemsa (G)-banded karyotypes were prepared by standard protocol, as described previously (12).

**Results**

**Immortalization of RC-165N cells following hTERT transduction.** To determine whether RC-165N cells were immortalized by the overexpression of hTERT, we introduced a retrovirus construct expressing hTERT into secondary RC-165N cells through overnight infection in the presence of polybrene (10 μg/ml). Non-infected cells could not be propagated serially beyond 3 subcultures (Table 1). In contrast, the hTERT-infected RC-165N cells have apparently unlimited life-span and have been successfully subcultivated for >100 population divisions.
doublings (>50 subcultures) over the course of one year with no evidence of decreased proliferation capacity.

Telomerase activity in RC-165N/hTERT cells. To confirm that the immortalized RC-165N/hTERT cells do contain the transduced hTERT, the telomerase repeat amplification protocol (TRAP) (10) was carried out. A high level of telomerase activity was detected in RC-165N/hTERT cells (Figure 1).

Phenotype of RC-165N/hTERT cells. The RC-165N/hTERT cells have the typical epithelial cell morphology (Figure 2). RC-165N/hTERT cells, like parent RC-165N cells, expressed an epithelial cell-specific cytokeratin 8, androgen-regulated prostate-specific gene NKX 3.1, AR, PSCA, p16 and GAPDH, but no detectable PSA was expressed (Figure 3). The RC-165N/hTERT cells did not grow in soft agar. In addition, the

Figure 4. Androgen receptor (AR) protein detection. Lysate (20 µg protein) of each cell line were electrophoresed, electroblotted and then detected with the Anti-AR monoclonal antibody and a secondary antibody. Lanes 1: LNCaP cells, 2: LNCaP cells (5 µg proteins), 3: 267B1, cells, 4: MLCSV40 cells, 5: DU-145 cells, 6: RC-165N/hTERT cells. 267B1 and MLCSV40 cells are previously immortalized human prostate cell lines.

Figure 5. Androgen sensitivity assay in a box plot format. Sensitivity to androgen (DHT) was assessed for RC-165N/hTERT cells after growth in serum-free K-SFM media with 0.1% BSA in the presence of 0, 0.1, 1.0, 10.0 and 100.0 nM DHT for 4 days. Significant difference in cell growth was observed at 10.0 nM DHT (p<0.05) in stimulation and 10.0 nM DHT (p<0.05) in inhibition. The numbers of cell counts were expressed as the mean value of triplicate observations (n=3).

Figure 6. A. The effect of 50 µM flutamide on the proliferation of RC-165N/hTERT cells in the presence of DHT doses in a box plot format. The addition of 50 nM flutamide undifferentiated the cell growth despite the presence of DHT doses (Group p=0.069). Thus, a single dose of flutamide (50 nM) blocked the stimulatory effects of DHT on the proliferation of RC-165N/hTERT cells. At 1.0 nM DHT, that showed the maximum stimulation effect (Figure 5), no difference from the control sample was observed in the presence of 50 nM flutamide (p=0.771). The values of relative proliferation (%) were computed by the ratio of the absorbance at 490 nm of the untreated control sample to those of the treated samples, and were expressed as the mean value of triplicate observations. B. Inhibition of cell proliferation by flutamide in the absence of exogenous DHT in a box plot format. The proliferation of RC-165N/hTERT cells was significantly inhibited by the presence of flutamide in a dose-dependent fashion (p<0.05). The dose with initial proliferation inhibition was observed at 10 nM flutamide (p<0.001). The inhibition doses (ID) with 50% and 25% effectiveness were calculated as 45 and 290 nM for flutamide, respectively. The values of relative proliferation (%) were computed by the ratio of the absorbance at 490 nm of the untreated control sample to those of the treated samples, and were expressed as the mean value of triplicate observations.
RC-165N/hTERT cells were non-tumorigenic for up to 6 months after subcutaneous inoculation in SCID mice (Table I).

Androgen receptors. To determine whether RC-165N/hTERT cells express detectable AR protein, Western immunoblot analysis was carried out. LNCaP and DU145 cells were used as a positive and a potential negative control, respectively. The results (Figure 4) indicate production of androgen receptor in RC-165N/hTERT cells. A strong-band for LNCaP and weaker band for the other cell lines, including the RC-165N/hTERT cells, were observed. No AR production was detected in DU-145.

After careful investigation of cDNA sequences, no mutation was found in all 8 exons, including codon 877, which mutated in LNCaP and some clinical samples (3,13). The number of CAG and GGC repetition is 26 and 17, respectively. Based on our sequencing data, we concluded that the RC-165N/hTERT cells express wild-type AR.

Androgen sensitivity of RC-165N/hTERT cells. To examine if the cell growth was sensitive to androgen, the RC-165N/hTERT cells were grown in the KGM/BSA medium at different doses of DHT for 4 days. The BPE and rEGF supplements of K-SFM were replaced by HPLC purified 0.1% bovine serum albumin solution to minimize endogenous androgen or AR-activating components. As the concentration of DHT increased to 1.0 nM, the cell count increased, but cell growth decreased as the DHT concentration further increased up to 10 nM. The cell growth showed modest biphasic profile, which was similar to that of LNCaP cells with respect to DHT doses (14, 15). Statistical analysis indicates that the mean value difference between control and 1.0 nM or 100 nM DHT treated was significant ($p<0.05$). The RC-165N/hTERT cells responded with maximum proliferation to 1.0 nM DHT, but cell proliferation returned to the control level at 10.0 and was significantly lower at 100.0 nM DHT (Figure 5).

AR antagonist responsiveness of RC-165N/hTERT cells. The functionality of the cells with wild-type AR was further characterized by an AR antagonist (flutamide) in terms of cell proliferation inhibition. The summary of inhibition by flutamide is presented in Figure 6A,B. Fifty nM flutamide was spiked to the same setup as the DHT dose dependence test (Figure 6A). A single dose of flutamide (50 nM) successfully reversed the stimulation of cell proliferation by stimulatory doses of DHT ($p=0.069$). This indicates that molar excess of an antagonist competes with DHT to AR proteins and inhibits the agonistic activity of 1.0 nM DHT in the RC-165N/hTERT cells. In contrast to the LNCaP cells that express mutated AR and are ineffectively responsive to flutamide (16), the competition between DHT and flutamide to wild-type AR in the RC-165N/hTERT cells is evident.
For further examination of the antagonistic effect on cell proliferation in the basal KGM medium with supplements (BPE and rEGF), the cells were treated by flutamide doses (Figure 6B). The proliferation of the cells in the KGM medium was inhibited in a dose-dependent fashion. A significant inhibition of proliferation was observed at 10 nM flutamide and greater ($p<0.0001$). The computed inhibition doses (ID) with 50% and 25% effectiveness were 45 and 290 nM for flutamide, respectively. After linearization of the inhibitory doses of flutamide with logarithm transformation and linear regression of the relative cell proliferation, ID values were calculated. Figure 6B also indicates the existence of either trace amounts of endogenous androgen or of AR-activating components in the K-SFM medium with the BPE and EGF due to no prior addition of exogenous DHT. These data suggest that the cell line not only grows in a DHT-dependent fashion, but also flutamide can block the AR-signaling pathway (Figure 6A,B). Based on the functional characterization of both agonistic (DHT) and antagonistic (flutamide) effects on cell proliferation, we concluded that the RC-165N/hTERT cell line responds to the presence of agonist (DHT) in stimulation, and proliferation in the basal KGM medium with supplements (BPE and rEGF), the cells were treated by flutamide doses (Figure 6B). The proliferation of the cells in the KGM medium was inhibited in a dose-dependent fashion. A significant inhibition of proliferation was observed at 10 nM flutamide and greater ($p<0.0001$). The computed inhibition doses (ID) with 50% and 25% effectiveness were 45 and 290 nM for flutamide, respectively. After linearization of the inhibitory doses of flutamide with logarithm transformation and linear regression of the relative cell proliferation, ID values were calculated. Figure 6B also indicates the existence of either trace amounts of endogenous androgen or of AR-activating components in the K-SFM medium with the BPE and EGF due to no prior addition of exogenous DHT. These data suggest that the cell line not only grows in a DHT-dependent fashion, but also flutamide can block the AR-signaling pathway (Figure 6A,B). Based on the functional characterization of both agonistic (DHT) and antagonistic (flutamide) effects on cell proliferation, we concluded that the RC-165N/hTERT cell line responds to the presence of agonist (DHT) in stimulation, and of AR antagonist in inhibition of cell proliferation mediated by functional and wild-type androgen receptors.

**Chromosome analysis.** Chromosome study was performed at passage 24 on 30 metaphases. The cell line is aneuploid human male (XO), with most chromosome counts in the 80’s to 90’s range. The model number is 90. There are observed 2X’s in each karyotype. Looking at the normal chromosomes and markers, there is loss of Y, 1q (1q42>qter) in marker M8/M8A, 4q (q26>qter) in marker M5, 10q (q22>qter) in M9, 12p (12p12.1>qter) in marker M7, some loss of N22. There is 7p loss in marker M2 but most likely this is translocated to marker M7. There are further gains of chromosome 15 and 20 and unknown material in M1 ([3q+]), M3([3p+]), M4 ([t(14q:?)], M6[16q+] and M8 [der (1)t(1q41:+hsr)] (Figure 7).

**Discussion**

The present study appears to represent the first documented case of the establishment of a human prostate epithelial cell line from high-risk African-American benign prostate tissue immortalized by telomerase. The immortalized RC-165N/hTERT cells show epithelial morphology and are grown in serum-free medium. They showed no cell growth in soft agar and no tumor formation in SCID mice. They expressed CK8, NKX3.1, PSCA, p16 and AR, but did not express PSA. The cell line was aneuploid and showed losses of chromosomes Y, 1q, 4q, 10q, 12p, and 22 and gains of chromosomes 15 and 20.

Some of the alterations of chromosomes 1q, 4q, 10q, 20 and Y observed in the RC-165N/hTERT cell line have been already reported in the literature (17). It is interesting to note that a predisposing gene for early-onset prostate cancer was mapped to locus 1q 42.2-43 (18, 19) and appeared to represent the most frequent known locus predisposing to hereditary prostate cancer in Western and Southern Europe (20). Possible evidence of a prostate cancer susceptibility locus on chromosomes 4q and 20 has been reported (21, 22). There are reports of loss of heterozygosity for both 10p and 10q in prostate cancer described in the literature (23,24).

We and others have previously developed reliable methods for generating and characterizing human prostate epithelial cell lines from normal prostate tissue by viral DNA oncoproteins such as SV40 and high-risk HPVs (25-28). However, no such virally-induced African-American prostate epithelial cell line has been established. These virally-induced cell models are not ideal, because the immortalizing cells frequently contain viral oncogenic DNA and accompany major cytogenic alterations. Development of in vitro human cell models that mimic human prostate cancer progression would be ideal. As described above, we have succeeded in immortalization by the introduction of telomerase into primary prostate epithelial cells derived from African-American benign prostate tissue. As shown by other human cells (4, 6-8), we anticipated that the hTERT-immortalized cell would maintain the phenotypic and genetic characteristics of its primary cells. Conceptually, the successful establishment of spontaneous immortalized human prostate epithelial cells derived from benign prostate tissue would be ideal and a major breakthrough in prostate cancer research. However, the generation of such cell lines is an extremely rare event and, to date, has not been reported. Establishment of a spontaneously immortalized primary androgen-responsive African-American prostate cancer cell line has recently been reported (29). However, this cell line does not express the full neoplastic phenotype.

As described, this RC-165N/hTERT cell line expresses several important biomarkers including wild-type androgen receptor. The most valuable feature of this cell line is its capability of androgen sensitivity and blockage of AR signaling pathway by an AR antagonist (flutamide). The novel cell line may provide a useful in vitro model for the study of prostate cancer biology, and for molecular characterization of prostate carcinogenesis, especially for the high-risk African-American population. We have, for the first time to our knowledge, succeeded in the immortalization of primary African-American benign prostate epithelial cells in culture by transduction with a retrovirus vector expressing hTERT. The telomerase expression in primary African-American benign prostate epithelial cells can itself induce immortalization and does not induce changes associated with a transformed phenotype. These immortalized cells express prostate-specific markers including androgen and androgen receptor antagonist responsiveness. These cells provide a useful tool to study the biology and the pathology of benign prostate epithelial cells, to understand the steps leading to malignant prostate transformation, especially for high-risk African-American men.
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References


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