ELSEVIER

Genetic Characterization of Immortalized Human Prostate Epithelial Cell Cultures: Evidence for Structural Rearrangements of Chromosome 8 and i(8q) Chromosome Formation in Primary Tumor-Derived Cells

Jill A. Macoska, Ben Beheshti, Johng S. Rhim, Bharati Hukku, Jeff Lehr, Kenneth J. Pienta, and Jeremy A. Squire

ABSTRACT: *We have utilized a combination of conventional and spectral karyotyping (SKY) techniques and allelotype analysis to assess numerical and structural chromosome alterations in two cell lines derived from normal human prostatic epithelium, and three cell lines derived from human prostate primary tumor epithelium, immortalized with the E6 and E7 transforming genes of human papilloma virus (HPV) 16 or the large T-antigen gene of simian virus 40 (SV40). These studies revealed trisomy for chromosome 20 and rearrangements involving chromosomes 3, 4, 8, 9, 10, 16, 17, 18, 19, 21, or 22. In addition, the four HPV-immortalized cell lines exhibited extensive duplications or translocations involving the 11q chromosomal region. Interestingly, allelotyping data disclosed loss of 8p sequences in two of the three primary tumor-derived cell lines, and SKY data revealed that the loss of 8p sequences was directly due to i(8q) chromosome formation and/or other structural alterations of chromosome 8. This provides intriguing evidence that 8p loss in primary human prostate tumors may, in some cases, result from complex structural rearrangements involving chromosome 8. Moreover, the data reported here provide direct evidence that such complex structural rearrangements sometimes include i(8q) chromosome formation.*

INTRODUCTION

Many genetic and epigenetic events are likely involved in prostate tumorigenesis. In particular, several cytogenetic and molecular studies from our laboratory and others have suggested that deletion or rearrangement of sequences that map to the short arm of chromosome 8 (8p) may be critically permissive for tumorigenesis in the prostate gland [1–7].

Deletion of 8p sequences is observed at comparable frequencies in low- and high-grade tumors, as well as in localized and invasive/regionally metastatic prostate cancers [3, 5, 7]. Moreover, the frequency of 8p loss is almost equivalent in prostate tumors and prostatic intraepithelial neoplasia (PIN), a putative premalignant lesion of the prostate [6, 8]. Taken together, these data suggest that 8p losses are frequent events during the initiation or early promotion of prostate tumorigenesis.

Other studies have also reported loss of 8p concurrent with gain of the long arm of chromosome 8 (8q) sequences in advanced prostatic cancer [9–13]. This combination of events occurring on the same chromosome—loss of 8p sequences and gain of 8q sequences—suggests formation of i(8q) chromosomes in advanced prostate tumors. Although i(8q) chromosomes have been detected in uncultured metastatic prostate tumors using conventional karyotypic analyses, no direct evidence for the existence of

From the Department of Surgery, Section of Urology (J. A. M.), the Department of Internal Medicine, Section of Hematology/Oncology (J. L., K. J. P.), and the Comprehensive Cancer Center, (J. A. M., K. J. P.), The University of Michigan, Ann Arbor, Michigan, USA; The Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, and Department of Laboratory Medicine and Pathobiology, and Medical Biophysics, Faculty of Medicine, University of Toronto (B. B., J. A. S.), Toronto, Ontario, Canada; The Center for Prostate Disease Research, The Uniformed Services University of the Health Sciences (J. S. R.), Rockville, Maryland, USA; and the Cell Culture Laboratory of the Children's Hospital of Michigan (B. H.), Detroit, Michigan, USA.

Address reprint requests to: Jill A. Macoska, Ph.D., Department of Surgery, Section of Urology, The University of Michigan, 7306 CCGC, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0946.

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655 Avenue of the Americas, New York, NY 10010

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i(8q) chromosomes in primary prostate tumors has been obtained, possibly due to the inability of interphase fluorescence in situ hybridization (FISH) techniques used with clinical specimens to accurately and precisely identify these chromosomes [10–13]. Therefore, we have utilized a combination of conventional and spectral karyotyping (SKY) techniques and allelotype analysis to assess numerical and structural alterations of chromosome 8 in two cell lines derived from normal human prostate epithelium, and three cell lines derived from primary human prostate tumors. The specific objective of these studies was to determine whether losses of 8p sequences previously reported for two of the cell lines, 1532T and 1542T [14], were directly due to i(8q) chromosome formation and/or other structural alterations of chromosome 8.

MATERIALS AND METHODS

Cell Lines and Culture

The 1535N, 1532T, 1535T, and 1542T cell lines were produced through immortalization of primary prostate epithelial cultures by transduction with a recombinant retrovirus encoding the E6 and E7 genes of HPV 16, as previously described [14]. The 1535N cells were produced from immortalization of normal prostatic epithelium, whereas the 1532T, 1535T, and 1542T cells were produced through immortalization of malignant epithelium from primary prostate tumors. The PrEC-T cells were produced through immortalization of normal human prostate epithelial cells (Clonetics, Inc.) by transfection with the pMT10D plasmid (Japanese Cancer Research Resources Bank, Tokyo) containing sequences encoding the simian virus 40 (SV40) Large T-antigen. All cell lines were grown in defined keratinocyte-SFM (GIBCO/BRL), 5% FBS, and 1% penicillin/streptomycin/fungizone antibiotic mixture (BioWhittaker) in a humidified incubator at 37°C with 5% CO_2.

G-Banding and Karyotypic Analysis

For the 1535N cells, chromosome counts, ploidy distributions, and GTG-banded karyotypes were prepared as previously described [15]. Briefly, exponentially growing cultures were treated with 0.04 ug/mL Colcemid for 1–2 hours, trypsinized, treated with 0.0375 M KCl for 9 minutes, then fixed in 3:1 methanol: glacial acetic acid. The resulting cell nuclei were pelleted by centrifugation, dropped onto cold, wet slides, then air-dried and stained using a 4% Giemsa solution. Chromosomes were examined and counted to establish ploidy distribution and constitutional alterations. Specific numerical and structural chromosomal alterations were established after the slides were aged at 60°C on a slide warmer for 18 hours, immersed in 0.025% trypsin for 11 seconds, stained with 4% Gurr-Giemsa solution for 11 minutes, washed in buffer, then air-dried and mounted in permount. Well-banded metaphase spreads were photographed at 800× magnification with Technical Pan Film 2415 (Kodak) and printed on Rapidoprint FP 1-2 (Agfa-Gevaert), or studied on the AKSII image analysis system.

SKY Analysis

Spectral karyotyping analysis was carried out on the 1532T, 1535T, 1542T, and PrEC-T cells using previously G-banded slides. Images were captured and the microscope coordinates were noted. Residual oil was removed with xylenes followed by destaining with methanol. The slides were then rehydrated in a descending ethanol series and fixed with 1% formaldehyde in a 50 mM $MgCl_2$/phosphate buffer solution for 10 minutes. Slides were dehydrated and denatured for 30–45 seconds at 75°C in 70% formamide/2 × SSC (saline sodium citrate), followed by a final dehydration. The SKY paints (Applied Spectral Imaging, Carlsbad, CA, USA) were denatured for 7 minutes at 75°C, reannealed at 37°C for 1 hour, then placed on the slide and covered with a glass coverslip. The coverslip was sealed with rubber cement, and hybridization was carried out in a humidified chamber for 24 hours at 37°C. Post-hybridization washes were carried out using established techniques and according to the manufacturer's instructions [16]. Ten metaphase images were captured per preparation using an SD 200 spectral bioimaging system (ASI Ltd., MigdalHaemek, Israel) attached to a Zeiss microscope (Axioplan 2) and stored on a SKY image-capture workstation. The images were analyzed using the SKY-

Table 1 Karyotype analysis of immortalized normal and malignant prostate epithelial cells

Cell lines	Karyotype
1532T	44∼47,XY,i(8)(q10),+20[2]/46∼47,idem,dup(11)(q13q23)[3]/ 46∼47,idem,dup(11)(p11.2p13),ins(17;11)(q21p11.2p13)[5]
1535T	46,XY,der(11)?qdp(q13q23)t(11;20)(q23;q11),der(20)t(11;20)(q13;q13.3)qdp(11)(q13q23)or hsr(11)[6] 46,idem,der(3)t(3;11)(p21;q13),del(18)(q21)[4]
1542T	46,XY,der(8;20)(q10;p10),der(11)qdp(q13q23)t(11;20)(q23,q11)[4]/46∼47,idem,i(8)(q10)[2] 36∼51,idem,der(22)t(11;22)(q14;p11)t(11;20)(q23;q11.2)[2] 45∼47,idem,−der(8;20),der(8;21)(p10;q10)[2]
1535N	45∼48,XY,der(18)[9]/45∼48,idem,add(19)(q13)[8] 45∼48,idem,der(11)dup(11)(q11q23)t(10;11)(q22;q23)[4]/45∼48,idem,del(10)(q21)[3]
PrEC-T	44,XY,der(3;15)(q10;q10),der(4)t(4;?14)(q35;p10),der(8)dup(8)(q11.2q24.3)t(8;8)(q24.3;q24.3), del(10)(q24),der(14;17)(q10;q10),der(16)t(9;16)(q10;q10),del(18)(q21), der(22)dup(??)t(22;22)(p13;?)t(8;22)(?;?),der(22)t(17;22)(p11.2;q11.2)[10]

Nonclonal chromosomal changes were also noted in the cell lines.

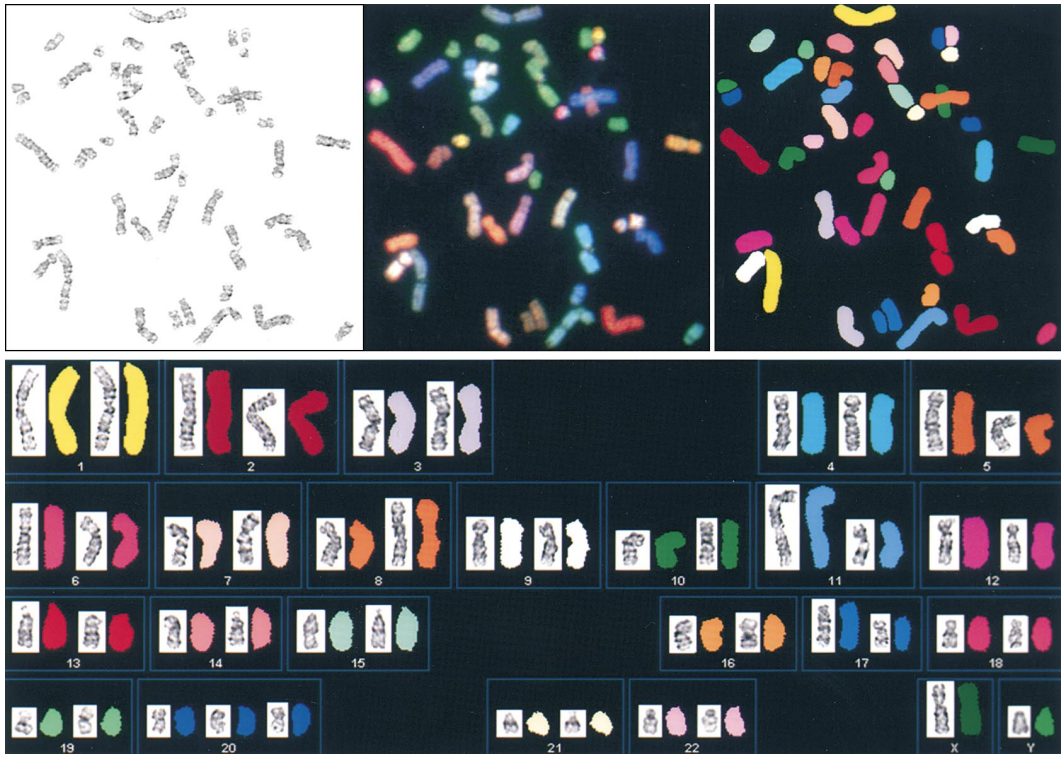
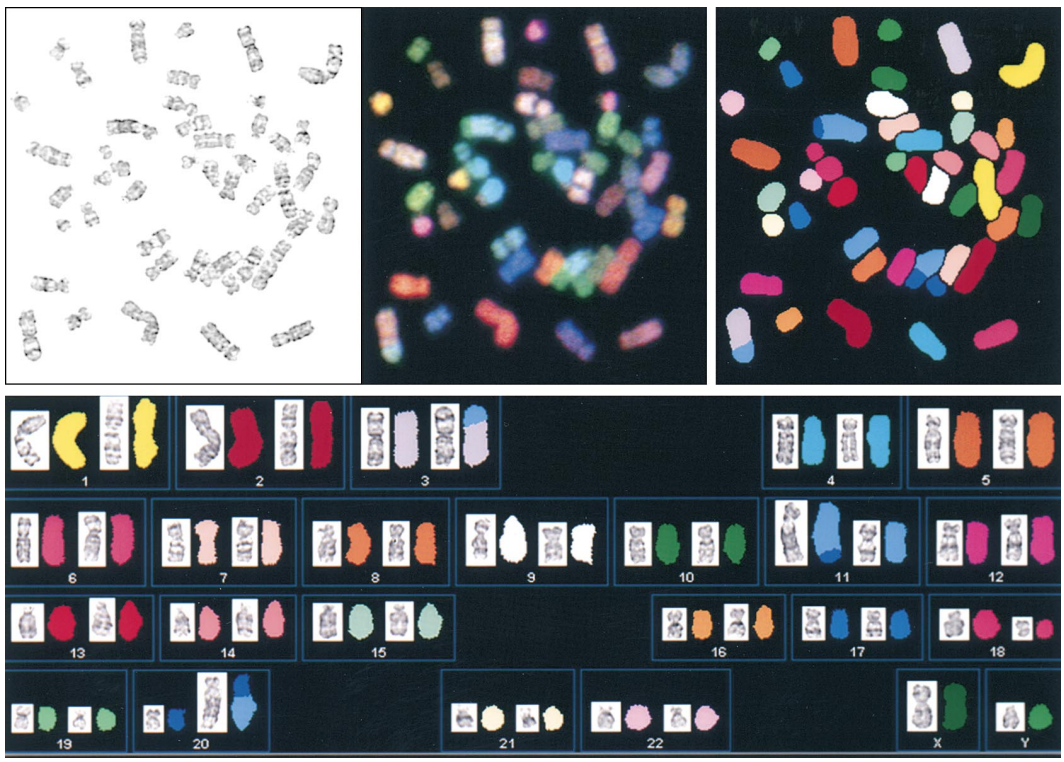


Figure 1 Spectral karyotype composite of the 1532T cell line. Upper panel: G-banded preparation of metaphase chromosomes from 1532T cells (left), hybridized to SKY paints (middle), and after pseudocolor application (right), as described in the text. Lower panel: Composite karyotype showing G-banded and pseudocolored chromosomes. The karyotype for the cell shown is: 47,XY,i(8)(q10),+20.

Figure 2 Spectral karyotype composite of the 1535T cell line. Upper panel: G-banded preparation of metaphase chromosomes from 1535T cells (left), hybridized to SKY paints (middle), and after pseudocolor application (right), as described in the text. Lower panel: Composite karyotype showing G-banded and pseudocolored chromosomes. The karyotype for the cell shown is: 46,XY,der(11)?qdp(q13q23)t(11;20)(q23;q11),der(20)t(11;20)(q13;q13),qdp(11)(q13q23),der(3)t(3;11)(p21;q13),del(18)(q21).



View software version 1.3. G-banding and SKY analyses were performed sequentially on each of the cell lines with the same ten metaphase images captured for G-banding also analyzed by SKY.

Allelotyping

Cells were trypsinized and DNA was purified using the Oncor (Gaithersburg, MD, USA) nonorganic DNA extraction kit according to the manufacturer's protocols. Polymerase chain reaction (PCR) reactions were performed as previously described [7]. The loci examined by PCR spanned 8p (12 loci) or localized to 8q12 (2 loci), and contained highly polymorphic microsatellite repeats. The linkage order of these markers has been reported as pter – D8S504 – D8S277 – D8S549 – D8S261 – NEFL – D8S540 – D8S513 – D8S535 – D8S505 – D8S87 – D8S1121 – D8S255 – D8S531 – D8S519 – qter (Table I). Primer sequences, additional linkage and contig information, and genetic mapping information were obtained from public databases maintained by the Center for Genome Research at the Whitehead Institute for Biomedical Research (http://www-genome.wi.mit.edu/), and the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), as accessed through the Internet.

RESULTS

Cytogenetic Analysis

Metaphase analysis showed that the five prostate-derived cell lines were pseudodiploid, with modal numbers ranging from 43 to 49 chromosomes/cell. The karyotypes of each cell line are described below and in Table 1.

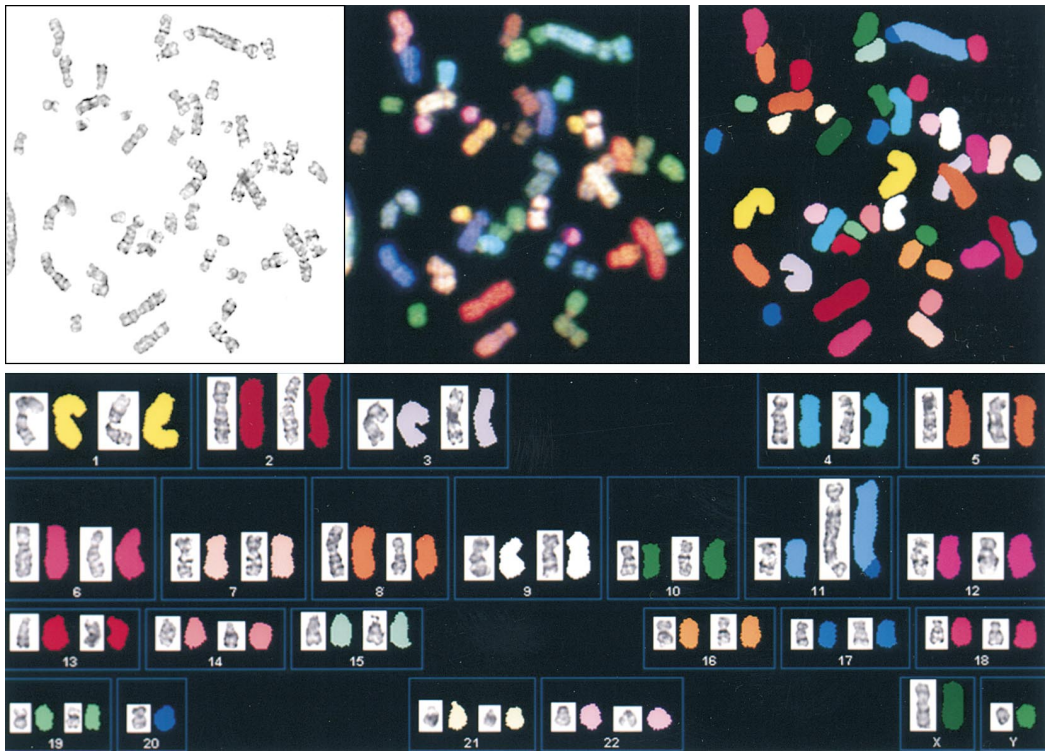
1532T

Ten karyotypes were analyzed from passage 44 of this cell line using SKY techniques. The consensus karyotype was 44∼47,XY,i(8)(q10),+20. Eight cells also demonstrated duplication of the (q13–q23) region of chromosome 11, and five cells demonstrated a duplication of the p11.2–p13 region of chromosome 11 and insertion into the q21 region of chromosome 17. Figure 1 shows a representative karyotype for this cell line.

1535T

Ten karyotypes were analyzed from passage 12 of this cell line using SKY techniques. The consensus karyotype was 46,XY,der(11)?qdp(q13q23)t(11;20)(q23;q11)der(20)t(11;20)(q13;q13.3)qdp(11)(q13q23) or hsr(11), with four cells also demonstrating der(3)t(3;11)(p21;q13),del(18)(q21). Figure 2 shows a representative karyotype for this cell line.

Figure 3 Spectral karyotype composite of the 1542T cell line. Upper panel: G-banded preparation of metaphase chromosomes from 1542T cells (left), hybridized to SKY paints (middle), and after pseudocolor application (right), as described in the text. Lower panel: Composite karyotype showing G-banded and pseudocolored chromosomes. The karyotype for the cell shown is: 45,XY,i(8)(q10),der(11)qdp(q13q23)t(11;20)(q23;q11).



1542T

Ten karyotypes were analyzed from passage 44 of this cell line using SKY techniques. The consensus karyotype was 46,XY,der(8;20)(q10;p10),der(11)qdp(q13q23)t(11;20)(q23;q11). In addition, two cells also demonstrated an i(8)(q10); 2 cells demonstrated these changes as well as der(22)t(11;22)(q14;p11)t(11;20)(q23;q11.2), and 2 cells were characterized by these accumulated changes except that the der(8;20) was absent and a der(8;21)(p10;q10) was apparent instead. Figure 3 shows a representative karyotype for this cell line.

1535N

Nine karyotypes were analyzed from passage 13 of this cell line using G-banding techniques. The consensus karyotype was 45∼48,XY,der(18), with eight cells also demonstrating an add(19)(q13) chromosome, and four cells demonstrating a complex derivative of chromosome 11 involving t(10;11). Three cells also displayed a deletion of chromosome 10 involving band q21. Figure 4 shows a representative karyotype for this cell line.

PrEC-T

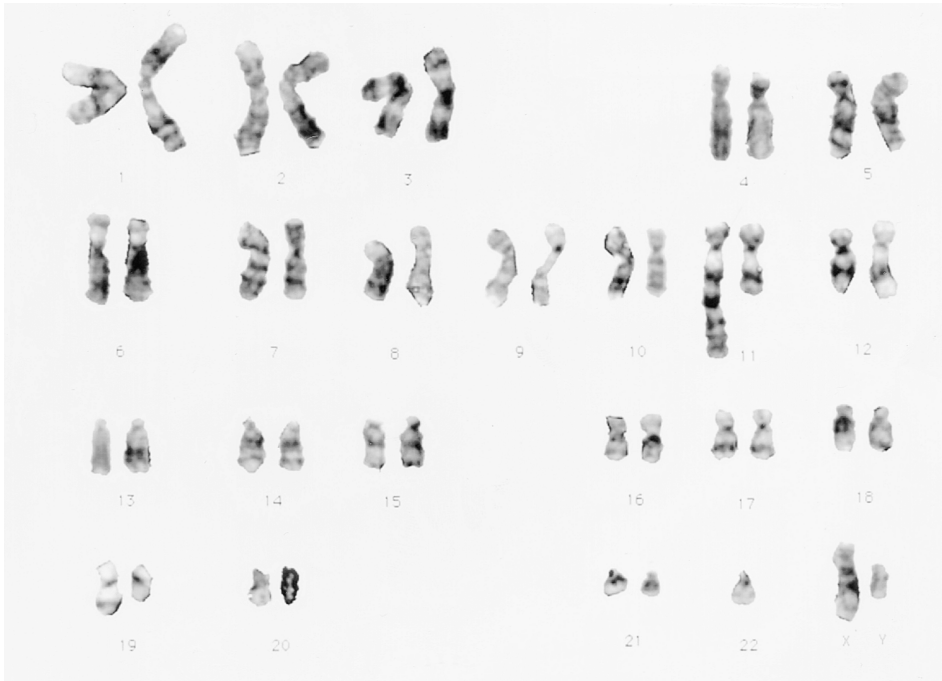
Ten karyotypes were analyzed from passage 27 of this cell line using SKY techniques. The consensus karyotype was 44,XY,der(3;15)(q10;q10),der(4)t(4;?14)(q35;p10),der(8)dup(8)(q11.2;q24.3)t(8;8)(q24.3;q24.3),del(10)(q24),der(14;17)(q10;q10),der(16)t(9;16)(q10;q10),del(18)(q21),der(22)dup(??)t(22;22)(p13;?)t(8;22)(?;?),der(22)t(17;22)(p11.2;q11.2)[10]. Figure 5 shows a representative karyotype for this cell line.

Interestingly, duplications, translocations and other structural changes involving 11q13q22q23 were observed in all four HPV-immortalized cell lines. The 1542T cells exhibited a distinctive quadruplication (abbreviated as "qdp" using ISCN nomenclature), suggesting a low-level amplification of the q13 to q23 region in this cell line.

Allelotyping

The 1532T, 1535T, and 1542T cell lines were allelotyped at 14 chromosome 8 loci, 12 spanning 8p, and 2 mapping to the pericentromeric region of 8q. Table 2 summarizes these data. As shown in Table 2, the 1532T cell line was homozygous for all 8p loci examined, consistent with the cytogenetic data revealing one normal chromosome 8 and one i(8)(q10) chromosome in these cells (Fig. 6). The 1542T cell line demonstrated one allele for all 8p loci, but two alleles for each of the pericentromeric 8q loci, D8S531 and D8S519. These data were also consistent with the cytogenetic findings for one normal chromosome 8 accompanied by any of three different structural alterations of chromosome 8:i(8)(q10),der(8,20)(q10;p10), and der(8;21)(p10;q10) in these cells (Fig. 6). In contrast, the 1535T cell line demonstrated two alleles for 9 of 12 8p, and both 8q loci examined, with no evidence for extended regions of homozygosity by allelotyping. These results were consistent with the SKY data, which did not reveal clonal numerical or structural alterations of chromosomes 8 in these cells.

Figure 4 G-banded karyotype of the 1535N cell line. G-banded metaphase chromosomes from 1535N cells were prepared as described in the text. The karyotype for the cell shown is: 45,XY,der(11)dup(11)(q11q23)t(10;11)(q22;q23),der(18),add(19)(q13),−22.



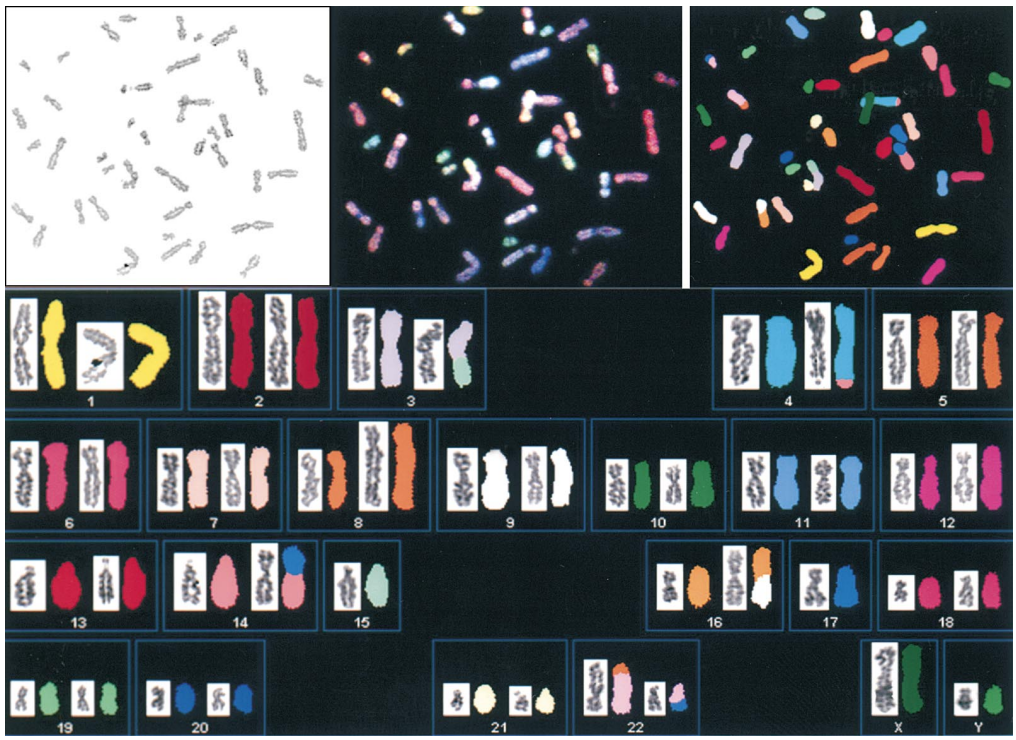


Figure 5 Spectral karyotype composite of the PrEC-T cells line. Upper panel: G-banded preparation of metaphase chromosomes from PrEC-T cells (left), hybridized to SKY paints (middle), and after pseudocolor application (right), as described in the text. Lower panel: Composite karyotype showing G-banded and pseudocolored chromosomes. The karyotype for the cell shown is: 44,XY,der(3;15)(q10;q10),der(4)t(4;?14)(q35;p10),der(8)dup(8)(q11.2;q24.3)t(8;8)(q24.3;q24.3),del(10)(q24),der(14;17)(q10;q10),der(16)t(9;16)(q10;q10),del(18)(q21),der(22)dup(??)t(22;22)(p13;?)t(8;22)(?;?),der(22)t(17;22)(p11.2;q11.2).

DISCUSSION

Cell lines provide a unique resource for the investigation of numerical and structural chromosomal alterations present in the tissues from which they were derived. However, the most intensively studied prostate-derived cell lines—PC3, DU145, and LNCaP—were all established from metastatic lesions. These cell lines possess highly aberrant karyotypes characterized by numerous structural and numerical chromosomal alterations [17]. As such, it is unlikely that these cell lines accurately recapitulate the genetic composition of human primary prostate tumors. Unfortunately, human prostate tissues, whether normal or malignant, survive only short term in culture, and rarely immortalize spontaneously. The use of viral transforming proteins to immortalized normal and primary tumor epithelium from human prostate has allowed the continual propagation of normal and malignant-derived cells in vitro [14]. The cell lines examined in the present study were created by Bright et al. through the transduction, and subsequent immortalization, of normal and primary tumor prostatic epithelium with the E6 and E7 transforming genes of HPV 16, or in our laboratory through the immortalization of normal prostatic epithelium with the large T-antigen gene of SV40 [14].

The cell lines demonstrated several numerical and structural chromosomal alterations, including trisomy for chromosome 20 (1532T cells) and rearrangements involving 3p (1535T cells) or 3q (PrEC-T cells), chromosome 9 (PrEC-T cells), 10 (1535N and PrEC-T cells), 16 (PrEC-T cells), 17 (1532T and PrEC-T cells), 18 and 19 (1535N), 20 (1542T cells), or 21, and 22 (1542T and PrEC-T cells). The PrEC-T cells also exhibited structural alterations of chromosomes 4 and 14. All of these alterations have been reported for epithelial cells from diverse tissue types, including uroepithelial and prostate, immortalized through transduction with all or part of the HPV 16 or 18 genomes [18-21]. Some of these alterations, notably gain of chromo-

Figure 6 Structural alterations involving chromosome 8. Structural alterations of chromosome 8, including i(8q) chromosomes, are shown for 1542T cells (left), 1532T cells (middle), and PrEC-T cells (right).

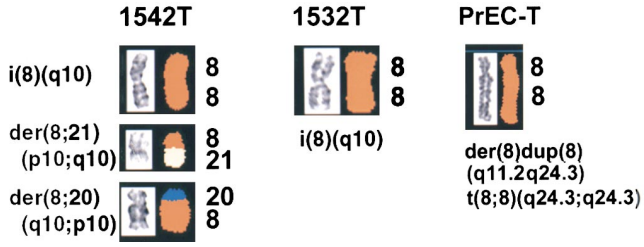


Table 2 Allelic status of chromosome 8 loci[a]

Locus	D8S504	D8S277	D8S549	D8S261	NEFL	D8S540	D8S513	D8S535	D8S505	D8S87	D8S1121	D8S255		D8S531	D8S519
cM	0.0	8.4	30.7	35.8		60.0	60.0	60.0	60.0			64.0		65.7	65.8
Chromosome location	8pter	8p23	8p23	8p22	8p21	8p12				8p12			8c	8q12	8q12
1532T	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]		1	1
1542T	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]		2	2
1535T	1	1	2	2	2	2	2	2	1	2	2	2		2	2

[a]The allelic status of each locus is denoted by 1 (homozygous) or 2 (heterozygous). Where known, the genetic distance (cM) and cytogenetic localization are shown.

[b]Extended regions of homozygosity defined as the observation of three or more adjacent homozygous loci.

some 20 and structural alterations involving chromosomes 3, 10, and 18, have also been observed through karyotypic analysis of short-term or uncultured primary prostate tumors [13]. It is therefore difficult to determine which genetic alterations were originally present in the prostatic tissues, and which arose subsequent to cellular immortalization. The presence of some of these genetic alterations in 1535N cells, however, suggests at least a subset of the observed karyotypic aberrations arose consequent to cellular immortalization. In particular, all four HPV-immortalized cell lines exhibited extensive duplications or translocations involving the 11q13q22;q23 chromosomal region. 11q+ alterations have been reported in cells immortalized with the HPV 16 or 18 genomes [22, 23], and the 11q23 region has been classified both as a fragile site and possible viral modification site [24, 25]. It appears that the 11q+ alteration observed in the cell lines examined comprises the common chromosomal aberration directly due to immortalization with the E6 and E7 genes of HPV 16.

Allelotype analysis demonstrated loss of 8p sequences in the 1532T and 1542T primary prostate tumor-derived cell lines. The four HPV-immortalized cell lines examined in the present study were partially allelotyped for 8p sequences by Bright et al., who reported loss for a limited number of markers mapping to 8p in the 1532T and 1542T, but not the 1535N or 1535T, cell lines [14]. Interestingly, the 8p loss pattern observed in the tumor tissues and resulting immortalized cell lines was concordant for the 1542T, but not 1532T or 1535T, cell lines. We have confirmed these results for the cell lines, and report a more precise allelotyping, with 12 markers spanning 8p and 2 markers pericentromeric to 8q (Table 2). Complete loss of one copy of 8p in the 1532T and 1542T cell lines was observed, with loss extending pericentromerically into 8q in 1532T cells. These findings are remarkably similar to those reported by others describing reduction to homozygosity for all or part of 8p in prostate tumor tissues [1–10]. Moreover, conventional G-banding and SKY data revealed that loss of 8p sequences in the 1532T and 1542T cell lines was associated with complex structural alterations of chromosome 8 (Fig. 6). The 1532T cells exhibited an i(8q) chromosome in all ten metaphases examined by spectral karyotyping. The 1542T cells also demonstrated an i(8q) chromosome, as well rearrangement of chromosome 8 material with either chromosome 20 or 21. The SKY data confirm reports of an i(8q) chromosome in 1542T cells demonstrated by CGH, FISH, and allelotyping data by Virgin et al., though other aspects of the 1542T karyotype—monosomy 4 and trisomy 11—reported by Virgin et al. differed from the SKY results reported here, possibly due to differences in the passage number and/or clones examined in these studies [26]. The PrEC-T cells, derived from normal prostatic epithelium immortalized after stable transfection with the large T-antigen gene of SV40, also exhibited extensive structural alterations of chromosome 8. These alterations included duplication of q arm material, from q11.2 to q24. In contrast to the 1532T and 1542T cells, the gain of 8q arm material observed in PrEC-T cells was not in the form of an i(8q) chromosome, although the net gain of 8q arm material was the same for all three cell lines.

In conclusion, the data presented here provide intriguing evidence that 8p loss in primary human prostate tumors may not result from simple deletion of all or part of the short arm, as has been previously inferred from allelotyping data [1–8]; rather, 8p loss may, in fact, result from complex structural rearrangements of chromosome 8, often resulting in gain of 8q material, which occurs during tumorigenesis. Moreover, the data reported here provide direct evidence that such complex structural rearrangements sometimes include i(8q) chromosome formation. These studies also suggest that gain of 8q sequences may occur as a consequence of i(8q) chromosome formation in some instances, but may also occur independently of either 8p loss or i(8q) chromosome formation.

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REFERENCES

1. Bergerheim USR, Kunimi K, Collins VP, Ekman P (1993): Deletion mapping of chromosomes 8, 10 and 16 in human prostatic carcinoma. Genes Chromosom Cancer 3:215–220.
2. Bova GS, Carter BS, Bussemakers JG, Emi M, Fujiwara Y,

Kyprianou N, Jacobs SC, Robinson JC, Epstein JI, Walsh PC, Isaacs WB, (1993): Homozygous deletion and frequent allelic loss of chromosome 8p22 loci in human prostate cancer. Cancer Res 53:3869–3873.

3. MacGrogan D, Levy A, Bostwick D, Wagner M, Wells D, Bookstein R (1994): Loss of chromosome arm 8p loci in prostate cancer: mapping by quantitative allelic imbalance. Genes Chromosom Cancer 10:151–159.
4. Trapman J, Sleddens HFBM, van der Weiden MM, Dinjens WNM, Konig JJ, Schroder FH, Faber PW, Bosman FT (1994): Loss of heterozygosity of chromosome 8 microsatellite loci implicates a candidate tumor suppressor gene between the loci *D8S87* and *D8S133* in human prostate cancer. Cancer Res 54:6061–6064.
5. Macoska JA, Trybus TM, Benson PD, Sakr WA, Grignon DJ, Wojno KD, Pietruk T, Powell IJ (1995): Evidence for three tumor supressor gene loci on chromosome 8p in human prostate cancer. Cancer Res 55:5390–5395.
6. Vocke C, Pozzatti RO, Bostwick DG, Florence CD, Jennings SB, Strup SE, Duray PH, Liotta LA, Emmert-Buck MR, Linehan WM (1996): Analysis of 99 microdissected prostate carcinomas reveals a high frequency of allelic loss on chromosome 8p12–21. Cancer Res 56:2411–2416.
7. Prasad MA, Wojno KJ, Macoska JA (1998): Homozygous and frequent deletion of proximal 8p sequences in human prostate cancers: identification of a potential tumor suppressor gene site. Genes Chromosom Cancer 23:255–262.
8. Sakr WA, Macoska JA, Benson PD, Grignon DJ, Wolman SR, Pontes JE, Crissman JD (1994): Allelic loss in locally metastatic, multisampled prostate cancer. Cancer Res 54:3273–3277.
9. Macoska JA, Trybus TM, Sakr WA, Wolf MC, Benson PD, Powell IJ, Pontes JE (1994): Fluorescence in situ hybridization (FISH) analysis of 8p allelic loss and chromosome 8 instability in human prostate cancer. Cancer Res 54:3824–3830.
10. Van Den Berg C, Guan X-Y, Von Hoff D, Jenkins R, Bittner M, Griffin C, Kallioniemi O, Visakorpi T, McGill J, Herath J, Epstein J, Sarosdy M, Meltzer P, Trent J (1995): DNA sequence amplification in human prostate cancer identified by chromosome microdissection: potential prognostic implications. Clin Cancer Res 1:11–18.
11. Takahashi S, Alcaraz A, Brown JA, Borell TJ, Herath JF, Bergstralh EJ, Lieber MM, Jenkins RB (1996): Aneusomies of chromosomes 8 and Y detected by fluorescence in situ hybridization are prognostic markers for pathological stage C ($pT_3N_0M_0$) prostate carcinoma. Clin Cancer Res 2:137–145.
12. Jenkins RB, Qian J, Lieber MM, Bostwick DG (1997): Detection of c-myc oncogene amplification and chromosomal aneusomies in metastic prostatic carcinoma by fluorescence in situ hybridization. Cancer Res 57:524–531.
13. Webb HD, Hawkins AL, Griffin CA (1996): Cytogenetic abnormalities are frequent in uncultured prostate cancer cells. Cancer Genet Cytogenet 88:126–132.
14. Bright K, Vocke CD, Emmert-Buck MR, Duray PH, Solomon D, Fetsch P, Rhim JS, Linehan WM, Topalian SL (1997): Generation and genetic characterization of immortal human prostate epithelia cell lines derived from primary cancer specimens. Cancer Res 57:995–1002.
15. Hukku B, Rhim JS (1993): Role of chromosome 5 in immortalization and tumorigenesis of human keratinocytes. Cancer Genet Cytogenet 68:22–31.
16. Schrock E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T (1996): Multicolor spectral karyotyping of human chromosomes. Science 273:494–497.
17. Beheshti B, Karaskova J, Park PC, Squire J, Beatty BG (in press): Identification of a high frequency of chromosomal rearrangements in the centromeric regions of prostate cancer cell lines by sequential Giemsa-banding and spectral karyotyping. Mol Diagn.
18. Coursen JD, Bennett WP, Gollahon L, Shay JW, Harris CC (1997): Genomic instability and telomerase activity in human bronchial epithelial cells during immortalization by human papillomavirus-16 E6 and E7 genes. Exp Cell Res 235:245–253.
19. Weijerman PC, van Drunen E, Konig JJ, Teubel W, Romijn JC, Schroder FH, Hagemeijer A (1997): Specific cytogenetic aberrations in two novel prostatic cell lines immortalized by human papillomavirus type 18 DNA. Cancer Genet Cytogenet 99:108–115.
20. Oda D, Bigler L, Mao EJ, Disteche CM, (1996): Chromosomal abnormalities in HPV-16 immortalized oral epithelial cells. Carcinogenesis 17:2003–2008.
21. Savelieva E, Belair CD, Newton MA, DeVries S, Gray JW, Waldman F, Reznikoff CA (1997): 20q gain associated with immortalization: 20q13.2 amplification correlates with genome instability in human papillomavirus 16 E7 transformed human uroepithelial cells. Oncogene 14:551–560.
22. Sutherland GR, Baker E, Callen DF (1998): A BrdU-enhanceable fragile site or viral modification site at 11q23.1 in lymphoblastoid cultures. Cytogenet Cell Genet 47:201–203.
23. Seki N, Tsuji H, Takahashi E, Yamauchi M, Saito T, Hashimoto T, Yanamoto K, Hori T (1992): Induction of a BrdU-enhanceable fragile-like lesion and sister chromatid exchanges at 11q23.1 in EBV-transformed lymphoblastoid cell lines. Cytogenet Cell Genet 61:95–98.
24. Hoffschir F, Ricoul M, Dutrillaux B (1988): SV40-transformed human fibroblasts exhibit a characteristic chromosomal pattern. Cytogenet Cell Genet 49:264–268.
25. Ray FA, Peabody DS, Cooper J, Scott-Cram L, Kraemer PM (1990): SV40 T antigen alone drives karyotype instability that precedes neoplastic transformation of human diploid fibroblasts. J Cell Biochem 42:13–31.
26. Virgin JB, Hurley PM, Nahhas FA, Bebchuk KG, Mohamed AN, Sakr WA, Bright RK, Cher ML (1999): Isochromosome 8q formation is associated with 8p loss of heterozygosity in a prostate cancer cell line. Prostate 41:49–57.