Malignant transformation of human benign prostate epithelial cells by high linear energy transfer α-particles

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Abstract. Although epidemiological studies have suggested a positive correlation between environmental radon exposure and prostate cancer, the mechanism involved is not clear. In the present study, we examined the oncogenic transforming potency of α-particles using non-tumorigenic, telomerase-immortalized human benign prostate epithelial cells. We report the malignant transformation of human benign prostate epithelial cells after a single exposure to 0.6 Gy dose of α-particles. Transformed cells showed anchorage-independent growth in soft agar and induced progressively growing tumors when transplanted into SCID mice. The tumors were characterized histologically as poorly differentiated adenocarcinomas. The cell line derived from tumor (SCID 5015), like the unirradiated cells, expressed cytokeratin 5, 8 and 18, NKX3.1 and AMACR. The malignant cells showed increased secretion of MMP2. Stepwise chromosomal changes in the progression to tumorigenicity were observed. Chromosome abnormalities were identified in both irradiated and tumorigenic cells relative to the non-irradiated control cells. Prominent changes in chromosomes 6, 11 and 16, as well as mutations and deletions of the p53 gene were observed in the tumor outgrowth and tumor cells. These findings provide the first evidence of malignant transformation of human benign prostate epithelial cells exposed to a single dose of α-particles. This model provides an opportunity to study the cellular and molecular alterations that occur in radiation carcinogenesis in human prostate cells.

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Abbreviations: HBPE, human benign prostate epithelial cells; LET, linear energy transfer; RT-PCR, reverse transcription-PCR; hTERT, human telomerase reverse transcriptase; K-SFM, keratinocyte-serum-free medium; MMP, matrix metalloproteinase; AMACR, α-methyl-CoA racemace

Key words: human benign prostate epithelial cells, immortalization, malignant transformation, carcinogenesis, α-particles

Introduction

Ionizing radiation can cause as well as cure human cancers. There is abundant evidence that α-particles are potent carcinogens. Exposure of the lung to α-emitting radon progeny represents the largest component of background radiation to the US general public (1). Epidemiological studies have shown that uranium miners exposed to high levels of radon progeny show the largest number of radiation induced lung cancers found in any exposed population (2,3). The US Environmental Protection Agency has estimated that the number of radon related lung cancer deaths amounts to ~21,000 annually (4). Furthermore, plutonium ingestion in experimental animals has also highlighted the extreme effectiveness of α-particles in tumor induction (5). The relative short range and high linear energy transfer (LET) nature of α-particles that characterize their high relative biological effectiveness in lung cancer induction among uranium miners and people who lived in high radon background areas might also account for their effectiveness in the treatment of cancer.

Carcinoma of the prostate is the most common male cancer in the United States. Interstitial brachytherapy using radium seeds that decay to emit predominantly γ-rays has been used in the treatment of localized lesions. More recently, targeted α therapy, using an α-particle emitting radioisotope such as ^{213}Bi to label monoclonal antibodies that are specific for prostate tissues has gained considerable attention (6,7). These studies have shown that targeted α therapy is highly cytotoxic to prostate cancer cells *in vitro* and can inhibit tumor growth in animal models as well.

Using population-averaged radon values from fourteen countries, Eatough and Henshaw have shown a positive correlation between radon level in houses and the incidence of prostate cancer (8). The carcinogenic mechanism, however, is not known. While α-emitting antibody presents an appealing targeted approach in prostate cancer treatment, the risk of

radiation induced second malignancy is a factor of concern. In the present study, the oncogenic transforming potency of high LET α-particles was evaluated using the human benign prostate epithelial (HBPE) cells exposed to a single dose of accelerator generated 150 keV/μm ^{4}He ions. We have demonstrated herein, and for the first time, the capacity of α-particle irradiation to transform HBPE RC-170N/hTERT clone 7 cells to a malignant phenotype.

Materials and methods

Cell culture. The non-tumorigenic benign HBPE cell line, designated RC-170N/hTERT clone 7 (9), was used at passage 19 for these transformation studies. This clonal cell line was derived from human benign prostate epithelium and immortalized by telomerase. Cells were grown and maintained in keratinocyte serum-free medium (K-SFM) supplemented with bovine pituitary extract (50 μg/ml), recombinant epidermal growth factor (5 μg/ml), and antibiotics (Life Technologies, Inc. Gaithersburg, MD) (KGM). The malignant transformed cells were adapted to grow in DMEM (Ca^{2+} higher 650 μM) + 10% FBS + Ins. and RPMI + 10% FBS (Ca^{2+} higher 650 μM) by stepwise fashion. The cells were grown for 2 weeks with a mixture of KGM and DMEM + 10% FBS + Ins. or KGM and RPMI-1640 + 10% FBS medium at a ratio of 1:1, 1:3 and 1:7 for two passages each.

Irradiation. Exponentially growing human prostate epithelial cells were plated in a specially constructed stainless ring with a 6-m mylar bottom at a density of $2x10^5$ cells 2 days before irradiation. Cells were irradiated with graded doses of 150 keV/μm ^{4}He ions accelerated in the 4 MeV van de Graff accelerator at the Columbia University Radiological Research Accelerator as described (10-12). Irradiated cells were sub-cultured immediately to determine survival levels and expanded in cultures to assay for transformed phenotypes. End-points such as growth kinetics, anchorage-independent growth and tumorigenicity in SCID mice were used to assays the transformation stage of these cells.

Colony formation in soft agar. A cell suspension ($1x10^5$ cells/ml) in 5 ml of 0.35% Noble agar with K-SFM was overlaid into a 60-mm dish containing a 0.5% agar base. Colonies >0.2 mm in diameter were counted on day 21.

Tumor formation in SCID mice. Tumorigenicity assay was carried out in SCID mice. Briefly, viable cells ($1x10^7$) in 0.2 ml of PBS were injected subcutaneously into the mid-dorsal intrascapular region of adult SCID mice to determine tumorigenicity. The SCID mice were observed for 6 months for the appearance of tumor developments.

RT-PCR assay. 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) and quantified with Beckman DU640 (Beckman, Somerset, NJ). One microgram of total RNA was reverse transcribed into cDNA with an RNA PCR-kit (Perkin-Elmer, Foster, CA) and 1/10 of the reverse-transcribed product from each sample was used for PCR to amplify NKX 3.1, cytokeratin (CK) 8 respectively. The primer sequences and the expected size of PCR products were the same as described previously (9).

Western blot analysis. Cell lysates were prepared from confluent cells by adding T-PER tissue protein extraction reagent (Pierce, Rockford, IL) containing the protease inhibitor cocktail (Calbiochem, San Diego, CA). Protein concentration was determined with a BCA protein assay kit (Pierce). Twenty micrograms of protein from each lysate was electrophoresed in 4-12% Tris-Bis SDS polyacrylamide gels and then electro-transferred to PVDF membrane (Invitrogen, Carlsbad, CA). The PVDF membrane was blocked in 5% non-fat milk in 1X PBS and 0.05% Tween-20 and then blotted with MMP2 and MMP9 antibodies (1:1000) (Cell Signaling, Danvers, MA). The membrane was stripped and reblotted with anti-tubulin monoclonal antibody (Sigma-Aldrich, St. Louis, MO) (1:5000).

Cytogenetic analysis. Chromosome counts, ploidy distribution and Giemsa (G)-banded karyotypes were prepared by standard protocol as described previously (13). M-FISH analyses were performed as reported previously (14,15).

Invasion assay. The cell invasion assay was performed by the method described by Lochter *et al* (16) and modified at the invasion cell counting step. Matrigel was diluted to 1 mg/ml in serum-free cold cell culture media and 100 μl was placed into the upper chamber (8 μm BD, San Jose, CA) of a 24-well transwell. The transwell was incubated at 37˚C for 4 h for gelling. The cells were harvested from tissue culture flasks by trypsinizing, washed 3 times with culture medium containing 1% FBS, and resuspended in media containing 1% FBS at a density of 10^6 cells/ml. Cell suspension (100 μl) was placed onto the Matrigel. The lower chamber of the transwell was filled with 600 μl of culture media and incubated at 37˚C for 24 h. The non-invaded cells on the top of the transwell were removed with a cotton swab and invaded cells were counted by coulter after trypsinizing.

p53 analysis. p53 DNA sequencing was carried out by using a method described previously (17). Exons 4 through 10 of the p53 gene were amplified in 3 independent PCR reactions. The primers were designed as following: exons 4 and 5, 3F/4R for amplification and sequencing; exon 6, 5F/5R for amplification and sequencing; exon 7, 7F/8R for amplification and 7F/7R for sequencing; exons 8-10, 7F/8R for amplification and 8F/8R for sequencing: p53 3F, 5'-GAA GCG AAA ATT CAT GGG ACT GAC -3'; 4R, 5'-CAT TGA AGT CTC ATG GAA GCC A -3'; 5F, 5'-CGT GTT CCA GTT GCT TTA TCT G-3'; 5R, 5'-GGA GGG CCA CTG ACA ACC A -3'; 7F, 5'-AGG CGC ACT GGC CTC ATC TT-3'; 7R, 5'-AGG GGT CAG CGG CAA GCA GA-3'; 8F, 5'-TTG GGA GTA GAT GGA GCC T-3'; 8R, 5'-AAC TTT CCA CTT GAT AAG AGG TC-3'.

The PCR reactions were carried out using 100 ng of DNA, 1X concentration of a PCR buffer, 0.2 mM dNTPs, 0.5 U pfu polymerase, and 1 μM each of a forward and reverse primer. The total reaction volume was 50 μl. Amplification occurred in a thermocycler under the following conditions: 5 min at 95˚C; 35 cycles of 30 sec at 95˚C, 1.5 min at 60˚C, and 1.5 min

at 72˚C; 1 cycle of 5 min at 72˚C. Ten percent of the PCR reaction was run on a 1% agarose gel to confirm product size and amplification. The PCR product was purified using a Qiaquick gel extraction kit (Qiagen, Chatsworth, CA). The purified PCR products were dye labeled using dye terminator cycle sequencing reactions with DNA polymerase (ABI). Purification of the dye attached PCR products was carried out on columns (Performa gel filtration cartridges Edge Biosystems PN) and loaded onto a 3100 Genetic analyzer sequencer (Hitachi). The data was collected and analysed using software (Edit Seq). The entire sequence of exons 5-10 were analysed and compared to normal p53 sequence (GeneBank).

Results

Transformation of RC-170N/hTERT clone 7 cells by α-particles. Telomerase-immortalized human prostate epithelial cells retain their epithelial morphology, contact inhibition of growth and are anchorage dependent (9). Upon exposure to a single 0.6-Gy dose of α-particles, the RC170N/hTERT clone 7 cells showed altered morphology and growth pattern by the 4th sub-cultivation after 8 weeks of post-irradiation growth (Fig. 1E). Similar changes were not observed in the unirradiated cells or in the cells irradiated once with 0.3-Gy α-particles (Fig. 1A and C). The cells began to pile up in the focal areas, forming small projections and then releasing small cells from the foci. The transformed cells exhibited the typical polygonal arrangement of epithelial cells (Fig. 1E).

Characterization of α-particle-transformed RC-170N/hTERT clone 7 cell line. The α-particle-transformed cells were further characterized by quantitative differences in growth properties, such as saturation densities and soft agar colony forming ability. Saturation densities of the α-particle-transformed cells were 5 times higher than those of the parental RC-170N/hTERT clone 7 cells, respectively (Table I). Moreover, the α-particle transformants grew to larger colonies in soft agar medium (Table I and Fig. 1F), whereas the unirradiated cells and cells irradiated once with 0.3-Gy α-particles grew to small colonies in soft agar (Table I and Fig. 1B and D).

When SCID mice were inoculated subcutaneously with $1x10^7$ of RC-170N/hTERT/clone 7 cells and then irradiated once with a 0.6-Gy dose of α-particles, tumors developed in 4 out of 5 SCID mice at the site of inoculation after 3.5-4 months (Table I). Histopathological examination of these tumors revealed poorly differentiated adenocarcinomas comprised of pleomorphic cells with a few keratinizing clusters consistent with prostate cancer (Fig. 1G). Karyotypic analyses of cultures derived from these tumors (SCID5105) confirmed their human origin. In contrast, the unirradiated RC-170N/hTERT clone 7 cells as well as the cells irradiated once with 0.3-Gy α-particles were non-tumorigenic for up to 6 months after s.c. inoculation in SCID mice (Table I).

RNA samples from the parental RC-170/hTERT clone 7 cells, the two α-particle-irradiated lines (0.3 and 0.6 Gy), the primary tumor cell line generated in SCID mice (SCID 5105), and the well-established human prostate cancer cells, LNCaP, were analyzed to determined the expression level of prostate specific markers by RT-PCR and immunostaining (data not shown). LNCaP cells, known to express a variety of prostate epithelial cell-associated genes are used as a positive control. All the cell lines, RC-170N/hTERT clone 7 cells, α-particle-irradiated (0.3 and 0.6 Gy) RC-170N/hTERT clone 7 cells and SCID 5105 cells expressed CK5, CK8, CK18 and NKX3.1. Since the gene, α-methyl-CoA racemace (AMACR), is often differentially expressed in prostate cancer versus normal prostate epithelium (18), the expression of AMACR was also examined. All the cell lines expressed AMACR.

Matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) is the type IV collagen/gelatin protease that plays an important role in cancer invasion and metastasis. The protein expression and activity are associated with prostate cancer progression (19-21). In our cell models, the MMP-2 protein was highly up-regulated in the transformed and tumor cells when grown in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum and 5 μg/ml insulin (Fig. 2). In contrast, MMP9 proteins were not detectable in our cells (data not show). The MMP-2 protein levels are coincidentally associated with invasion activity in the transformed cells and tumor cells (Fig. 2B). The results indicated that our cell model mimics the prostate cancer progression in the prostate cancer patients.

The chromosomal alterations in the α-particle-irradiated RC-170N/h TERT clone 7 cells. The chromosomal count, ploidy, modal number and composite karyotype are given in Table II and Fig. 3. From Table II, it is evident that as the cell line progressed towards the tumorigenic stage, the chromosomal modal number decreased correspondingly (91, 89 and 78 respectively) or in other words, there is loss of normal chromosomes.

The control cell line is near tetraploid. Chromosomes 1, 3, 7, 11, 12, 13 and 14 are involved in marker formation. According to M-FISH results, there is gain of chromosome 8 material in M1 as q+ part, chromosome 1p material in M6C. There is no loss of 22 as it is present in markers M3 and M8 (Table II). Based on the M-FISH analyses, there was a gain of chromosome 8 fragment in M1 as q+ part and chromosome 1p fragment in M6C. In addition, there was no loss of chromosome 22 as it was presented in markers M3 and M8 (Table II). After taking into consideration the data obtained using M-FISH and the configuration of marker chromosomes, it was evident that there were chromosomal losses involving 4, 6, 10 and 21. Similarly, there were losses of chromosome 1q (q32>qter) in M8, 3q (q26>qter) in M1A, 4q (M11) and 12p12.pter in M7. Finally, an extra copy of 5p in M10 and 9q? in M11 was identified.

Compared to the control clone 7 cell line, radiation-treated cells showed much pulverization, obviously due to the result of radiation. In addition, there was further gain of a copy of 15 and loss of one copy of 17, 18 and 19, one copy of 16 as 16q+ (M13) and interstitial deletion of 6q14-q24 (marker M14).

The primary tumor cell from the SCID mouse tumor showed further changes (Fig. 3). There was loss of a copy of chromosomes 2, 3, 9, 13, 17, 20 and 21. There was further loss in marker M14 as M14A leading to deletion 6q12-q14. Marker M8 showed further change as M8C leading to loss of material (1p32-p36) and translocation of unknown material instead. Similarly, marker M2 showed further change as M2C leading to loss of 7p10-p14 and possible translocation of 9q?

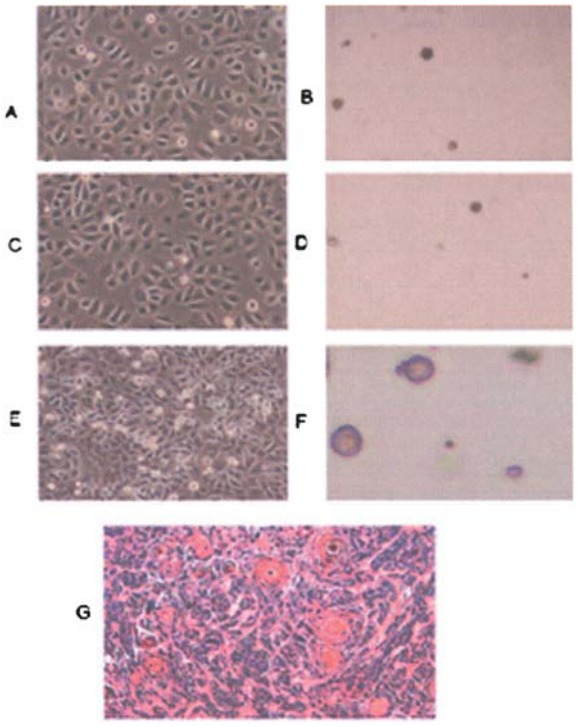

Figure 1. RC-170N/hTERT clone 7 cells irradiated with a single dose of α-particles, followed by subculturing 4 times. (A) Unirradiated RC-170N/hTERT clone 7 cells; (B) soft agar of unirradiated RC-170N/hTERT clone 7 cells; (C) irradiated RC-170N/hTERT clone 7 cells with 0.3 Gy; (D) soft agar of irradiated RC-170N/hTERT clone 7 cells (0.3 Gy); (E) irradiated RC-170N/hTERT clone 7 cells with 0.6 Gy; (F) soft agar colonies of RC-170N/hTERT clone 7 (0.6 Gy); (G) poorly differentiated adenocarcinoma produced by inoculating the RC-170N/hTERT clone 7 cells irradiated with α-particles (0.6 Gy) in SCID mice (SCID5105) (x100).

Table I. Biological properties of RC-170N/hTERT clone 7 cell lines irradiated with α-particles.

Cell line irradiated once	Saturation density (cells/cm^2 x 10^5)	Soft agar colony formation (%)	Tumorigenicity in SCID mice
Control	2.2	<0.01	0/4
0.3 Gy	2.3	<0.01	0/4
0.6 Gy	9.5	0.12	4/5[a]

[a]Tumors were reestablished in tissue culture and confirmed as human cells. Their resemblance in the cells of origin was determined by karyological analysis.

There was translocation of material with chromosome 3, 4 and 5 as 3q+, 4p+ and 5p+ as markers M1D, M15 and M16 respectively. The translocated material may be of chromosome 2 and 3 origin. 16q+ marker M13 is more prominent compared to the radiation-treated cell line (3/8 radiation treated, versus 6/9 in the tumor-derived cell line). Chromosome 11 showed prominent changes as markers M6C1, M6D and M6G. Markers M6C1 and M6G showed an addition at 11q22 (Fig. 3B).

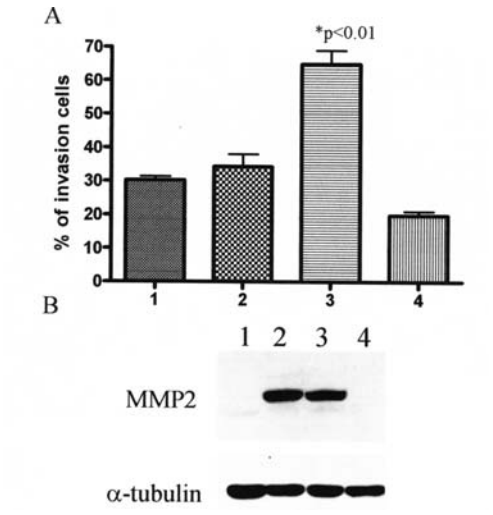

Figure 2. Invasion ability of A-particle-transformed cells and tumor cells. (A) The cell invasion analysis was performed by following the protocol (16). Briefly, cells were trypsinized and counted with cell coulter and using 10^5 cells for each well invasion analysis. The results shown in the figure are averages from triple analysis and similar results in 3 individual experiments. A-particle-transformed cells and tumor cells showed higher invasion ability. 1, RC-170N/hTERT clone 7 cells; 2, RC-170N/hTERT clone 7 (0.6 Gy) cells; 3, SCID 5105 cells; 4, PC-3 cells. (B) Expression of MMP2 protein in radiation-transformed cells and tumor cells. Western blot analysis was described in Materials and methods. Briefly, loading 20 μg protein from total cell lysate onto 4-12% Bis-Tris Gel and transferred to PVDF membrane. The MMP2 antibody (Cell signaling) was 1:1000 diluted. The MMP2 protein (MW 72 kDa) was higher expressed in radiation transformed cell and tumor cells. 1, RC170N/h/clone 7; 2, RC170N/h/clone 7/0.6Gy (D); 3, SCID5105 (D); 4, PC-3 cells.

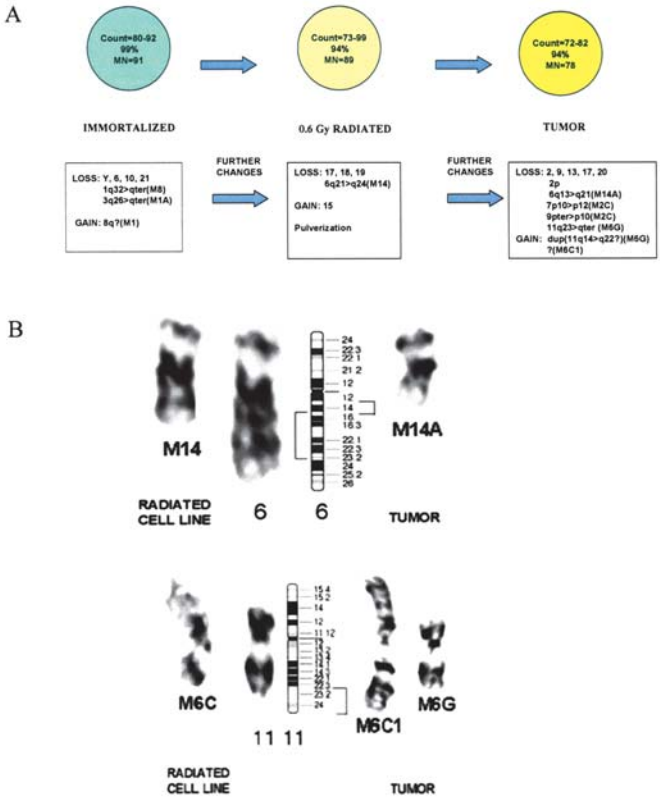

Figure 3. (A) Stepwise genetic changes in immortalized RC-170N/hTERT clone 7, A-particle-irradiated RC-170N/hTERT clone 7, and tumor cell line. (B) Changes observed in chromosome 6 and 11 in A-particle-irradiated (0.6 Gy) and tumor cell lines.

Table II. Cytogenic characteristics of RC-170N/hTERT clone 7 cells unirradiated and irradiated with A-particles and the tumor cell line following xenografting of the irradiated cells into SCID mice.

A,

Cell line	Count	2N	4N	Modal no.	Karyotype	
					Loss or gain of chromosomes	Markers (M)
RC-170N/h/0 p14	80-92, XXOO	99	1	91	86-91 XX, -(1, 3, 3, 4, 6, 7, 7, 10, 11 12, 12, 13, 13, 14, 14, 14, 21, 22, 22) (6K)	+[M1, M1A, M2X2, M3X2, M4X2, M6C, M7, M7A*, M8, M9X2, M10X2, M11X2, M12]
RC-170N/h/0.6 Gy p12	73-99, XXOO	94	6	89	80-99 XX, -(1, 3, 3, 4, 6, 6, 7, 7, 10, 11 12, 12, 13, 13, 14, 14, 14, 17, 18, 19, 22, 22)+15 (8K)	+[M1, M1A, M2X2, M3X2, M4X2, M6C, M6C1*, M7, M7A*, M8, M9X2, M10X2, M11X2, M12, M13*, M14]
SCID 5015 p17	72-82, XXOO	94	6	78	72-81 XX, -(1, 2, 3, 3, 3, 4, 4, 6, 6, 7, 7, 9, 10, 11, 11, 12, 12, 13, 13, 13, 14, 14, 14, 16, 17, 17, 18, 19, 20, 21, 22, 22) +15 (9K)	+[M1, M1A, M1D, M2, M2C, M3, M4X2, M6C1, M6D, M6G, M7, M7A, M8C, M9X2, M10X2, M11X2, M12, M13, M14A, M15, M16]

+, Present in >50%; *, present in <50%; -, absent; M, deletion, translocation or derivative chromosome; k, karyotype.

B, Marker description.

Marker	M-FISH[a]
M1=add(3)(q+)	q+ chromosome 8 material
M1A=del(3)(q25/q26)	
M1B=del(3)(q10)	
M1B1=del(3(p10)	
M1D=3q+ (15q?)	
M2=del(7)(p14)	Chromosome 19 translocation
M2C=t(7q;?)	
M3= der(13)t(13:?)(q10;?)	?=Chromosome 22 material
M4=der(14)t(14;?)(q10;?)	?= 20 or 18,20
M6=i(11q)	
M6B=add (11)(p14)	
M6C=add (11)(p14)	Add=1p?
M6C1=add (11)(p14)(q+)	
M6D=der(19)t(11;19)(q14;p/q13)	
M6F=del(11)(q10)	
M6G=dup/del(11q14/q22?)	
M7= der(12)t(7?;12)(p20;p12.1)	
M7A=12p-	
M7B=12q+	
M8=del(1)(q32)	t(1;22)(q32;22)
M8C=der(M8)p+	
M9=minute	
M10=unknown	t(5p;10)
M11=unknown	t(4p;9)
M12=i(?)	t(22;6;14)
M13=16q+	
M14=del(6)(q14q24)	
M14A=del(6)(q12q26)	
M15=der(4) 4p+	
M16=der(5) 5p+	

[a]This data is from M-FISH study of control cell line RC170N/h p1+14.

Table III. Sequencing analysis of p53 mutations of RC-170N/hTERT clone 7 cell line irradiated with α-particles.

	Control	0.6 Gy	SCID5105
Exon 4	Normal	Normal	Normal
Exon 5	Normal	Normal	Mutation at 314 G-T resulting, Pro. To Leu, 383 G-T resulting Ala-Ser
Exon 6	Normal	Normal	Normal
Exon 7	Normal	Normal	Normal
Exon 8	Normal	Normal	Deleted (total exon 8)
Exon 9	Normal	Normal	Normal
Exon 10	Normal	Normal	Mutation at 1090 C-T resulting Pro. To Leu.

p53 analysis. The p53 tumor suppressor gene has been shown to be inactivated either by gene mutations or loss of function of the gene product in a wide variety of human tumors including human prostate cancer. To detect the p53 gene mutation in radiation-transformed prostate epithelial cells, we carried out direct sequencing of exons 4-10 (Table III). DNA sequence analysis showed that the tumor cells SCID 5105 had deletion in exons 8 and 5 containing a G→T transition in codon 314 (Pro-Leu), 383 (Ala-Ser) and exon 10 containing a C→T transition in codon 1090 resulting in the substitution Pro by Leu whereas in the parental cells no mutation was detected. Direct sequencing of the p53 gene is the only sure way to assess whether the mutation event inactivates this gene. Our results suggest that mutation and deletion of the p53 gene is involved in the neoplastic transformation process of human prostate epithelial cells by radiation.

Discussion

Our results demonstrate that malignant transformation of an immortalized HBPE clonal cell line (RC-170N/h clone 7) by a single exposure to a 0.6-Gy dose of α-particles. The RC-170N/h clone 7 cells were initiated by hTERT transduction into prostate epithelial cells obtained as an outgrowth of a benign tissue explant (9) and were derived from a clonal line. They are anchorage dependent and non-tumorigenic in SCID mice. The α-particle transformed cells showed morphological alterations, formed colonies in soft agar, and formed adeno-carcinomas when transplanted into SCID mice. The tumor (SCID 5105) produced in SCID mice expressed CK5, 8, 18 and NKX3.1 except PSA. The AMACR, a gene known to express prostate-specific marker, was also expressed. In addition, the transformant and tumor cells showed increased secretion of MMP-2 protein, a marker linked to aggressive tumorigenic behavior. In the present study, we show, for the first time, that immortalized human prostate epithelial cells can be malignantly transformed by a single dose of high LET α-particles. Earlier reports also showed the malignant transformation of BEP2D (HPV-immortalized human bronchial epithelial cells) (10) and MCF-10F (a human mammary epithelial cells) cells. There are no other reported studies using α-particles for human cells. However, there are several reports of transformation studies using hamster or rodent cells with α-particles (22).

Here we report the genetic characterization of stepwise chromosome changes observed in immortalized prostate epithelial cells before and after radiation treatment, and its conversion to tumorigenicity using classical cytogenetics techniques.

In the present study, there is observed loss of chromosomes 4, 6, 10 and 21, and part of 1q(q32>qter) in M8, 3q(q26>qter) in M1A, 4q(M11), 12p12>pter in M7 and 12p in M7A in the parental immortalized cell line. There is extra copy of 5p in M10 and 9q in M11, and gain of chromosome 8 material in M1 as q+ part. All these chromosomes are already implicated in prostate cancer (23-31).

The radiation-treated cell line showed further gain of a copy of 15 and loss of one copy of 17, 18 and 19, one copy of 16 as 16q+ (M13) and interstitial deletion of 6q14-q24 (marker M14). This loss of 6q is reported in literature (25,28,30,31) and Slager *et al* found a strong evidence of linkage, using Gleason score as a measure of severity of disease, 6q23 as the region. In the present study also interstitial deletion of 6q14-q24 was the only prominent marker in radiation-treated cells.

The tumorigenic cell line showed further changes, of which chromosome 11 showed the most prominent changes as markers M6C1, M6D and M6G. Both markers M6C1 and M6G showed additional fragments at 11q22. Gain of 11q has been reported in literature (23-26,28,30). Das *et al* reported gain of 11q13 with higher Gleason score and statistically significant, and as more common in metastatic disease (24). In the present study, not only were there observed changes in chromosome 11q, but also further loss of material from marker M14 as M14A leading to deletion of 6q12-q14 and loss of 7p10-p14 and 9p in M2C. Both 6q deletion and 11q amplification are implicated in aggressive behaviors of tumor cells and both genetic changes are observed in the present study.

Ionizing radiation is known to cause DNA double-strand breaks, which can lead to deletions and rearrangements within cellular DNA and modulation of gene expression. A potential target for this action is the tumor suppressor gene p53. This gene is susceptible to small rearrangements, deletions, and point mutations, and thus is a potential site of action for the mutagenic effects of ionizing radiation. Mutations in this gene have been associated with malignant transformation in human and animal systems (32,33) and implicated in the pathogenesis of prostate carcinoma (34,35). Loss of p53 function after radiation transformation of primary human mammary epithelial cells has been reported (36). Alterations of p53 in tumorigenic HPV-immortalized human bronchial epithelial (BEP2D) cells induced by a single low dose of either α-particles or 1 GeV/nucleon ^{56}Fe were reported (37). PCR-SSCP and sequencing analysis found band shifts and gene mutations in all four of the secondary tumors. A G→T transversion in codon 139 in exon 5 that replaced Lys with Asn was detected in two tumor lines. One mutation each, involving a G→T transversion in codon 215 in exon 6 (Ser→ Ile) and a G→A transition in codon 373 in exon 8 (Arg→His), was identified in the remaining two secondary tumors. These results

suggested that p53 alterations correlate with tumorigenesis in the BEP2D cell model and that mutations in the p53 gene may be indicative of metastatic potential. Our analysis of exons 4-10 of the p53 gene in the parent α-particle-irradiated and tumor cells showed gene mutations in the tumor cells (SCID 51058). In the tumor cells, the deletion in exon 8 a G→T transmission in codon 314 (Pro→Leu) and in codon 383 (Ala→Ser) in the tumor cells were detected. A C→T transversion in codon 1090 (Pro→Leu) was also detected in the tumor cells. These results suggest that p53 alterations correlate with tumorigenesis in the RC-170N/hTERT clone 7 cell model. Our data also suggest that mutation in the p53 gene is likely to be a late event in the malignant transformation process of human prostate epithelial cells by radon. An alternative explanation would be that there are p53 mutations in the polyclonal parent line, but that none are prevalent enough in the population to be detected during sequencing. In contrast, the tumor that ends up growing in an SCID mouse could be composed of just one or a few clones, thus allowing mutation detection.

Interstitial brachytherapy using α-emitting radium seed has been used in the localized prostate cancer lesions. The human prostate epithelial cell system described here may be a useful *in vitro* tool for dissecting the cellular and molecular alterations that occur in radiation carcinogenesis in human prostate cells.

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