Neoplastic Transformation and Cytogenetic Changes after Gamma Irradiation of Human Epithelial Cells Expressing Telomerase

Author(s): Andrew Riches, Clare Peddie, Simon Rendell, Peter Bryant, Horst Zitzelsberger, Jochen Bruch, Jan Smida, Lui Hieber, and Manfred Bauchinger
Source: Radiation Research, 155(1):222-229. 2001.
Published By: Radiation Research Society
DOI: http://dx.doi.org/10.1667/0033-7587(2001)155[0222:NTACCA]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1667/0033-7587%282001%29155%5B0222%3ANTACCA%5D2.0.CO%3B2

RADIATION RESEARCH **155**, 222–229 (2001)
0033-7587/01 $5.00

Neoplastic Transformation and Cytogenetic Changes after Gamma Irradiation of Human Epithelial Cells Expressing Telomerase

Andrew Riches,[a,1] Clare Peddie,[a] Simon Rendell,[a] Peter Bryant,[a] Horst Zitzelsberger,[b,c] Jochen Bruch,[b] Jan Smida,[b] Lui Hieber[b] and Manfred Bauchinger[b]

[a] *School of Biology, Medical Science & Human Biology, University of St. Andrews, St. Andrews KY16 9TS, Scotland, United Kingdom;* [b] *Institute of Radiobiology, GSF-Forschungszentrum für Umwelt Gesundheit, GmbH, D-85764, Neuherberg, Germany; and* [c] *Institute of Radiation Biology, Ludwig Maximilians University, D-80336 München, Germany*

Riches, A., Peddie, C., Rendell, S., Bryant, P., Zitzelsberger, H., Bruch, J, Smida, J, Hieber, L. and Bauchinger, M. Neoplastic Transformation and Cytogenetic Changes after Gamma Irradiation of Human Epithelial Cells Expressing Telomerase. *Radiat. Res.* 155, 222–229 (2001).

Neoplastic transformation of human epithelial cells by radiation has previously been investigated using cell lines immortalized with viral vectors. There are disadvantages to this approach, and we report here the results of studies using a human retinal pigment epithelial cell line (340RPE-T53) immortalized by treatment with telomerase. After exposure of the cells to fractionated doses of γ radiation, there was a marked increase in anchorage-independent growth of the surviving cells. The cloned cell lines derived from these anchorage-independent cultures exhibited an increased growth rate *in vitro* and were serum-independent compared with the parent cell line. The parent cell line maintained a stable diploid karyotype. The cell lines cloned after irradiation with the lower doses (10 × 2 Gy) were hypodiploid with loss of chromosome 13 and a high level amplification of 10p11.2 associated with a deletion of the remaining short arm segment of chromosome 10 distal to 10p11.2. In contrast, the cell lines cloned after irradiation with the higher doses (15 × 2 Gy) were near-tetraploid with derivative chromosomes present characterized by SKY analysis. Thus this human epithelial cell line immortalized with telomerase provides an improved model to investigate mechanisms of radiation carcinogenesis.

INTRODUCTION

The transformation of normal human epithelial cells to cancer cells is associated with three major classes of phenotypic change: immortalization, aberrant growth control, and malignancy. The understanding of the multistage nature of human carcinogenesis is limited by the absence of human models with which to elucidate genetic changes that accompany neoplastic transformation.

Rodent cells, particularly C3H 10T½ cells, have been exploited very successfully to establish dose–response relationships, dependence of transformation after exposure to ionizing radiation and RBE (*1*, *2*). In contrast, it has been difficult to establish models of human carcinogenesis *in vitro*. Previous models have used immortalized human cell lines to induce transformation with ionizing radiation. However, to immortalize these cell lines, viral vectors (SV40, Epstein Barr, adenovirus and HPV) have been used to transfect primary cultures. Using this approach, studies of radiation carcinogenesis using human thyroid epithelial cells (*3*, *4*), human fibroblasts (*5*), human keratinocytes (*6*), human prostatic epithelium (*7*), and human bronchial epithelium (*8*) have been reported. Cell lines immortalized in this manner are often genetically unstable, with variable chromosome numbers and polyploid karyotypes. These complicating factors limit the use of these models, as the instability confounds studies of genetic change in carcinogenesis. Consequently, investigators studying radiation-induced cancers have been searching for an improved *in vitro* model of human neoplastic transformation.

Primary cultures of mammalian cells undergo senescence *in vitro*. This seems to be associated with gradual shortening of the telomeres until they become critically short, resulting in cell death. Telomerase is repressed in most normal human somatic cells, limiting the potential number of cell divisions available, but is almost universally expressed in cancer cells (*9–12*) and in stem cells. The mechanism by which telomerase is normally repressed appears to operate at the level of transcriptional regulation of the catalytic subunit (i.e. the protein component) of the telomerase ribonucleoprotein. The life span of primary cultures of human cells has been extended substantially by transfecting these cells with a vector expressing TERT (the catalytic subunit of telomerase) (*13*). These cells express telomerase and maintain their telomere length during culture.

While these cells express telomerase, this does not seem to induce a malignant phenotype on its own (*14*, *15*). Thus

[1] Author to whom correspondence should be addressed at School of Biology, Medical Science & Human Biology, University of St. Andrews, St. Andrews KY16 9TS, Scotland, UK.

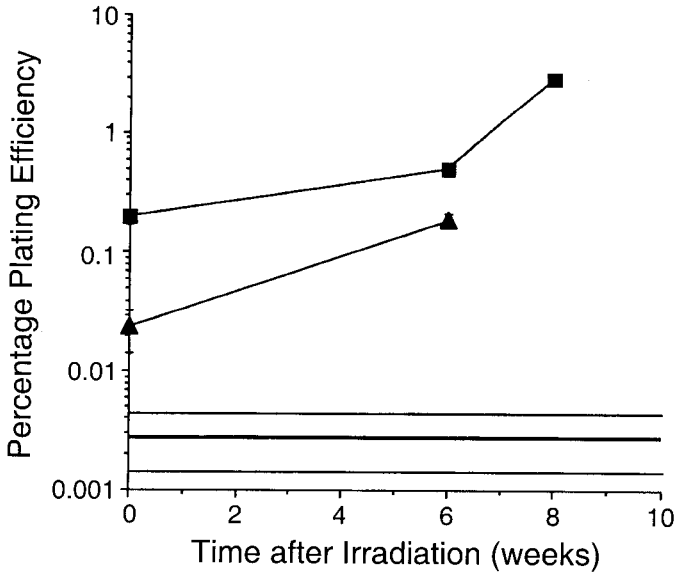


FIG. 1. Anchorage-independent colony formation of subconfluent cultures of retinal pigment epithelial cells immortalized with TERT and exposed to fractionated doses of γ radiation of 10 × 2 Gy (■) or 15 × 2 Gy (▲). After irradiation, cells were cultured for 2 days, 6 weeks or 8 weeks and then 10^4 cells were plated in 0.3% agar over a 0.5% underlayer and finally cultured for 21 days before scoring of anchorage-independent colonies. The plating efficiency is plotted as a function of the time after irradiation. Results are plotted as mean ± standard error.

it has been concluded that induction of an immortal phenotype was a necessary prerequisite and probably an early event in malignant transformation but was not sufficient in itself. Combining TERT, oncogenic *RAS* (*HRAS* V12), and human papilloma virus-16 E6/E7 viral oncoproteins failed to elicit transformation of human fibroblasts (*14*), while the combination of TERT, large T antigen, and an oncogenic *RAS* (*HRAS* V12) was successful in converting human epithelial and fibroblast cells into tumor cells (*16*). In both cases, inactivation of the TP53 and RB signaling pathways ensued but clearly gave different results.

A human retinal pigment epithelial cell line has been transfected successfully and stably with TERT (*13*). The line exhibits a diploid karyotype and responds to cytotoxic agents including radiation by cell cycle arrest (*15*). This paper describes the transformation of this cell line by fractionated doses of γ radiation and the properties of the irradiated cell lines derived.

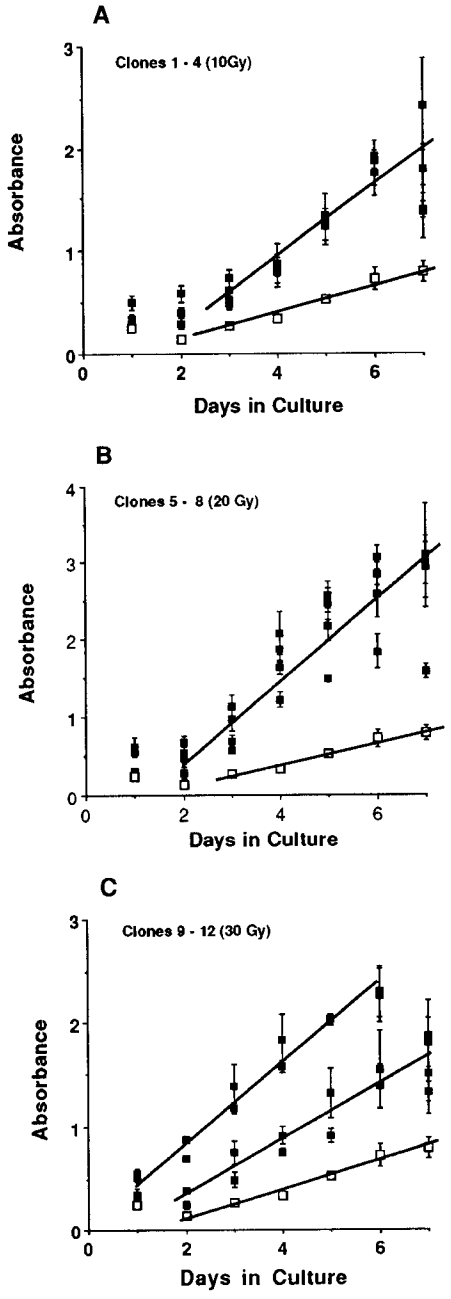
MATERIALS AND METHODS

Cell Culture

The 340RPE-T53 cell line was generated by transfection with pGRN145, which expresses TERT from the MPSV promoter, and was kindly supplied by Dr. Andrea Bodnar, Geron Inc. (*13*). Cells were routinely cultured at 37°C in 10% CO_2 in air. The culture medium was DMEM/F12, 10% fetal bovine serum, 2 m*M* glutamine, 0.348% (w/v) $NaHCO_3$, 15 m*M* Hepes, 10 μg/ml gentamicin, and 10 μg/ml hygromycin B. Subconfluence was normally maintained by splitting cultures at a ratio of 1:8 using trypsin.

Irradiation

Subconfluent cultures of the 340RPE-T53 clone were irradiated in T-75 tissue culture flasks with γ radiation from a ^{137}Cs source. Cells were



→

FIG. 2. Comparison of the growth kinetics of the parent human retinal epithelial cell line in culture (□) with (panel A) clones 1–4 derived from anchorage-independent colonies after irradiation of the parent cells with 5 × 2 Gy (■); (panel B) clones 5–8 derived from anchorage-independent colonies after irradiation of the parent cells with 10 × 2 Gy (■); and (panel C) clones 9–12 derived from anchorage-independent colonies after irradiation of the parent cells with 15 × 2 Gy (■). Cells were grown in 96-well plates and the growth kinetics was monitored using a spectrophotometric method (MTT assay). The absorbance was measured as the optical density at 570 nm minus the reference optical density at 690 nm.

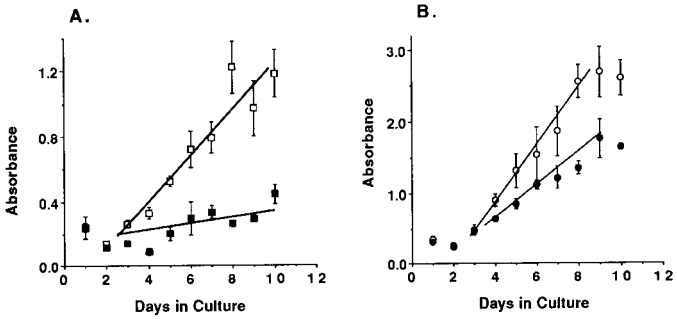


FIG. 3. Comparison of the growth kinetics of (panel A) the parent human retinal epithelial cell line in culture in the presence (□) and absence (■) of 10% fetal calf serum and (panel B) one of the cloned tumor cell lines (clone 9) derived from it after exposure to 15 × 2-Gy fractions of γ radiation in the presence (○) and absence (●) of 10% fetal calf serum. Cells were grown in 96-well plates and the growth kinetics was monitored using a spectrophotometric method (MTT assay). The absorbance was measured as the optical density at 570 nm minus the reference optical density at 690 nm.

exposed to 2 Gy at a dose rate of 4.6 Gy/min once every 72 h. Cultures reaching confluence during irradiation protocols were passaged in the normal manner to maintain cells in a proliferative state. Cells were harvested after accumulated doses of 10, 20 and 30 Gy. Control cultures were maintained in identical conditions but with no irradiation.

Cell Proliferation and Serum Independence

Cells from the parent RPE-hTERT cell line and the transformed clones were plated in 200-μl aliquots into 96-well tissue culture plates at a concentration of 2000 cells per milliliter. After 72 h culture, the plates were rinsed three times with sterile PBS before culture in serum-free medium. Identical plates were measured each day for 6 days after removal of serum. Control wells were treated identically, but the cells in the rinsed wells were cultured in medium containing 10% serum. Cell growth was measured using a rapid colorimetric assay (*17*). The yellow water-soluble tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] is reduced by live but not dead cells to a purple formazan product. Cultures were incubated in the dark for 4 h with 50 μl MTT (50 mg/ml in filter-sterilized PBS). The medium and MTT were removed from the wells and the formazan crystals dissolved in 200 μl DMSO with 25 μl Sørensen's glycine buffer (0.1 *M* glycine, 0.1 *M* NaCl adjusted to pH 10.5 with 1 *M* NaOH). The absorbance at 570 nm was read immediately with an ELISA microtiter plate reader.

Anchorage-Independent Growth

Cells were resuspended at the required concentrations in 0.3% agar (Difco). The cell suspension (2 ml) was added to 60-mm plates (Nunclon) over an underlay of 4 ml medium with 0.5% agar (Difco). The cells were cultured for 21 days and stained for 24 h with p-iodonitrotetrazolium violet (Sigma), and viable colonies of 50 or more cells that stained red were counted using a binocular microscope.

Chromosome Analysis

Chromosome analysis was carried out after *in vitro* culture of 10 to 20 population doublings. After G-banding with Wright's staining solution, karyotyping was performed according to the International System for Human Cytogenetic Nomenclature (*18*).

Comparative Genomic Hybridization (CGH)

CGH analysis was performed as described previously (*19, 20*). Tumor and reference DNA were directly labeled using nucleotide mixtures of

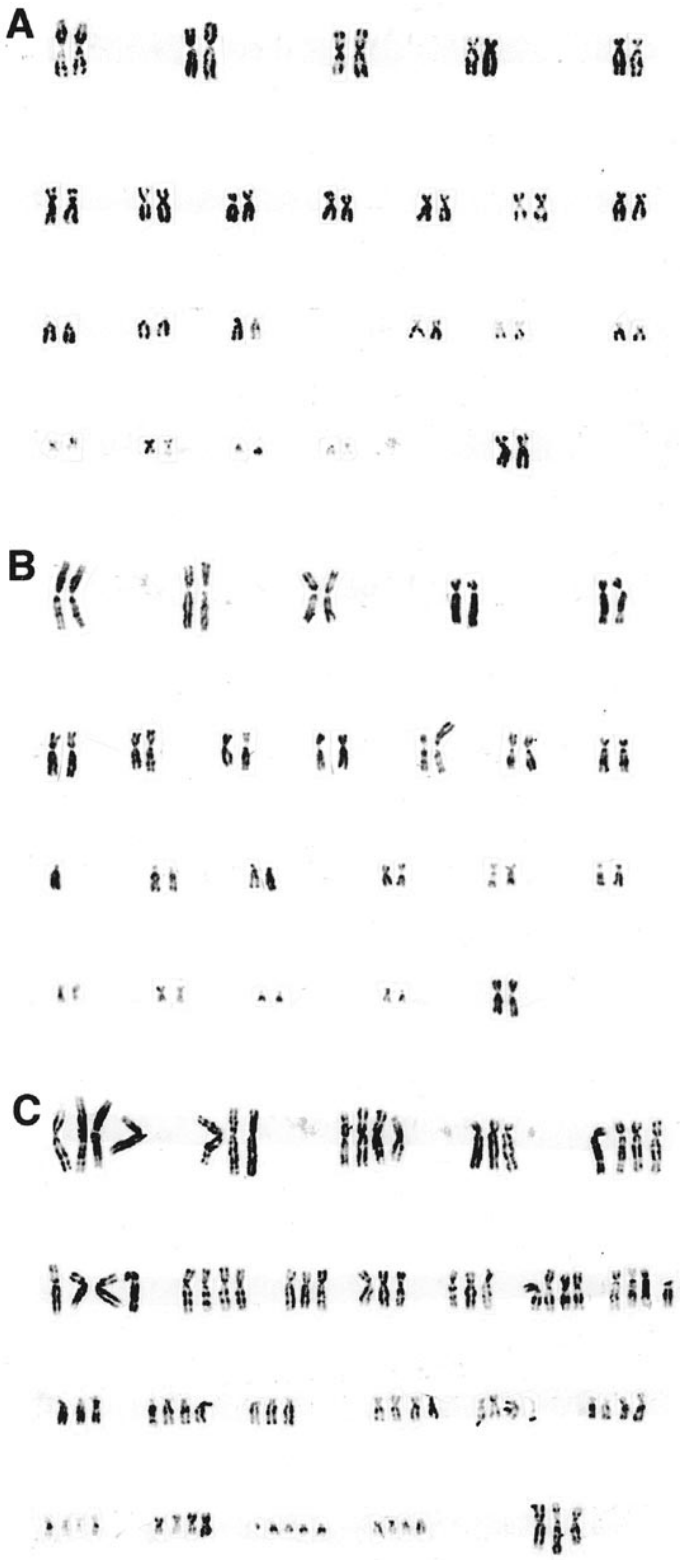


FIG. 4. Karyotypes derived from (panel A) the RPE parent cell line illustrating a stable karyotype with a normal female diploid chromosome complement (46, XX); (panel B) clone 7 (10 × 2 Gy) illustrating the loss of chromosome 13 and a large marker chromosome 10 in the hypodiploid clone; and (panel C) clone 10 (15 × 2 Gy) illustrating the near-tetraploid chromosome complement.

TABLE 1
Comparison of the Cytogenetics of the RPE Parental Cell Line and the Cloned Tumor Cell Lines Derived from it after Exposure to Fractionated Doses of Gamma Radiation (clones 5–8, 10 × 2 Gy; clones 9–12, 15 × 2 Gy) Characterized using G-banding, Comparative Genomic Hybridization (CGH), and Spectral Karyotyping (SKY)

RPE clone	Chromosome number (no. metaphases analyzed)	Polyploidy index (%)	G-banding	SKY	CGH
RPE parental	46 (15)	4	46,XX	n.d.	no changes
RPE C5	45 (7)	2	45,XX,-13,mar(10)	n.d.	losses: 10p12-pter, 13 gain: 10p11.2 (high-level amplification)
RPE C7	45 (11)	6	45,XX,-13,mar(10)	n.d.	losses: 10p12-pter, 13 gain: 10p11.2 (high-level amplification)
RPE C8	45 (10)	2	45,XX,-13,mar(10)	n.d.	losses: 10p12-pter, 13 gain: 10p11.2 (high-level amplification)
RPE C9	45 (11) 84-88 (5)	75	45,XX,-13,mar(10) near-tetraploid with various marker chromosomes	45, XX, -13, der(10), 83, XX, del(6q), del(9q), der(1)t(1;10;17)(q11;21;p11), der(2)t(2;5)(q31;q13), der(X;3), der(17;10), der(11;13), der(19;17)	losses: 6, 13, 17p gain: 18, Xq12-q25
RPE C10	75-86 (5)	96	near-tetraploid with various marker chromosomes	83-86, XX, del(4p), del(6q), der(2)t(2;10)(p11;q11), der(4)t(4;12)(q11;q11), der(X)t(X;17)(p11;p11), der(5;2), der(8;6), der(16;12), der(13;5), der(19;22), der(X;20)	losses: 4p14-pter, 10pter-q21, 17p
RPE C11	67-91 (5)	98	near-tetraploid with various marker chromosomes	83-85, XX, del(7q), der(1)t(1;16)(p35;q23), der(1)t(1;17)(q11;q21;p11), der(4)t(4;5;5)(q31;q13;p13), der(4)t(4;16) (q21;q24), der(11)t(11;13)(q21;q14), der(3)t(3;6)(q11;q11), der(6)t(6;7)(p11;q11), 2Xi(9q), der(6;17)	losses: 6q12-25, 9p, 17p gain: 9q
RPE C12	80-105 (5)	97	near-tetraploid with various marker chromosomes	n.d.	losses: 6q11-q22, 9p, 13, 17p, 18 gain: 9q

FITC-dUTP/FITC-dCTP (tumor DNA) and Lissamine-dUTP/Lissamine-dCTP (reference DNA) in a nick translation reaction. Hybridization was carried out with 500 ng of labeled DNA from transformed tumorigenic cell lines and reference DNA which were cohybridized for 72 h at 37°C with 25 μg CotI DNA to denatured metaphases from a healthy donor. For CGH analysis, at least 10 metaphases were imaged and karyotyped after visualization with a Zeiss Axioplan 2 fluorescence microscope equipped with filter sets (single-band excitation filters) for 4′-6-diamidino-2-phenylindole, FITC and TRITC. Averaged profiles were generated by CGH analysis software (ISIS 3, V2.84; MetaSystems, Altlussheim, Germany) from at least 10 to 15 homologous chromosomes and interpreted according to published criteria (*21*) using statistical confidence limits for the CGH ratios based on *t* statistics. An overexpressed area was classified as a high-level amplification if the ratio exceeded a value of 1.5 or if the green fluorescence showed a strong, distinct signal by visual inspection in combination with a diagnostic ratio profile for overrepresentation (*22*).

Spectral Karyotyping (SKY)

For SKY analysis (*23, 24*), metaphase preparations were pretreated with RNase A (0.1 mg/ml in 2× SSC) and pepsin solution (12 μg/ml 0.01 *M* HCl) and fixed in 1% formaldehyde. Pepsin digestion was performed under microscopic control and slides were placed in denaturing solution [70% formamide (FA) in 2× SSC] at 72°C for 90 s and dehydrated in a 70%, 80%, 100% ethanol series. Subsequently, they were hybridized with a probe mixture supplied by Applied Spectral Imaging (ASI, San Diego, CA). The probe mixture contains 24 painting probes specific for each human chromosome, labeled with combinations of five different fluorescent dyes (Spectrum Green, Spectrum Orange, Texas Red,

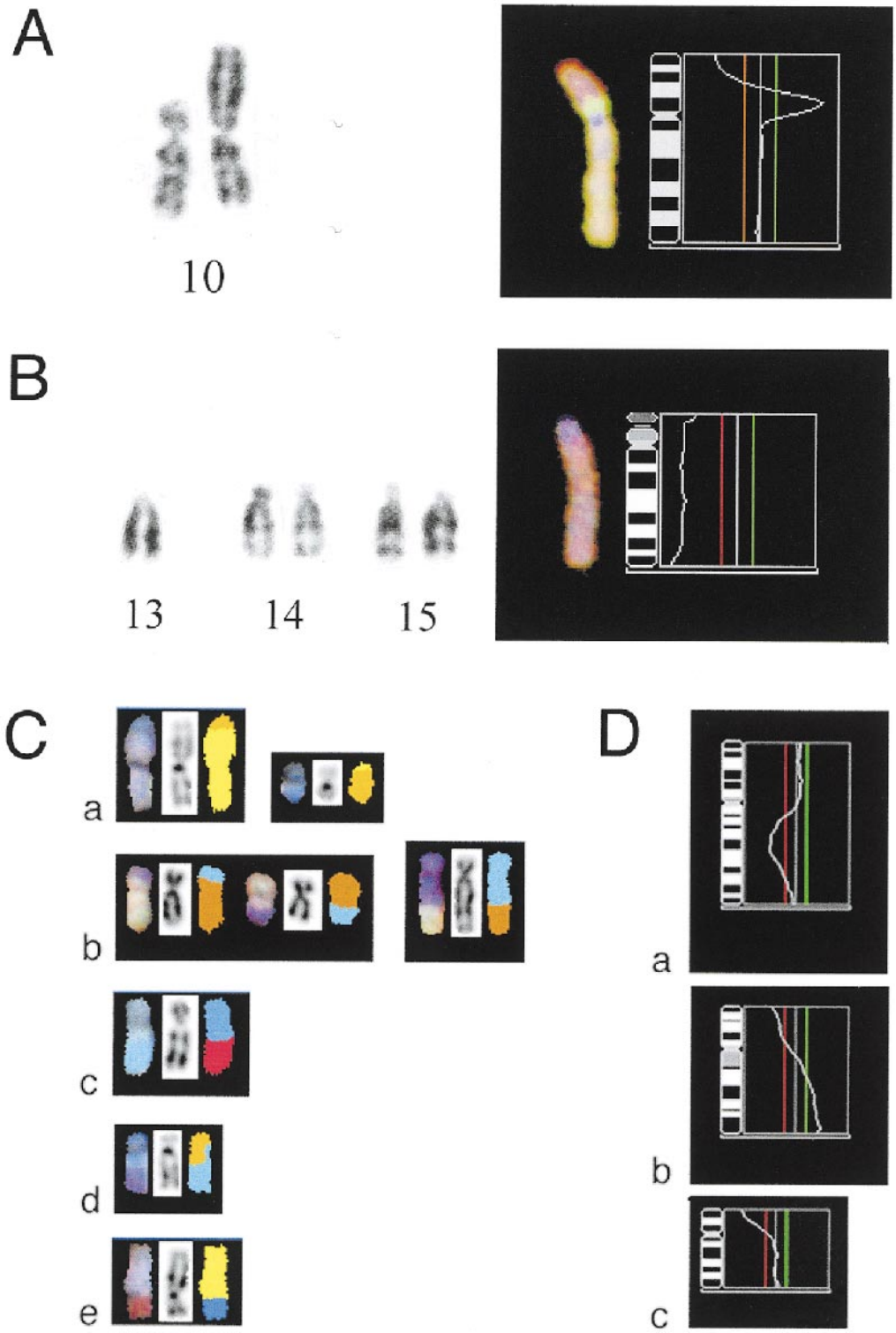


FIG. 5. G-banding and comparative genomic hybridization analysis of clone 7 illustrating (panel A) a high-level amplification on 10p11.2 and a deletion of the remaining short arm segment of chromosome 10 distal to 10p11.2 resulting in a huge marker chromosome 10 and (panel B) the loss of chromosome 13. Panel C: SKY analysis of clone 11 illustrating recurrent derivative chromosomes observed. a, der(t(1;16)(p35;q23)); b, der(t(4;5;5)(q31;q13; p13)); c, der(t(11;13)(q21;q14)); d, der(t(4;16)(q21; q24)); e, der(t(1;17)(q11; p11)). Panel D: Comparative genomic hybridization analysis of clone 11 illustrating (a) losses on chromosome 6q11-q22, (b) losses on chromosome 9p and gains on 9q, and (c) losses on chromosome 17p.

Cy5 and Cy5.5). The probe (SKY™ mixture) was denatured at 75°C for 7 min and incubated at 37°C for 1 h. The hybridization solution was applied to the denatured metaphases and incubated for 2 days at 37°C. Posthybridization washes were performed in 50% FA/2× SSC (three times for 5 min), 1× SSC (twice for 5 min at 45°C), and 4× SSC/0.1% Tween 20 (2 min at room temperature). Detection of biotinylated and digoxigenin-labeled probes was performed with avidin Cy5 and goat anti-mouse antibody conjugated to Cy5.5. Metaphase preparations were stained with DAPI solution (150 ng/ml DAPI in 2× SSC) and covered with antifade solution (Vectashield mounting medium, Vector Laboratories, Burlingame, CA). Metaphases were acquired by use of a SpectraCube system (ASI) and analyzed with the SKYView imaging software.

RESULTS

Anchorage-Independent Growth of Irradiated RPE Cells

The parent control cells formed only one or two colonies with a few clusters of less than 50 cells present. When the cells were plated a few days after irradiation, anchorage-independent growth was not observed in cultures exposed to 1 × 2-Gy, 2 × 2-Gy, 3 × 2-Gy, 4 × 2-Gy or 5 × 2-Gy fractions. At the same time, a modest increase in cloning efficiency from 0.0029 ± 0.0016% in unirradiated controls was observed in the groups exposed to 10 × 2 Gy and 15 × 2 Gy (0.20 ± 0.03% and 0.024 ± 0.009%, respectively; Fig. 1, $T = 0$). After passaging of the 10 × 2-Gy irradiated group, the cloning efficiency increased at 6 weeks to 0.49 ± 0.05% and at 8 weeks to 2.88 ± 0.14% (Fig. 1).

Growth Properties of Cloned Cell Lines

Individual colonies were isolated from the agar and expanded to produce 12 cloned cell lines, 4 from 5 × 2-Gy, 4 from 10 × 2-Gy, and 4 from the 15 × 2-Gy irradiated groups.

The growth properties of the irradiated cloned cell lines were compared with that of the parent cell line using the MTT assay. In each case, all the irradiated cloned cell lines grew more rapidly than the parent cell line (Fig. 2A, clones 1–4; Fig. 2B, clones 5–8; Fig. 2C, clones 9–12). Growth of cells of the parent line was inhibited by serum deprivation (Fig. 3A), whereas the irradiated cloned cell lines grew at virtually the same rate with and without serum, as illustrated by clone 9 (Fig. 3B).

Cytogenetic Characterization of the Irradiated Cloned Cell Lines

Cytogenetic characterization of the RPE parental cell line using G-banding demonstrated that the line exhibited a stable karyotype with a normal female diploid chromosome complement (46, XX) (Fig. 4A). Cloned cell lines grown from cells irradiated with 10 × 2-Gy fractions (clones 5, 7 and 8) were hypodiploid and exhibited loss of chromosome 13 (Fig. 4B). These changes in clones 5, 7 and 8 were observed in all metaphases analyzed (RPE clone 5, 7/7 metaphases; RPE clone 7, 11/11 metaphases; RPE clone 8, 10/10 metaphases). The cloned cell lines derived from cells irradiated with 15 × 2-Gy fractions (clones 9–12) were found to have a more complex pattern and were near-tetraploid (Fig. 4C). Loss of chromosome 13 was also observed in some of the near-tetraploid clones (Table 1). CGH analysis confirmed the loss of chromosome 13 in the hypodiploid clones and further demonstrated a high-level amplification on 10p11.2 associated with a deletion of the remaining short-arm segment of chromosome 10 distal to 10p11.2 (Fig. 5A and B). This amplification on chromosome 10 resulted in a huge marker chromosome 10 that was observed by G-banding (Fig. 4B). Complex karyotypes were studied using SKY analysis. A number of derivative chromosomes were characterized in the near-tetraploid cell lines (Table 1). An example of the SKY analysis of clone 11 illustrates derivative chromosomes involving the following chromosomes: der(t(1;16)(p35;q23)), der(t(4;5;5)(q31; q13;p13)), der(t(11;13)(q21;q14)), der(t(4;16)(q21;q24)), and der(t(1;17)(q11; p11)) (Fig. 5C). CGH analysis of clone 11 revealed losses on chromosome 6q11-q22, 9p and 17p and gains on 9q (Fig. 5D).

DISCUSSION

Marked changes in the properties of the parent human epithelial cell line have been induced after fractionated doses of radiation. Repeated doses of radiation seem necessary to induce changes. In previous studies using human prostate epithelial cells (*7*) and human keratinocytes (*6*), the investigators were also unable to transform cells with single exposures. With the RPE cells, anchorage-independent colonies could be detected at low frequency within a few days of completion of the appropriate fractionated dose of radiation. After fractionated doses of 10 × 2 Gy, there was a 150-fold increase in anchorage-independent colony formation relative to control cultures after 6 weeks of passaging postirradiation and 1000-fold at 8 weeks after irradiation. As fractionated doses were used and no anchorage-independent colonies were detected after 1 × 2 Gy, 2 × 2 Gy, 3 × 2 Gy, 4 × 2 Gy or 5 × 2 Gy, it thus was not possible to calculate transformation frequencies.

Cloned cell lines were selected from the anchorage-independent colonies derived from irradiated cultures. These transformed lines grew more rapidly than the parent cell line, with a doubling time approximately twice that of the parent cell line. Presumably the increase in anchorage-independent colony formation assayed at different times after irradiation after passaging of the cells would result from the selective growth advantage of the irradiated clones. The frequency of transformed clones might thus be expected to double approximately every 7 days. The increase observed was much less than this, which suggests that some fixation time is required to express the transformed phenotype.

The RPE parent cell line was nontumorigenic in athymic nude mice, as has been reported for other cell lines immortalized with TERT (*14, 15*). Thus expression of telo-

merase alone is not sufficient to induce a tumor phenotype. All of the irradiated cloned lines were tumorigenic in athymic nude mice.

A number of primary cell lines transfected with TERT have been shown to retain normal growth control (*14, 15*). Their growth rate decreases markedly in serum-free medium, and they also respond to cell cycle checkpoints after exposure to cytotoxic agents including radiation (*14, 15*). In our study, the growth of the RPE parent cells also slows in response to serum deprivation, whereas the irradiated cloned lines show only a small change in growth rate in serum-free medium. The growth rate of the irradiated cloned lines had also increased relative to the parent line. These changes in growth characteristics are indicative of transformation.

The RPE parent cell line retained a stable diploid karyotype during the course of passaging in this series of experiments. This is a major advantage over cell lines immortalized by other agents, as they usually exhibit an unstable polyploid karyotype (*25–27*). This makes analysis of radiation-induced changes more complex (*28*). The results from this study show a clear dose-dependent increase in ploidy and structural aberrations. At the lower doses, hypodiploid clones were detected with single chromosome loss and a large amplification on chromosome 10. At the higher doses, complex karyotypes emerged which could be analyzed using SKY techniques demonstrating a complex set of derivative chromosomes. Of particular interest was the observation that recurrent chromosome changes could be detected in different clones originating in part from different irradiation experiments with different doses. This may point to early changes being induced reproducibly after irradiation. These chromosome aberrations may also provide suitable starting points for positional cloning of genes involved in radiation-induced carcinogenesis. Our cytogenetic analysis would support the suggestion that there is inactivation of the *RB* gene (loss of chromosome 13) and the *TP53* gene (loss of 17p) in the radiation-induced clones. Of course, there will be loss of many other genes associated with the loss of whole chromosomes or chromosome fragments. This is consistent with changes reported in transformed human epithelial cells and fibroblasts using TERT, large T antigen, and oncogenic *RAS* (*HRAS* V12) (*16*).

Thus human epithelial cells can be transformed by exposure to a single physical agent coupled with immortalization with telomerase. The TERT-immortalized cell lines thus provide an exciting model with which to investigate and link cytogenetic changes to molecular events induced by ionizing radiations.

ACKNOWLEDGMENTS

We thank Dr. Andrea Bodnar at Geron Corporation for kindly supplying the RPE cell line. The work was supported in part by the EC Nuclear Fission Safety Programme, Nuclear Electric and Scottish Nuclear.

Received: October 26, 1999; accepted: March 3, 2000

REFERENCES

1. E. J. Hall and T. K. Hei, Oncogenic transformation of cells in culture: pragmatic comparisons of oncogenicity, cellular and molecular mechanisms. *Int. J. Radiat. Oncol. Biol. Phys.* **12**, 1909–1921 (1986).
2. T. K. Hei, K. Komatsu, E. J. Hall and M. Zaider, Oncogenic transformation by charged particles of defined LET. *Carcinogenesis* **9**, 747–750 (1988).
3. A. C. Riches, Z. Herceg, P. E. Bryant and D. Wynford-Thomas, Radiation-induced transformation of SV40-immortalized human thyroid epithelial-cells by single and fractionated exposure to gamma-irradiation *in-vitro*. *Int. J. Radiat. Biol.* **66**, 757–765 (1994).
4. A. C. Riches, Z. Herceg, P. E. Bryant, D. L. Stevens and D. T. Goodhead, Radiation-induced transformation of SV40-immortalized human thyroid epithelial cells by single exposure to plutonium alpha-particles *in vitro*. *Int. J. Radiat. Biol.* **72**, 515–521 (1997).
5. T. L. Morgan, D. J. Yang, D. G. Fry, P. J. Hurlin, S. K. Kohler, V. M. Maher and J. J. McCormick, Characteristics of an infinite life span diploid human fibroblast cell strain and a near-diploid strain arising from a clone of cells expressing a transfected v-myc oncogene. *Exp. Cell Res.* **197**, 125–36 (1991).
6. P. Thraves, Z. Salchi, A. Dritschilo and J. S. Rhim, Neoplastic transformation of immortalised human epidermal keratinocytes by ionizing radiation. *Proc. Natl. Acad. Sci. USA* **87**, 1174–1177 (1990).
7. K. Kuettel, P. Thraves, M. Jung, S. Varghese, S. Prasad, J. Rhim and A. Dritschilo, Radiation-induced neoplastic transformation of human prostate epithelial cells. *Cancer Res.* **56**, 5–10 (1996).
8. T. K. Hei, C. O. Piao, J. C. Willey, S. Thomas and E. J. Hall, Malignant transformation of human bronchial epithelial cells by radon-simulated α particles. *Carcinogenesis* **15**, 431–437 (1994).
9. N. W. Kim, M. A. Piatyszek, K. R. Prowse, C. B. Harley, M. D. West, P. L. Ho, G. M. Coviello, W. E. Wright, S. L. Weinrich and J. W. Shay, Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**, 2011–2015 (1994).
10. S. Bacchetti and C. M. Counter, Telomeres and telomerase in human cancer. *Int. J. Oncol.* **7**, 423–432 (1995).
11. J. W. Shay and W. E. Wright, Telomerase activity in human cancer. *Curr. Opin. Oncol.* **8**, 66–71 (1996).
12. J. W. Shay and S. Bacchetti, A survey of telomerase in human cancer. *Eur. J. Cancer* **33**, 787–791 (1997).
13. A. Bodnar, M. Ouellette, M. Frolkis, S. Holt, C. Chiu, G. Morin, C. Harley, J. Shay, S. Lichtsteiner and W. Wright, Extension of life span by introduction of telomerase into normal human cells. *Science* **279**, 349–352 (1998).
14. C. Morales, E. Holt, M. Ouellette, K. Kaur, Y. Yan, K. Wilson, M. White, W. Wright and J. Shay, Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. *Nat. Genet.* **21**, 118 (1999).
15. X. Jiang, G. Jimenez, E. Chang, M. Frolkis, B. Kusler, M. Sage, M. Beeche, A. Bodnar, G. M. Wahl and C. Chiu, Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. *Nat. Genet.* **21**, 111–114 (1999).
16. W. C. Hahn, C. M. Counter, A. S. Lundberg, R. L. Beijersbergen, M. W. Brooks and R. A. Weinberg, Creation of human tumour cells with defined genetic elements. *Nature* **400**, 464–468 (1999).
17. T. Mossman, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63 (1983).
18. F. Mitelman, Ed., *An International System for Human Cytogenetic Nomenclature.* S. Karger, Basel, 1995.
19. A. K. Walch, H. F. Zitzelsberger, M. Aubele, M. Bauchinger, S. Candidus, H. W. Prauer, M. Werner and H. Hofler, Typical and atypical carcinoid tumors of the lung are characterized by 11q deletions as detected by comparative genomic hybridization. *Am. J. Pathol.* **153**, 1089–1098 (1998).
20. H. Zitzelsberger, U. Kulka, L. Lehmann, A. Walch, J. Smida, M. Aubele, T. Lorch, H. Hofler, M. Bauchinger and M. Werner, Genetic heterogeneity in a prostatic carcinoma and associated prostatic intra-

epithelial neoplasia as demonstrated by combined use of laser-microdissection, degenerate oligonucleotide primed PCR and comparative genomic hybridization. *Virchows Arch.* **433**, 297–304 (1998).

21. O. P. Kallioniemi, A. Kallioniemi, J. Piper, J. Isola, F. M. Waldman, J. W. Gray and D. Pinkel, Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. *Genes Chromosomes Cancer* **10**, 231–243 (1994).

22. S. Solinas-Toldo, C. Wallrapp, F. Muller-Pillasch, M. Bentz, T. Gress and P. Lichter, Mapping of chromosomal imbalances in pancreatic carcinoma by comparative genomic hybridization. *Cancer Res.* **56**, 3803–3807 (1996).

23. E. Schröck, S. du Manoir, T. Veldman, B. Schoell, J. Wienberg, M. A. Ferguson-Smith, Y. Ning, D. H. Ledbetter, I. Bar-Am and T. Ried, Multicolor spectral karyotyping of human chromosomes. *Science* **273**, 494–497 (1996).

24. H. Zitzelsberger, L. Lehmann, L. Hieber, H-U. G. Weier, C. Janish, J. Fung, T. Negele, F. Spelsberg, E. Lengfelder and M. Bauchinger, Cytogenetic changes in radiation-induced tumors of the thyroid. *Cancer Res.* **59**, 135–140 (1999).

25. N. R. Lemoine, E. S. Mayall, T. Jones, D. Sheer, S. McDermid, P. Kendall-Taylor and D. Wynford-Thomas, Characterisation of human thyroid epithelial cells immortalised *in vitro* by simian virus 40 DNA transfection. *Br. J. Cancer* **60**, 897–903 (1989).

26. R. R. Reddel, S. E. Salghetti, J. C. Willey, Y. Ohnuki, Y. Ke, B. Gerwin, J. F. Lechner and C. C. Harris, Development of tumorigenicity in simian virus 40-immortalized human bronchial epithelial cell lines. *Cancer Res.* **53**, 985–991 (1993).

27. B. J. Christian, L. J. Loretz, T. D. Oberley and C. A. Reznikoff, Characterization of human uroepithelial cells immortalized *in vitro* by SV40. *Cancer Res.* **47**, 6066–6073 (1987).

28. H. Zitzelsberger, J. Fung, C. Janish, G. McNamara, P. E. Bryant, A. C. Riches and H. U. G. Weier, Spectral karytotyping analysis of heritable effects of radiation-induced malignant transformation. *SPIE Proc.* **3605**, 325–331 (1999).