Role of SV40 Integration Site at Chromosomal Interval 1q21.1 in Immortalized CRL2504 Cells

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Abstract

We have applied a functional gene transfer strategy to show the importance of viral integration site in cellular immortalization. The large tumor antigen of SV40 is capable of extending the cellular life span by sequestering tumor suppressor proteins pRB and p53 in virus-transformed human cells. Although SV40 large T antigen is essential, it is not sufficient for cellular immortalization, suggesting that additional alterations in cellular genes are required to attain infinite proliferation. We show here that the disruption of human chromosomal interval at 1q21.1 by SV40 integration can be an essential step for cellular immortalization. The transfer of a 150-kb bacterial artificial chromosome (BAC) clone, RP364B14, corresponding to viral integration site in CRL2504 cells, reverted their immortal phenotype. Interestingly, the BAC transfer clones of CRL2504 cells displayed characteristics of either senescence as shown by β-galactosidase activity or apoptosis as revealed by positive staining with M30 CytoDEATH antibody. The SV40 integration at 1q21.1, in the vicinity of epidermal differentiation complex (*EDC*) genes, resulted in the down-regulation of the *filaggrin* (*FLG*) gene that is part of the *EDC*. *FLG* gene expression was increased in BAC transfer senescent and apoptotic clones. Our results suggest that the disruption of native genomic sequence by SV40 may alter expression of genes involved in senescence and apoptosis by modulating chromatin structure. These studies imply that identification of genes located in the vicinity of viral integration sites in human cancers may be helpful in developing new diagnostic and therapeutic strategies. [Cancer Res 2009;69(19):7819–25]

Introduction

The mechanisms of neoplastic transformation have been addressed by using human cells immortalized with DNA tumor viruses or viral sequences, such as SV40 large T antigen (SV40-LT), human papillomavirus (HPV) 16 E6 and E7, and adenovirus E1A and E1B (1). A two-stage model consisting of M1 (mortality stage 1) and M2 (mortality stage 2) has been proposed for SV40-LT–induced immortalization of human cells (2). The cells challenged with SV40-LT bypass M1 stage and continue to multiply until they reach M2 stage (2). Subsequently, the entire cell population enters a state of crisis that is characterized by massive cell death through apoptosis or necrosis (3). However, a rare variant cell (1 in 10^7 to 10^8) survives the crisis state and continues to multiply indefinitely (2). These studies suggest that M1 and M2 are regulated through separate genetic controls, and immortalized cells have either inactivated or bypassed regulatory pathways involved in both M1 and M2 stages.

The presence of SV40 has been reported in some human tumors, such as mesothelioma, osteosarcoma, ependymomas (4–7), bronchopulmonary carcinoma, and nonmalignant pulmonary disease (8). Despite these observations, the role of SV40 in human cancers remains controversial. On the other hand, HPV has been detected in >99% of cervical cancer cases (9). Although the above observations suggest viral etiology of these cancers, the contribution of disrupted host genes due to integration of viral genome has not been addressed.

It has been shown that SV40-LT releases the host cell from the G_1 checkpoint (M1 stage) and extends its life span by sequestering pRB and p53 family of tumor suppressor proteins (10–12). In order for cell to attain indefinite growth potential, however, additional mutations in cellular genes are essential to bypass M2 stage (2). Although SV40 is known to integrate randomly at multiple sites in the host genome, the immortalized cells often show its integration at a unique site. We propose that the disruption of a specific genomic site due to the viral integration may contribute to malignant transformation of human cells. Furthermore, the integration of SV40 into a specific genomic site may promote genomic instability and likely confer growth advantage.

We have applied a functional approach to restore senescence in SV40 immortalized cells and investigated the role of the integration site in cellular immortalization. We have characterized the genomic region in the vicinity of the site interrupted by SV40 integration. The introduction of a genomic clone, corresponding to the genomic site disrupted by SV40 at 1q21.1, restores senescence or apoptosis in CRL2504, an immortalized cell line.

Materials and Methods

Cell Lines and Growth Conditions

A single-cell subclone of a human bronchial epithelial cell line, immortalized with a cloned Ori$^-$ SV40 (13), was used in these studies. CRL2504 cells contain a single integrated copy of SV40 genome and do not contain any free virus.[4] The cell lines used for comparison included normal human diploid fibroblasts GMO3468A (Genetic Mutant Cell Repository, Camden, NJ) and FS-2 (generated in the lab). The cell lines were routinely cultured at 37°C in a 7.5% CO_2 atmosphere in DF/12 medium supplemented with 10% fetal bovine serum containing 1% penicillin and streptomycin. The

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doi:10.1158/0008-5472.CAN-09-1003

[4] J. Liu and R.S. Athwal, unpublished observations.

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gene transfer clones carrying *neo* marker were isolated and maintained in the medium containing G418 (400 μg/mL).

Isolation of Human DNA Flanking SV40 Insertion Site by Inverse PCR

The *Eco*RI-digested CRL2504 DNA (400 ng) was circularized by overnight incubation at 16°C in an appropriate reaction buffer containing 2 μL of T4 DNA ligase (New England Biolabs). The circularized DNA was amplified with the primer pair SV40 1726F/796R specific to the SV40 sequences flanking the human genomic DNA. The amplification was performed in a 25 μL reaction mixture containing 5 μL of ligation products, 2 mmol/L $MgCl_2$, 200 μmol/L each of deoxynucleotide triphosphates, 1 μmol/L of each primer, and 1 unit of Taq polymerase (Promega). The reaction conditions included initial denaturation at 96°C for 5 min followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, and 2 min at 72°C. The amplified products were fractionated by electrophoresis in a 2% agarose gel, visualized by ethidium bromide staining, and purified from the gel for cloning.

Transfer of Bacterial Artificial Chromosome Clone into CRL2504 Cells

The bacterial artificial chromosome (BAC) clones RP11-364B14 and 152L6 were retrofitted with MJ0X166 to incorporate *neo* marker using a vector exchange procedure (14). Briefly, human DNA inserts from the BAC clones were released by digestion with *Not*I restriction enzyme (New England Biolabs) and separated by pulsed-field gel electrophoresis (PFGE). The purified inserts were mixed separately with *Not*I-digested and dephosphorylated retrofitting vector pJMOX166 at a molar ratio of 2:1 or 4:1 and ligated using T4 DNA ligase at 16°C for 16 h. An aliquot (1 μL) of the ligated products was transformed into competent DH-5α cells, and cells were plated on LB agar plates containing chloramphenicol (12.5 μg/mL) and kanamycin (30 μg/mL). The DNA isolated from the retrofitted clones was digested with *Not*I, fractionated by PFGE, and blotted onto a membrane. The blot was hybridized with DNA probes for the colocalization of human insert and pJMOX166. The presence of *neo* marker in the retrofitted BAC clones was confirmed by PCR.

The DNA from retrofitted BAC clones 364B14 and 152L6 was transferred into mammalian cells by electroporation using published procedure (15). Briefly, exponentially growing cells were harvested by trypsinization, washed twice in medium without serum, and suspended in 0.4 mL of serum-free medium at a density of 2.5×10^7/mL. The BAC DNA (5 μg) was added to the cell suspension, mixed gently, and transferred into a cuvette (Bio-Rad) for electroporation using 350 V and 500 μFb that produced a time constant in the range of 10 to 20 s (Bio-Rad Gene Pulser Apparatus with Capacitance Extender). The cells were placed on ice for 5 min immediately after electroporation and then plated on five tissue culture dishes (100 mm) in complete medium. The medium was replaced after 24 h with selection medium containing G418 (400 μg/mL) and refreshed every 2 d on a regular basis. A control experiment was also performed by transferring empty vector pJMOX166 into cells, and the transfected cells were selected as above.

Analysis of BAC Transfer Clones

The BAC transfer colonies that grew in selection medium were either isolated individually or followed in plates. The growth of individual colonies was monitored and their morphology was visualized using a phase-contrast microscope and photographed at regular intervals.

Staining for senescence-associated β-galactosidase activity. The cells in culture dishes were washed with PBS and fixed in 2% formaldehyde/0.2% glutaraldehyde at room temperature. The staining solution [1 mg/mL 5-bromo-4-chloro-3-indolyl β-galactosidase, 40 mmol/L citric acid/sodium phosphate (pH 6.0), 5 mmol/L potassium ferrocyanide, 5 mmol/L potassium ferricyanide, 150 mmol/L NaCl, and 2 mmol/L $MgCl_2$] was overlaid on fixed cells and incubated at 37°C for 16 h (16). The stained cells were visualized using an inverted phase-contrast microscope and photographed.

Bromodeoxyuridine incorporation assay for DNA replication. The cells (2,000 per chamber) in an eight-chamber slide (Nalgene Nunc International) were incubated for 24 h at 37°C, and then bromodeoxyuridine (BrdUrd; 10 μmol/L) was added to the medium. The incubation with BrdUrd was carried out for 24 h, and the cells were subsequently fixed for 45 min in a fixative solution (7 volumes of ethanol and 3 volumes of 50 mmol/L glycine). The fixed cells were hybridized with a 1:600 dilution of mouse anti-BrdUrd monoclonal antibodies (Molecular Probes), and signal was detected by staining with a 1:200 dilution of the Alexa Fluor 488–conjugated donkey anti-mouse IgG. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI), and cells were visualized under a fluorescence microscope.

Analysis for Apoptosis

CytoDEATH antibody M30 (Roche Diagnostic Corp.), which recognizes a specific caspase cleavage site within CK18, was used to detect apoptosis (17). The cells were seeded on eight-chamber slides (Nalgene Nunc International) and fixed in ice-cold methanol at −20°C for 30 min. The fixed cells were treated with 0.25% Triton X-100 (Sigma) and then incubated for 2 h with donkey serum (Molecular Probes). The slides were overlaid with a solution (100 μL) containing M30 antibody and incubated at 4°C overnight. The signal was detected by staining with a 1:200 dilution of donkey anti-mouse IgG linked with Alexa Fluor 488 (Molecular Probes), and cells were photographed using Olympus AHVT3 fluorescence research photomicroscope system.

Sequences of Primers

Sequences of various primers used are as the follows:

SV40-1726F:	5′-ACA GTT TAC AGA TGA CTC TCC-3′
SV40- 796R:	5′-TTG CAG TAA AGC TGC AAA TCC -3′
SV40-1745F:	5′-CCA GAC AAA GAA CAA CTG CC- 3′
SV40- 477R:	5′- CCG TCA ACA GTA TCT TCC CC-3′
H-F87:	5′- GAT CCC AAC TAA AAC ATC ACC- 3′
H-R484:	5′-AGC CCT TAT TGT TTA AAA GAC C-3′
SV2504-IS-F165:	5′-CTT CTG TTA TAT CAT TTG ACC C-3′
SV2504-IS-R453:	5′-TAC TGT TTC ATT CCT TGA GCC-3′
Neo-F1289:	5′-TCA CGA CGA GAT CCT CGC C- 3′
Neo-R1705:	5′-TTG TCA AGA CCG ACC TGT CC-3′
GAPDH-F420:	5′- AGA AGG CTG GGG CTC ATT TG 3′
GAPDH-R1080:	5′- TCC ACC ACC CTG TTG CTG TA 3′

Fluorescence *In situ* Hybridization

Normal human mononuclear cells from peripheral blood were cultured in the presence of 0.05 μg/mL phytohemagglutinin (Life Technologies), treated with 0.05 to 0.1 μg/mL of colcemid (Life Technologies) for 1 h, exposed to standard hypotonic solution (75 mmol/L KCl), and fixed in 3:1 absolute methanol/glacial acetic acid. Fixed cells were transferred to glass slides to prepare metaphase chromosome spreads. The metaphase chromosome spreads were hybridized with a mixture of 1 μg of biotin-labeled BAC DNA and 100 μg of human Cot-1 DNA (Clontech Laboratories). The detection of the hybridized BAC DNA, digital image acquisition, processing, and direct visualization on G-banded chromosomes were all carried out as described earlier (18).

Results

Cloning of Human DNA Sequence Flanking the SV40 DNA

A restriction map for the integrated p129-SV40 in CRL2504 was first deduced by Southern blot analysis of the DNA digested with *Bam*HI, *Bst*XI, *Eco*RI, *Kpn*I, *Pst*I, and *Pvu*II either individually or with a combination of two enzymes. The digestion of CRL2504 DNA with *Eco*RI generated 2.2- and 5.1-kb fragments that were linked to SV40 DNA. The inverse PCR with primer pair SV40-1726F/796R directed to 2.2-kb fragment of *Eco*RI-digested CRL2504 DNA generated a 1,200-bp amplicon. The origin of this fragment from the SV40-human DNA junction was confirmed by PCR using primer pair SV40-1745F/477R that was internal to the first set of primers. The 1,200-bp PCR product, designated as SV2504-IS, was

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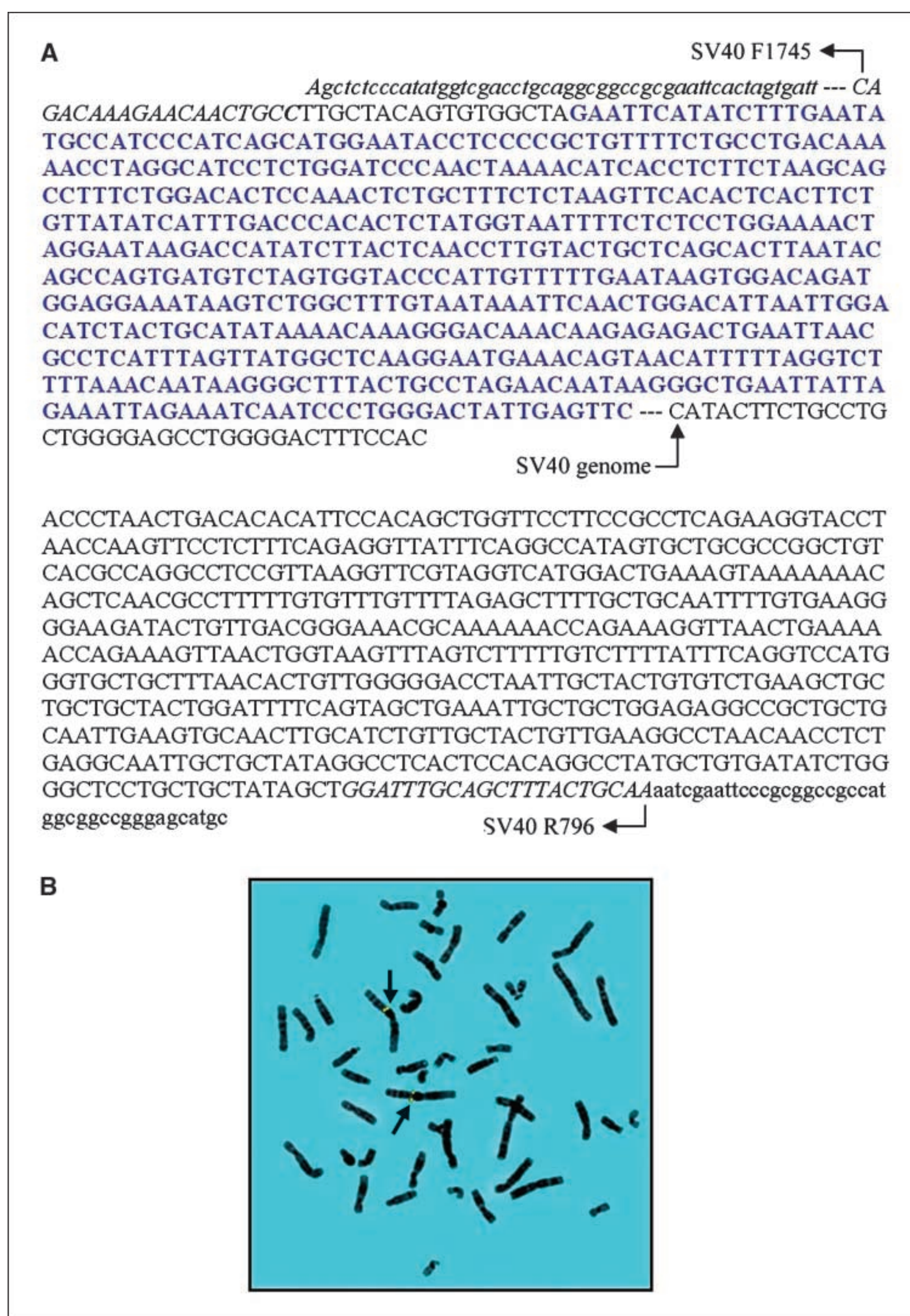

Figure 1. Sequence surrounding SV40 integration site and its chromosomal location. *A,* the human DNA sequence flanking the integration site was isolated by inverse PCR as described in Materials and Methods. *Arrows,* locations of SV40 primers used for amplification. *B,* primer sequences corresponding to the human DNA region were used to isolate the BAC clone RP11-364B14, and its location as determined by FISH on chromosome 1q21.2 is indicated by arrows.

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cloned into pGEM-T easy vector (Promega) and sequenced. The database search and BLAST comparison revealed this fragment to contain 552 bp of human DNA linked to 648 bp of SV40 sequence (Fig. 1*A*). The identity of the rescued DNA was further confirmed by PCR amplification of human DNA with primer pair H-F87/H-R484 designed from the recovered human DNA sequence. These primers amplified an expected 297-bp product from the human DNA template (data not shown).

Identification of a BAC Clone Corresponding to SV40 Integration Site in CRL2504 Cells

A human PCR-ready BAC library (Research Genetics) was screened with primer pair H-F87/H-R484 that led to the identification of a 115-kb clone RP11-364B14. This clone, obtained from Research Genetics, was found to represent the human-SV40 junction sequence. The position of the BAC was mapped to chromosome 1 by aligning the 552-bp cloned human DNA sequence against genome database. The location of the BAC at 1q21.1 was further confirmed by fluorescence *in situ* hybridization (FISH) to the chromosomes prepared from peripheral blood lymphocytes (Fig. 1*B*).

Functional Testing of BAC RP11-364B14 by Introduction into CRL2504 Cells

The *neo* marker containing retrofitted BAC clones RP11-364B14 and 152L6 (14) and empty vector pJMOX166 were transfected into CRL2504 cells by electroporation. The empty vector pJMOX166

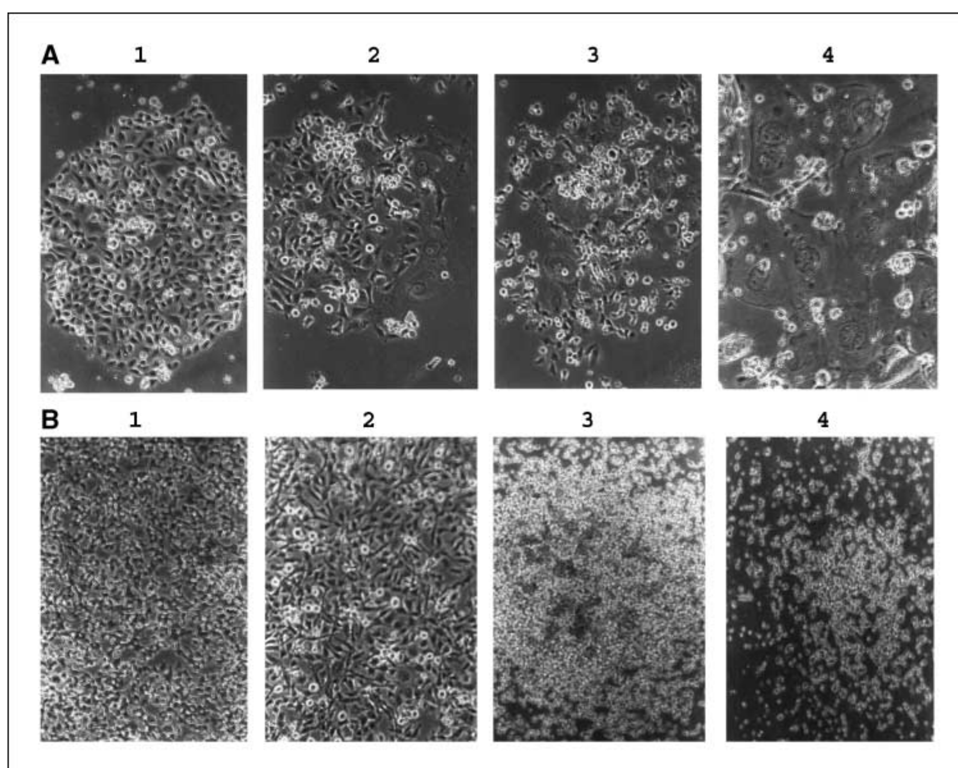

Figure 2. Morphology of senescent and apoptotic transfer clones. *A,* senescent transfer clones of CRL2504/BAC364B14 were grown and photographed after 21 d (*1*), 28 d (*2*), 35 d (*3*), and 42 d (*4*). *B,* CRL2504 cells 8 wk after transfection with the empty vector (*1*), 8 wk after transfer of an unrelated BAC 152L6 (*2*), 23 d after transfer of BAC364B14 (*3*), and 27 d after transfer of BAC364B14 (*4*) were photographed as described in Materials and Methods.

and BAC 152L6, known not to affect the growth of immortal cell lines,[5] served as control transfections. The BAC transfer colonies, which grew in G418 medium, were isolated individually into 60-mm dishes or followed in the plate. A total of 89 independent colonies were isolated following the transfer of RP11-364B14, and 50 colonies were isolated for each of the control transfections. The presence of the transferred DNA in these clones was ascertained by PCR using *neo*-specific primer pair F1289/R1705 (data not shown).

BAC transfer colonies and control colonies were classified as senescent, apoptotic, or immortal based on their growth characteristics and morphology. Eleven of 89 (12.4%) CRL2504/364B14neo colonies displayed characteristic senescent phenotype (Fig. 2*A*), 9 (10.1%) colonies became apoptotic (Fig. 2*B*), and 69 (77.5%) colonies retained the immortal parental cell phenotypes. Our data may be biased in favor of immortal colonies for the reason that some of the senescent colonies may have been eliminated at an early stage before being recognized and/or camouflaged by the fast-growing immortal colonies.

Analysis of BAC Transfer Colonies

Senescent colonies. Senescent colonies grew at a slow rate and the cells seemed enlarged, flattened, and highly vacuolated (Fig. 2*A*). The doubling time was assessed by counting cells at regular intervals either under a phase-contrast microscope or in photomicrographs. These cells displayed an initial doubling time of 30 to 36 hours that progressively increased to 4 to 7 days and terminated in complete growth arrest after a period of 6 to 8 weeks. These cells were attached to the plates, and their senescent phenotype was chemically detectable by the senescence-associated β-galactosidase (SA β-gal) activity (Fig. 3*B*).

[5] R.S. Athwal, unpublished results.

The lack of DNA replication, a hallmark of senescent cells, was assessed by BrdUrd incorporation in specific BAC transfer colonies and cells from control transfer experiments. The cells were subsequently stained with DAPI to visualize nuclei and determine the relative abundance of BrdUrd-positive cells. A small proportion (1–7%) of cells in the senescent colonies were positive for DNA replication, whereas >90% of cells in control cultures were undergoing DNA replication (Fig. 3*A*).

Apoptotic colonies. The proliferation rate for nine BAC transfer colonies was similar to the parental CRL2504 cells during the initial period lasting for about 21 to 30 days (data not shown). However, after ∼4 weeks, these cells became rounded, detached from the plate surface, and entered a phase of apoptosis (Fig. 2*B*). The apoptotic cells were collected from each colony and examined after staining with M30 CytoDEATH antibody (Roche Diagnostic). All nine colonies stained positive for M30 CytoDEATH antibody (Fig. 4), whereas colonies recovered from control experiments did not show any staining. These data show that BAC 364B11 induces apoptosis in CRL2504 cells.

Immortal colonies. The immortal *neo*-positive colonies recovered from 364B14neo transfer and control transfer experiments consisted of compact cells that were identical to untransfected CRL2504. The immortality of these colonies was confirmed by their ability to proliferate in culture beyond 15 passages. These cells did not show SA β-gal activity and were positive for BrdUrd staining (data not shown). Although the structure of integrated BAC in these cells was not characterized, we speculate that the BAC became rearranged or had lost DNA sequence during transfer or after integration into the host cell genome.

Expression of Filaggrin Transcript Is Altered in CRL2504 Cells

The chromosome interval at 1q21.1 encodes the epidermal differentiation complex (EDC) that consists of *S100A11, trichohyalin*

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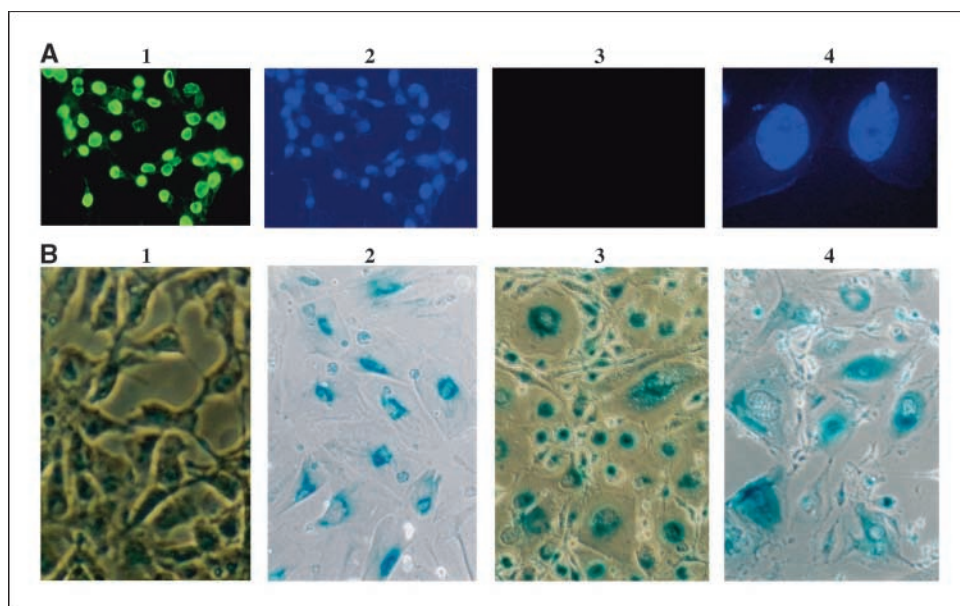

Figure 3. Visualization of cells after BrdUrd incorporation and SA β-gal assay. *A,* native CRL2504 cells (*1*) and BAC364B14 transfer clone 10 (*3*) were visualized with anti-BrdUrd antibody after growing the cells in the presence of BrdUrd. The nuclei of native cells (*2*) and the cells from BAC364B14 transfer clone 10 (*4*) were subsequently stained with DAPI. The cells were photographed as described in Materials and Methods. *B,* the cells from a confluent culture of native CRL2504 (*1*), GMO3468 cells after 62 passages (*2*), BAC364B14 transfer clone 10 after 7 wk (*3*), and BAC364B14 transfer clone sen II after 6 wk (*4*) were assayed for the presence of SA β-gal activity. The cells were photographed as above.

(*THH*), and *filaggrin* (*FLG*) genes. All three genes are expressed in terminally differentiated epithelial cells (19). A semiquantitative reverse transcription-PCR (RT-PCR) revealed that *FLG* transcript was expressed at very low levels in CRL2504 cells, whereas its expression was increased in the BAC transfer senescent cells (Fig. 5). These results suggest that *FLG* down-regulation may be associated with cellular immortalization.

Our data clearly show that the introduction of genomic DNA, corresponding to the viral integration site in SV40 immortalized cells, induces senescence and/or apoptosis in the parent cells. The results are consistent with the notion that the site of viral insertion in the host genome is important for immortalization of human cells. Our observations may help explain the virus-induced carcinogenesis in cancers such as cervical cancer.

Discussion

The transfer of a native 150-kb human BAC clone that spans the SV40 integration site at 1q21.1 in CRL2504 cells restores cellular senescence and/or apoptosis. The newly acquired senescent

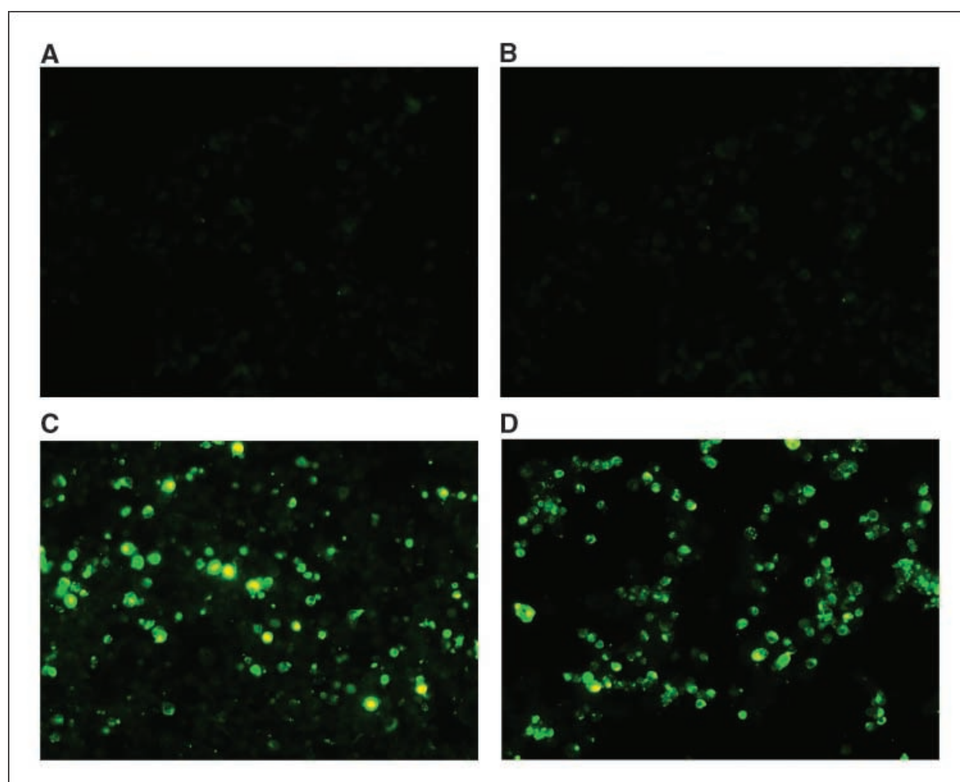

Figure 4. Staining of apoptotic cells with CytoDEATH M30 antibody. Native CRL2504 cells (*A*), vector transfer clone (*B*), BAC364B14 transfer clone (*C*), and floating cells from the dish containing BAC364B14 transfer clone (*D*) were stained with CytoDEATH M30 antibody and photographed as described in Materials and Methods.

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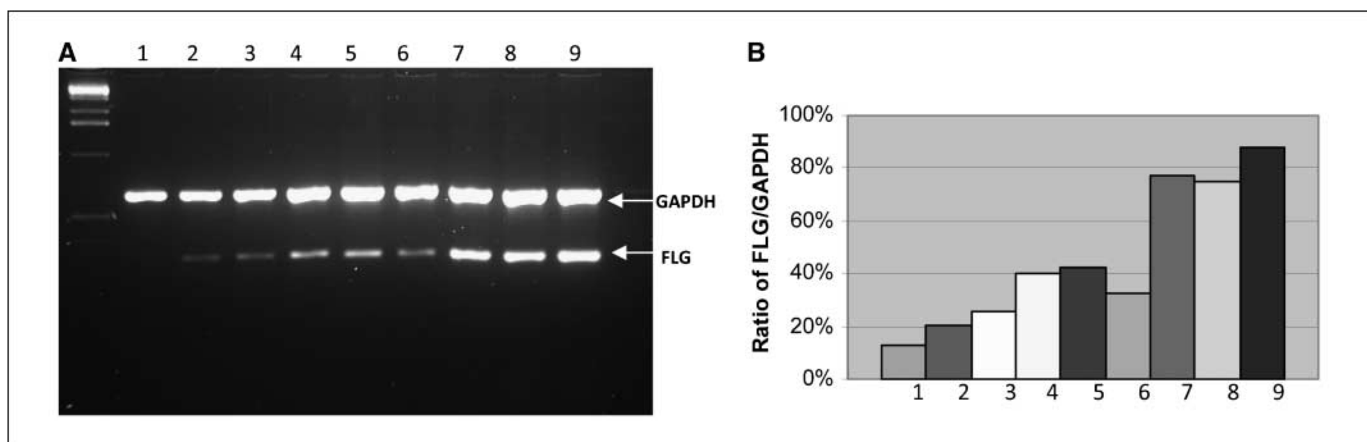

Figure 5. Semiquantitaive evaluation of *FLG* transcript by RT-PCR. *A,* RT-PCR was performed with *FLG*-specific primers and GAPDH primers on total RNA isolated from CRL2504 parent (*lane 1*), CRL2504/pJMOX166 vector control (*lane 2*), CRL2504/152L16 (*lane 3*), immortal CRL2504/364B14 clone 5.1 (*lane 4*), immortal CRL2504/364B14 clone 5.3 (*lane 5*), immortal CRL2504/364B14 clone 5.7 (*lane 6*), senescent CRL2504/364B14 clone 5.5 (*lane 7*), senescent CRL2504/364B14 clone 5.9 (*lane 8*), and senescent CRL2504/364B14 clone 5.17 (*lane 9*), and the amplified products were electrophoresed on an agarose gel as described. *Arrows,* amplified products. *B,* the *FLG* transcript was normalized by calculating the ratio of *FLG* and *GAPDH* products. The ratios are expressed as percentages.

phenotype in the transfer clones is likely due to the complementation of the loci disrupted by SV40 integration in CRL2504 cells. These findings support the hypothesis that integration of SV40 in the host genome may be a prerequisite for the acquisition and maintenance of the immortal phenotype in human cells. Furthermore, immortalization may likely involve altered expression of the senescence and/or apoptosis genes mapping proximal to the SV40 integration site.

Although SV40 T antigen–mediated inactivation of pRb and p53 pathways extends the life span of cells, these changes by themselves are not sufficient to promote infinite cell proliferation. To acquire infinite growth potential, the cells require additional mutations or altered expression of host genes. The disruption of the host genome structure due to the integration of SV40 sequences may contribute toward immortalization of host cells. However, the implication of SV40 integration site for immortalization is complicated by the presence of episomal virus that can self-replicate and cause multiple random integrations in the host genome (20–23).[2] The CRL2504 cells used in our studies carry a single integrated copy of the viral genome and contain no episomal viral particles (data not shown), thus making it feasible to address the role of a specific integration site in immortalization. We have clearly shown that functional reconstitution of the disrupted site is sufficient to restore normal growth pattern, senescence, and/or apoptosis in CRL2504 cells.

FRA1F, a common aphidicolin-inducible fragile site, maps near the SV40 integration site in CRL2504 cells. The disruption of fragile sites is known to mediate aberrant gene expression as evidenced by the amplification of *MET* oncogene and deletions of *FHIT* and *WWOX* tumor suppressor genes (24). Similarly, recombinant adeno-associated virus integrates at fragile sites harboring oncogene or tumor suppressor genes (25), and recombinant murine leukemia virus (MLV) has been found within *FRA11E* fragile site near the *LMO2* oncogene (26). The altered expression of specific genes is illustrated by integration of hepatitis B virus into *cyclin A* gene (27), MLV into transcription start sites (28), HIV-1 into transcriptionally active genes (29), TBLV near c-myc locus (30), and ecotropic MLV into *STAT5A* gene (31).

The SV40 integration site on chromosomal interval 1q21.1 in CRL2504 cells maps within the *EDC* (19) that comprises S100 proteins (32), cell envelop precursor proteins, and the fused-type proteins such as pro-*FLG* and *THH*-like 1 (33). These genes are clustered within 2.0 Mb of genomic DNA interval at 1q21.1. In particular, *S100A11* and *S100A10* genes, members of a large family of EF-hand calcium-binding proteins (34), are located in the close vicinity of BAC364B14. The altered expression of specific members of S100 family has been reported in colorectal cancers (35), breast cancer–derived metastatic axillary lymph nodes (36), and skin cancers (32). Interestingly, *S100A11* is one of the 376 genes specifically up-regulated during senescence in human fibroblasts (37). FLG, a major protein component of the keratohyalin granules of mammalian epidermis, has been implicated in several keratinizing disorders (38–40). We have shown relatively low expression of *FLG* transcript in CRL2504 cells as compared to in senescent BAC transfer clones. Our results conform to altered expression of *EDC* genes observed in cancers and other disorders and point to possible involvement of *FLG* protein in epithelial cell senescence. It is noteworthy that we have been unable to show the disruption of any specific gene through SV40 integration. The reduced expression of *FLG* transcript may be attributed to inactivation of one allele by alterations in a regulatory region upstream of the gene. Alternatively, disruption of the higher-order chromatin structure and epigenetic programming (41) mediated by SV40 insertion may also contribute to the decreased gene expression. Such decrease in transcript levels may lead to haploinsufficiency of the gene product as shown for tumor suppressor genes *Pten* and *Smad4/Dpc4* (42, 43).

The senescent and apoptotic phenotypes observed in BAC transfer clones of CRL2504 cells warrant a comparison of their biological relevance. The evolution of cellular senescence is considered to be a fail-safe mechanism against the risk for neoplastic transformation in normal cells. For this reason, the underlying significance of cellular senescence is quite similar to apoptosis. Whereas apoptosis eliminates potential cancer cells, cellular senescence irreversibly arrests their growth (44). The other similarities in underlying mechanisms may be drawn from the fact that the induction of both these phenotypes can be mediated by

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p53 and pRB pathways and a common set of stimuli, such as dysfunctional telomeres, DNA damage, disrupted chromatin structures, altered expression of certain oncogenes, and supraphysiologic mitogenic signals (44).

In conclusion, we have shown the importance of SV40 integration site in cell immortalization and characterized the ability of the native genomic sequence containing BAC clone to restore the senescent or apoptotic phenotype. Our results are consistent with the proposed ability of the integrated viral genome to modulate the chromatin structure and expression of senescence and apoptosis genes mapping to the chromosomal interval 1q21.1. These observations set the foundation for investigating the integration sites for other oncogenic viruses, such as HPV, and the role of disrupted genes in the development of human cancers.

Disclosure of Potential Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgments

Received 3/16/09; revised 7/24/09; accepted 8/6/09; published OnlineFirst 9/29/09.

Grant support: NIH grant CA74983, U.S. Army Breast Cancer Research Program grants DAMD17-99-1-9393 and DAMD17-02-1-0574, and Susan G. Komen Breast Cancer Foundation grants BCTR9830 and BCTR1092.

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