Malignant conversion of chemically transformed normal human cells

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ABSTRACT Two structurally unrelated chemicals, aflatoxin B1, and propane sulfone, transformed human foreskin cells to a stage of anchorage-independent growth. Isolation from agar and regrowth in monolayer culture of these transformed cells was followed by transfection with a cDNA library, which resulted in cells that exhibited an altered epithelioid morphology. Chemically transformed/nontransformed cells and transfected normal cells did not undergo a significant morphological change. These epithelioid-appearing, transfected cells, when inoculated into nude mice, form progressively growing tumors. The tumors are histopathologically interpreted as carcinomas. All of the first generation tumors in the surrogate hosts exhibited characteristic rates of growth similar to those of transplants of spontaneous human tumors. In the second generation of tumor xenografts, the progressively growing tumors derived from the transfected cells exhibited a more rapid rate of growth. Southern analysis and reverse transcription PCR confirmed that a 1.3-kb genetic element was integrated into the genome and was actively being transcribed. Examination of the metaphase chromosomes in normal human cells revealed that the genetic element responsible for this conversion was located at site 31–32 of the q arm of chromosome 7. The DNA sequence of this 1.3-kb genetic element contains a coding region for 79 amino acids and a long 3′-untranslated region and appears to be identical to CATR1.3 isolated from tumors produced by methyl methanesulfonate-converted, nontransplantable human tumor cells.

Since the initial reports in 1977 of the chemical transformation of human cells, several investigators have examined this process with varying levels of success (1–9). Treatment of human cells with a variety of chemical carcinogens—e.g., benzo[a]pyrene diol epoxide I, propane sulfone (PrS), aflatoxin B1 (AFB1), or N-methyl-N-nitro-N-nitrosoguanidine—will transform normal human fibroblast or epithelial cells into a stage in which the cells will grow in soft agar and produce localized, self-limiting nodules when injected into nude mice (1, 3, 5, 8, 9). However, the sequential process of human cell carcinogenesis from chemically transformed cells to fully malignant cells routinely appears abrogated in the early stages of progression, usually at the level of expression of anchorage-independent growth (AIG). In this article, we report the completion of the final stage in the tumorigenic process, development of cells that produce progressively growing tumors in a surrogate host.

We have previously isolated and characterized an AIG population of cells from human head and neck tumors that is similar in characteristics to the carcinogen-transformed normal human cells described above (9–12). In cells from some of these head and neck malignancies, the tumor-associated genes, c-myc and H-ras, exhibited an increased expression (9, 11, 12), and the presence of a mutation in the 12th/13th codon of H-ras was detected (ref. 9; H. Lee, C.S., and G.E.M., unpublished data). These tumors were characterized with respect to tumorigenicity in the nude mouse, and it was found that ~32% of the tumors will yield cells that clone in soft agar. Of these clones, ~40% have the capacity to produce progressively growing tumors in nude mice; the remainder of the clones either terminally differentiate in culture or produce cell lines that are not transplantable to nude mice. However, the presence or absence of mutations in the commonly described oncogenes or tumor suppressor genes did not appear to be causally related to either the nonmalignant AIG or the progressively growing tumorigenic stage in nude mice (12). In these spontaneously occurring human squamous cell carcinomas (SCC) tumors, <15% exhibit mutations in the ras genes, and mutations in the p53 gene are even more rare (Lee et al., unpublished data). Recently, we have found that a nontransplantable human cell line, derived from a human tumor with mutations in codons 126 of p53 and 12 of H-ras, had no additional mutations in these genes and no change in the level of H-ras mRNA following the conversion to a tumorigenic stage by methyl methanesulfonate treatment (refs. 9 and 14; Lee et al., unpublished data). Sequencing analyses of the DNA from this and other nonmalignant cell lines showed that the lesion in the 126 codon of the p53 gene was generally a nonsense/splice mutation; existence of the same type of lesion has been demonstrated in nonmalignant phenotypes isolated from different anatomical sites (Lee et al., unpublished data). Because these data are inconsistent with the causal role of oncogenes/tumor suppressor genes in the conversion to malignancy, it was suspected that other molecular changes must be involved.

Based on this assumption, a cDNA expression library was developed from the methyl methanesulfonate-converted tumor cells and used to transfect nontransplantable human cells derived from human SCC tumors (15); it was assumed that the cDNA library would contain copies of mRNA that were unique to the tumorigenic cells and responsible for expression of tumorigenicity. The transfected cells, when injected into nude mice, formed progressively growing tumors that increased in malignant vigor when serially passaged in vivo (ref. 15; Lee et al., unpublished data). From these tumors was isolated a genetic element, CATRI.3 (currently listed as CATRI in the Genome Data Bank), which was expressed in the malignant cells and derived from the cDNA expression library used for transfection. This cDNA library appeared to be responsible for imparting tumorigenicity to nonmalignant cells (15). Characterization/sequencing of the transfected genetic material has been accomplished (15), and by using specific vector sequences as "tags" (15), the chromosomal location of the relevant CATRI.3 genetic element was established. The DNA sequence of the CATRI.3 contains a coding region for 79 amino acids and a long 3′-untranslated region (15). As described (15), the nucleotide sequence of the CATRI.3 genetic element has not shown any homology with any known oncogenes or existing tumor suppressor genes (accession no. U25433, GenBank). Fluorescent in situ hybridization of meta-

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Abbreviations: AFB1, aflatoxin B1; AIG, anchorage-independent growth; FBS, fetal bovine serum; PrS, propane sulfone; RTPCR, reverse transcription PCR; SCC, squamous cell carcinoma.

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phase chromosomes indicated that the 1.3-kb fragment was localized on the q arm of chromosome 7 at site 31-32.

The purpose of the present study was to combine the methodologies for carcinogen-induced transformation of normal human cells to an AIG phenotype, the cDNA transfection-mediated tumorigenic conversion of nontransplantable human tumor cells, and the techniques for induction of tumors in nude mice to demonstrate the progression in vitro of normal human cells to fully tumorigenic cells, which heretofore had not been accomplished. Further, it was reasoned that this system would provide a means to evaluate the role of the newly discovered genetic element in the neoplastic process, in association with various oncogenes and tumor suppressor genes.

MATERIALS AND METHODS

Carcinogen Treatment. Human neonatal foreskins were minced into 1-mm² sections, digested with a 0.25% collagenase solution (13, 15), and seeded into 75-cm² flasks (1, 3). After passage, using 10,000 cells/cm², the cells were arrested in the G1 phase of the cell cycle by deletion of arginine and glutamine from IBR modified Dulbecco’s medium (3) supplemented with 10% dialyzed fetal bovine serum (FBS). The cell cycle block was relieved after 24 hr by feeding the cultures with complete MEM containing 10% FBS and 0.5 units of insulin (16, 17). Ten to 12 hr after release and 2-3 hr into early S phase, the carcinogens at their respective ED₅₀ dosages were added to the cultures and incubated overnight (18). The concentrations used were determined in other studies (19) – i.e., PrS at 20 µg/ml and AF₅₃ at 9 µg/ml. At the conclusion of the treatment period, the experimental medium was removed, the cells were washed three times with three volumes of MEM without FBS, and the cultures were then split at a 1:2 dilution in complete MEM supplemented with 8% amino acids, 2% vitamins, and 20% FBS. The cells were incubated in an air atmosphere enriched with 5% CO₂ at 37°C, and at each confluence, the cells were split 1:10 through 20 population doublings.

AIG. At the conclusion of the final 1:10 split outlined above, 50,000 cells in 1 ml were seeded into 2 ml of 0.5% soft agar and poured over a 5-ml 2.0% agar base. Both agar layers were supplemented with MEM and 20% FBS. These soft agar assays were incubated at 37°C in a 4% CO₂-enriched air atmosphere. Twenty-one days later, colonies >60 µm (AIG) were removed and seeded into growth medium in 75-cm² flasks. Cultures were propagated to yield >10⁷ cells for injection into nude mice for evaluation of tumorigenicity.

Tumorigenicity Assay. Male nude mice, 3–4 weeks of age, were splenectomized, and 24–72 hr after surgery, the mice were used to evaluate the tumorigenicity of the chemically transformed AIG and the transfected cells (1, 3). Cells were suspended in MEM at a density of 10⁷ cells per 0.5 ml and injected subcutaneously into the subcapsular or flank area of the mouse (14). Four to six mice were used for each cell type injected. Tumors >2.0 cm long were harvested for histopathological identification (3, 15).

cDNA Library Construction. In a recent publication, the construction of a cDNA library was described (15, 20). The expression cDNA library was made from SCC cells that had been converted to tumorigenicity in nude mice by methyl methanesulfonate (15). Initially, 5 µg of poly(A)⁺ RNA was used to synthesize cDNA using oligo(dT) primers and Superscript reverse transcriptase (GIBCO/BRL). The cDNA library was constructed by using the Librarian cDNA library construction kit (Invitrogen). Briefly, BstXI cloning linkers (5'-GAATTCCACACA/5'-GGGAGGATCC) were added to both ends of oligo(dT)-primed cDNA. The cDNA with linkers was then purified by cDNA spin column (Pharmacia) and ligated to the BstXI site of the eukaryotic expression vector pRC/RSV (Invitrogen). The ligation mixture was used to transform Escherichia coli strain DH10B. The cDNA library was plated out on Luria-Bertani (21) plates containing ampicillin at 50 µg/ml, after which 1.1 x 10⁶ colonies from primary plates were pooled in 200 ml of Luria-Bertani medium containing 7% (vol/vol) dimethyl sulfoxide and stored at -20°C as library stock.

Library Transfection Procedure. Plasmid DNA was prepared from the cDNA library as described elsewhere (20). The transfection was accomplished using LipofectAce (GIBCO/BRL) according to the manufacturer’s recommendations. Briefly, cultured, chemically transformed, AIG human cells at ~60% confluence were washed twice with 20 ml of PBS, and then 40 µg of LipofectAce and 30 µg of Sal I-linearized plasmid in 5 ml of serum-free medium were added to each plate. After overnight incubation, 5 ml of fresh medium containing 20% FBS was added. The plates were incubated for another 24 hr and the medium then replaced with fresh MEM containing 10% FBS. G418 (GIBCO/BRL) was added at 250 µg/ml for the selection of pRC/RSV-transfected cells. After 2 days, the cell cultures were split 1:4 in MEM with 10% FBS containing G418 at 250 µg/ml, and after 2 weeks the G418-resistant colonies were pooled and the cells grown to generate >10⁷ cells.

Reverse Transcription PCR (RTPCR). RTPCR amplification was carried out in a standard thermal cycler as described (15). The GeneAmp kit (Perkin-Elmer/Cetus) was used in the PCR process. The cloning linker-specific primer used in this PCR process was 5'-GCCAGTGGTTGGAATTC. The sample was incubated for 2 min at 95°C before addition of the Taq DNA polymerase.

FIG. 1. (A) Photograph of a monolayer culture of normal cells ~2 days after seeding 10,000 cells/cm². The monolayer cultures were prepared within 72 hr after seeding 2 g of collagenase-digested foreskins. This photograph represents a typical culture with fibroblast-like normal cells at 30% density. (Magnification, x66). (B) Photograph of carcinogen-treated, cDNA transfected cells at 60% density. The small foci of epithelioid-like cells were selectively trypsinized and repopulated in monolayer culture for tumorigenicity evaluation. This morphology is similar to that of cells isolated and repopulated from first generation tumors. (Magnification, x66).
Table 1. Expression of AIG and tumor incidence of chemically treated/transfected cells

<table>
<thead>
<tr>
<th>Transforming condition</th>
<th>Concentration, μg/ml*</th>
<th>AIG colonies†</th>
<th>Transfected cell tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>20</td>
<td>30</td>
<td>Before‡ 0/8 After§ 2/14</td>
</tr>
<tr>
<td>AFB₁</td>
<td>9</td>
<td>50</td>
<td>0/10</td>
</tr>
<tr>
<td>Normal cells</td>
<td>0</td>
<td>0</td>
<td>0/18</td>
</tr>
<tr>
<td>Transfected normal cells</td>
<td>0</td>
<td>&gt;100</td>
<td>0†</td>
</tr>
</tbody>
</table>

*Treatment concentration represents the ED₅₀ value for each chemical.
†The values in this column represent the number of colonies ≥60 μm formed in 21 days per 10⁶ seeded treated cells.
‡Tumor incidence of chemically treated cells selected from soft agar growth. Cells formed small nodules in the nude mouse that regressed within 96 hr after injection of 10⁷ cells.
§Incidence of tumors >1.0 cm at the primary site of injection. Chemically transformed/transfected cells were injected subcutaneously into the flank area of 4-week-old male nu/nu mice.
†Transfected cells can grow in soft agar, but the isolated colonies fail to grow to sufficient numbers in culture for tumorigenicity testing.

The amplification cycle used was 1 min at 95°C, 1 min at 56°C, and 2 min at 72°C through 35 cycles; 1/50 of the reaction mixture was used for a second round of PCR using CATRI.3 2F and CATRI.3 2R as primers.

**Southern Analysis.** DNA isolation, electrophoresis of the samples, and the hybridization protocol were as described (15). Briefly, 10 μg of restriction enzyme-digested genomic DNA was loaded per lane. The probes were labeled by random priming and a 1 × 10⁶ cpm/ml labeled probe was used for hybridization.

**Chromosomal Localization of the CATRI.3 Genetic Element.** Metaphase preparations of human cells were made according to standard cytogenetic procedures with and without mitotic inhibitors. Briefly, cells were harvested during logarithmic phase growth, placed in hypotonic KCl (0.075M), centrifuged, fixed in methanol/glacial acetic acid (3:1), dropped onto microscope slides, air-dried, and stored at −20°C. For the fluorescence in situ hybridization procedure, slides were removed from the freezer, treated with 100 μg of RNase per ml of 2× standard saline citrate (SSC, pH 7.0) for 1 hr at 37°C, rinsed 3 times for 10 min each in 2× SSC, dehydrated in a series of room temperature ethanol washes from 50 to 100% ethanol, and air-dried. The chromosomal DNA was then denatured at 70°C with formamide/2× SSC (70:30) at pH 7.0 for 3 min without agitation. The slides were then quickly rinsed in ice-cold 2× SSC and dehydrated with 50%, 75%, and 100% of ice-cold ethanol (22).

For hybridization, 10–15 ng of the digoxigenin-labeled CATRI.3 cDNA or 2 ng of the Oncor chromosome 7 alpha-satellite probe D7Z1, or a mixture of the two for simultaneous detection, were denatured in hybridization solution (formamide

![Image](image.png)

**Fig. 2.** (A) A typical tumor observed on nude mice that is reported in Table 1 of the text. The tumor length was in excess of 3.0 cm and represents a progressively growing tumor on the mouse that developed within three months of receiving an injection of 5 × 10⁶ cells of either PrS or AFB₁ chemically transformed/AIG cDNA transfected cells. (B) An 8-μm fixed cross section of one of the AFB₁/transfected cell malignant tumors stained with hematoxylin and eosin. The section was prepared from a 3.0-cm tumor growing in the subcutaneous flank region of a nude mouse. Several tumors were collected for histopathological examination from this and other carcinogen treatments. All tumors were quite similar in histological appearance. Microscopically, the tumor features polygonal, somewhat cuboidal, cells. The cells in this tumor, and others, appear to sit in an interstitial connective tissue background. The tumor cells have a prominent nucleus with minimal cytoplasm. They grow cohesively in a strand and cord arrangement. The interstitial connective tissue consists of elongated fibroblasts and delicate collagen bundles. Similar features were observed in two other AFB₁/cDNA library converted malignancies and in several PrS/transfected cell malignant tumors. (Magnification, ×40).
and dextran sulfate in Denhardt's solution containing salmon sperm DNA), and 20 μl of the solution applied to the slides. The slides were incubated overnight, washed 4 times, transferred to PBS, and held at 4°C until used. The presence of the incorporated dUTP–digoxigenin on the CATRI.3 insert was detected by fluorescein isothiocyanate–sheep anti-digoxigenin and counterstained with propidium iodide. Fluorescence was implemented by exciting the conjugated dye with a Zeiss epifluorescent excitation using a passband filter for fluorescein isothiocyanate at 490–520 μM (21).

RESULTS
Two chemicals, AFB₁ and PrS, were used to transform normal human cells (Figure 1A) to an anchorage-independent stage. The resultant transformed phenotypes at 20 population doublings exhibited AIG (Table 1). Six to 20 colonies per condition were combined in a 75-cm² flask and repopulated to yield >10⁷ cells. Cells (5 × 10⁶) in 0.5 ml were injected subcutaneously into the flank or subscapular area of the nude mouse. These injections resulted in formation of small local nodules but not in the formation of progressively growing tumors (>1.0 cm long). Most of the nodules that formed within 72 hours after receiving the cell inoculum regressed.

The sister cultures of the AIG chemically transformed cells were transfected with the cDNA expression library containing the CATRI.3 genetic element (15). After removal of the selection agent, epithelial-like cells developed among the surviving fibroblast-like cells (Fig. 1B). These cells were isolated and propagated in culture, and 5 × 10⁶ cells were inoculated into nude mice. Populations of chemically transformed cells transfected with the cDNA library produced progressively growing tumors within 3 months after cell injection (Table 1). Normal cells transfected with the cDNA library would grow in soft agar, but when isolated and seeded into a culture flask, cells did not proliferate sufficiently to test for tumorigenicity.

Two separate cultures of AFB₁ chemically transformed cells, that were transfected with the cDNA library, when injected into nude mice, formed eight tumors in all four mice. PrS-

![Fig. 3](image-url)

**Fig. 3.** A metaphase spread of normal foreskin cells. A digoxigenin-labeled (21) CATRI.3 cDNA probe was hybridized with metaphase chromosomes prepared from monolayers of human neonatal foreskin cells. The chromosomal localization of the 1.3-kb genetic element was detected using fluorescein isothiocyanate-conjugated rabbit anti-digoxigenin. The cells were then counterstained with propidium iodide. The localization of the signals were observed on the distal long arms of two of the number 7 chromosomes. Specifically, the signals were localized on sister chromatids in band 7q31–32 (inset i). This localization was determined after comparison with banding patterns observed after 4',6-diamidino-2-phenylindole staining (inset ii) of the same chromosome.

![Fig. 4](image-url)

**Fig. 4.** Idiogram representing the location of the CATRI.3 genetic element on the chromosome identified by in situ fluorescence in Fig. 3. The 1.3-kb segment is localized on the q arm of chromosome number 7, at the border of bands 31 and 32 (arrow).
The same localization of the 1.3-kb genetic element was found in the PrS-transformed/transfected cells as shown with normal cells in Fig. 3. The location of the 1.3-kb genetic element in these cells was verified as being on chromosome 7, due to the unique staining with the fluorescein isothiocyanate-conjugated probe for the chromosome 7 satellite centromere. An increase in the number of chromosomes was observed in both the AFB1 and PrS carcinogen-transformed/transfected cell-induced tumors that were classified as carcinomas. As discerned by Southern analysis, the expression library vector is integrated into the genomic DNA (Fig. 5). The integration of the cDNA library construct was further verified by demonstration of expression of the neo selection gene in tumor cell lines by RTPCR (Figure 6A). Also by RTPCR, it was observed that the 1.3-kb DNA fragment was integrated and actively transcribing mRNA (Fig. 6B) in all six cell lines isolated from tumors. This would imply that the conversion was a specific event related to CATR1.3 and not a random event caused by other components of the cDNA library. Further evidence (data not shown) for the involvement of the 1.3-kb genetic element in this conversion process was demonstrated by transfection of the CATR1.3 cDNA into nontumorigenic cells and the conversion of these cells to tumorigenic cells.

DISCUSSION

Several years ago, we and others demonstrated that human cells treated with chemical carcinogens in early S phase (0–4 hr after S phase entry) exhibited AIG (1–8) and formed localized tumors of limited growth potential in a surrogate host. These localized tumors rarely developed into progressively growing tumors [i.e., >1.0 cm long (19, 23)] and were compatible in appearance, but not in behavior, with a sarcoma (24) or an undifferentiated mesenchymal tumor (3). More often than not, transforming normal human cells to a progressively growing tumor stage could not be achieved (22, 25, 26). However, chemical transformation of human cells to a stage of AIG did result in the induction of a sarcoma tumor-associated, plasma membrane, cell-surface antigen that is identical to the antigen found on tumor cells isolated from primary human tumors (23).

Other attempts to use transfection procedures (27) have converted immortalized nontumorigenic cells to tumorigenic cells after transfection with an activated mouse oncogene, v-Ha-ras.

Fig. 6. RTPCR amplification of the expressed neo selection marker (A) and CATR1.3 (B), which are commonly expressed in six transfection-induced tumorigenic cell lines. One microgram of total RNA was used in each reverse transcription reaction. For neo gene expression, reverse transcription was performed using oligo (dT) as first strand primer. One-tenth of the reverse transcription reaction mixture was used for PCR amplification using primer Neo1 and Neo2, which would amplify an 831-bp neo-specific product. The expression of the neo gene further confirmed that the cells derived from the transfected cell-induced tumors are the same human cells transfected with the cDNA expression library (A). For CATR1.3 expression, reverse transcription was performed with a vector-specific downstream primer (RS2). One-fourth of the reaction was then used for PCR with vector-specific primers (RS1 and RS2) flanking the cDNA insert. To further identify CATR1.3, the reaction mixtures were subjected to a second round of amplification using CATR1.3-specific primers CATR1.3 2R and CATR1.3 2F. The expression of the 1.3-kb cDNA insert was a common feature in all transfected cell-induced tumors. Total RNA from untransfected normal human fibroblasts was used as a control. Very little endogenous CATR1.3 RNA was amplified in untransfected cells because of the use of vector-specific primers. In lane M, EcoRI- and HindIII-digested λ DNA was used as marker. The RNA origins are identified for each lane as follows: NHF, normal human fibroblast negative control; −, no RNA included as a second negative control; PCA1 and PCA2, two different tumor lines derived from the transfection of AIG cells transfected by PrS; and ACA1-4, four different tumorigenic cell lines derived from the transfection of AIG cells chemically transformed by AFB1. Arrow indicates the RTPCR product of CATR1.3 (B).

However, these transfected cells required 100–200 population doublings in culture before the oncogenic phenotype could be detected (28); other ras oncogene-vector sequences did not convert these cells to tumorigenicity (29). The mechanism of conversion to a tumor phenotype may be gene rearrangement, gene activation, amplification, overexpression, or mutations in tumor suppressor genes (12).

Many different nontumorigenic phenotypes from humans have been examined (12) for the existence of lesions in various oncogenes and tumor suppressor genes. We have found several inconsistencies between the presence of mutations in the p53 tumor suppressor, c-myc, and H-ras genes and the conversion of
nontumorigenic cells to progressively growing tumorigenic phenotypes—i.e., the nontumorigenic phenotypes prepared from human tumors were converted to the tumorigenic phenotype irrespective of the mutations in the different oncogenes or tumor suppressor genes. Some of these cells from human tumors had lesions in the c-myc and H-ras oncogenes; some had lesions in the p53 tumor suppressor gene; some had lesions in both the p53 tumor suppressor gene and oncogenes; and some had no detectable lesions (ref. 12, Lee et al., unpublished data). The nontumorigenic phenotype, SCC 83–01–82, with mutations in p53 and H-ras, could be converted to a tumorigenic phenotype by transfection with a cDNA library containing the CATRI.3 genetic element (15). Transfection of this same cDNA library into normal human cells has resulted only in the conversion to an AIG phenotype, and these cells, when isolated from agar and placed in culture, fail to replicate sufficiently for tumorigenicity testing (Table 1).

The 1.3-kb fragment maps to an area on chromosome 7 that is of current interest in cancer genetics. Chromosomal translocations and deletions involving 7q32 have been described in myeloplasia and acute myelogenous leukemia (30), leiomyosarcoma (31), and colorectal carcinoma (32). The exact mechanism of action of the CATRI.3 genetic element remains to be determined. However, it is our opinion, as expressed in our recent publication in Proceedings (15), that this CATRI.3 genetic element can be used as a tag in the transfection process to study the action of the relevant susceptible transforming genes in human cells. It may be that the CATRI.3 genetic element cooperates with other tumor-associated genes that have a spectrum of lesions in both oncogenes and tumor suppressor genes to establish a multidirectional pattern through the stages of malignant progression in human cells. Presently, this transfection procedure permits us to examine the process of tumorigenic conversion of normal cells in vitro from nontumorigenic AIG cells to fully malignant cells. Therefore, we believe that the acquisition of tumorigenicity must be mediated by either an alteration in the expression of CATRI.3 or an insertional activation or inactivation of a nearby specific gene.

A loss of heterozygosity on the q arm at site 31 on chromosome number 7 has been demonstrated in breast cancer cells, where a 41% loss of heterozygosity at 7q31 is associated with a poor prognosis in patients (13). These investigators concluded “that this region is associated with a tumor suppressor function and is a very early event in breast tumorogenesis.” They ascribe the activity associated with this area to be due to a deletion in DNA sequences. This area is also consistently correlated with the expression of human acute leukemia and myelodysplasia (30).

In conclusion, normal human cells that are successfully and reproducibly transformed to an AIG stage by chemical carcinogens can now be converted to an aggressive malignant tumorigenic stage by transfection with a cDNA expression library. This library was derived from a human tumor cell line that was converted to tumorigenicity following methyl methanesulfonate treatment. The genetic element CATRI.3 was the most commonly expressed mRNA in the tumorigenic cells (15). RTPCR analysis of the transfected cells confirmed the integration of the 1.3-kb insert into the genomic DNA, which continues to be expressed during serial passage of the transfected cells in nude mice.

Cloning, sequencing, and chromosomal localization of the 1.3-kb genetic element provide an excellent opportunity for investigation of the role of this genetic element, in concert with other oncogenes and tumor susceptibility genes, in human cell malignant progression. For the present, abrogation of the tumor suppressor gene functions and expression of AIG in human cells appear to be early events in this process. Now that we can identify and localize the genetic element to a specific chromosome, we can begin to examine the tumorigenic cells and the conversion process for molecular mechanisms associated with the CATRI.3 DNA fragment in the various stages of malignant progression.

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