Gene Expression, Vol. 8, pp. 129–139, 1999
Printed in the USA.

1052-2166/99 $20.00 + .00

Changes in Levels of Normal *ML-1* Gene Transcripts Associated With the Conversion of Human Nontumorigenic to Tumorigenic Phenotypes

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Evaluation of malignant human tumors in a xenobiotic nude mouse system has demonstrated that not all cells in tumors exhibit the capacity to form progressively growing tumors. However, nontumorigenic cells isolated from human tumors can be converted to a tumorigenic phenotype in nude mice by treatment with chemical carcinogens or by transfection with antisense to tumor suppressor genes. A newly discovered gene, designated *ML-1*, appears to be associated with tumorigenesis, because an *ML-1* antisense cDNA construct, transfected into nontumorigenic, anchorage-independent growth (AIG) cells, was sufficient to convert these cells into a tumorigenic phenotype. The AIG cells transfected with *ML-1* antisense cDNA constructs and converted to tumorigenic cells did not exhibit expression of normal *ML-1* mRNA transcripts in the converted cells when evaluated by Northern analysis, whereas premalignant and normal cells expressed *ML-1* transcripts at a high level. The converted cells exhibited a loss of growth control and produced tumors in a surrogate nude mouse that were greater than 2.0 cm in less than 2 months. The *ML-1* gene has a DNA sequence that is 2177 bp in size and is located on chromosome number 13 on the q arm at site 12-14. Sequence analysis and investigation of GenBank sequences indicate that this is a newly described human gene.

ML-1 Malignant conversion Antisense cDNA Chromosome 13

HEAD and neck squamous cell carcinomas (SCC) are common tumors that account for 6% of all solid tumors in humans (20). Although smoking and alcohol are often considered risk factors (2,5) for SCC of the oral cavity, the basis whereby epithelial cells acquire tumorigenic potential is not clear. At the molecular level, the activation of oncogenes and the inactivation of tumor suppressor genes are believed to play major roles in tumorigenicity (4). The most commonly altered genes in SCC are c-*myc*, *p53*, and H-*ras*, including an increased expression of c-*myc* (23) and mutations in *p53* and H-*ras* (1,7,29). We have previously isolated and characterized several cell lines derived from human head and neck tumors that were similar in characteristics to carcinogen-transformed normal human cells (15,16,24,25) in that they formed colonies in soft agar (AIG), but failed to form tumors in nude mice. In these cell lines, c-*myc*, *p53*, and H-*ras* expressions were increased (15,24,25) and mutations were detected in codon 12/13 of H-*ras* and codon 126 of *p53* (9,15,24). We have extensively investigated one of these cell lines, designated SCC 83-

Received February 8, 1999; revision accepted June 7, 1999.
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01-82, that was derived from a human oral squamous carcinoma. If treated with a chemical mutagen, such as methyl methanesulfonate (MMS), this cell line produced progressively growing tumors in nude mice (16). When this and other cell lines, with mutations in codons 126 of *p53* and 12 of H-*ras*, were converted to tumorigenicity by MMS, no additional mutations in these genes and no change in the level of H-*ras* mRNA expression were detected (9,16,24,25).

Despite an increasing number of newly discovered tumor susceptibility genes, the specific genetic events that result in squamous cell carcinomas are unknown. Complex interactions among many genes and different pathways are involved in the tumorigenesis of different human cell types. The majority of squamous cell carcinomas cannot be explained by alterations in existing tumor susceptibility genes; therefore, other genes must be associated with malignant progression and remain uncharacterized.

To identify the genetic factor(s) responsible for tumorigenic conversion of SCC 83-01-82 cells by MMS, a nondirectional expression cDNA library was constructed using total RNA isolated from the MMS-converted, tumorigenic cells and introduced into nontumorigenic SCC 83-01-82 cells by transfection (14). The transfected cells produced progressively growing tumors with a histopathology similar to that of the original patient tumor and to tumors produced by MMS-converted cells. From the library, extracted from the transfected cells, we recovered two major bands of 1.3 and 0.6 kb. The 1.3-kb band, designated *CATR1.3*, was directly associated with the cDNA library-induced tumorigenic conversion (10), because an antisense cDNA construct of this genetic segment would convert nontumorigenic cells from either human tumors or chemically transformed human cells into cells that were tumorigenic for nude mice (12). The *CATR1* gene is 3401 bp in size and is located on chromosome 7 on the q arm at site 31-32. Examination of the literature indicates that a number of different human cancers are associated with changes at this site on chromosome 7, such as head and neck malignancies, prostate cancer (19,33,34), breast tumors (32), T-cell lymphomas (3), and myeloid leukemia (21). All of the above malignancies have been found to have LOH or mutational changes at 7q31.

The presence of a second major band of 0.6 kb from the library suggested that another gene may have been involved in the malignant conversion process. This gene, designated *ML-1*, is approximately 2 kb in size and has no homologous sequences to the *CATR1* gene. Data presented herein demonstrate that the *ML-1* gene is located on chromosome 13 on the q arm at site 12-14. This region of chromosome 13 has been shown to be deleted in a number of diverse human neoplasms including: chronic lymphocytic leukemia, which frequently shows a loss of a region encompassing *BRCA2* at 13q12,3 (6) and familial and sporadic breast cancer with deletions at site 13q12-13 (31). There are also reports that describe structural rearrangements of 13q12-13 in atypical myeloproliferative disorder with T-cell leukemia and eosinophilia (28). Interestingly, LOH at 13q has also been described in head and neck squamous cell carcinomas, which appears to involve *BRAC2* (8), but other as yet unidentified candidate tumor suppressor genes may also be present in this region. *ML-1* appears to be one of the candidate genes in this region that exhibits a role in oral squamous cell tumor progression. The purpose of this investigation was to characterize and sequence the *ML-1* gene and to determine its role in tumorigenic conversion. The combination system of transfection-mediated expression cloning in SCC cells and tumorigenic selection in nude mice appears to be a useful way of isolating tumor susceptibility genes without prior knowledge of the gene family.

MATERIALS AND METHODS

cDNA Library Construction

A cDNA library was constructed using total RNA isolated from MMS-converted, tumorigenic, SCC-83-01-82CA (SCC-CA) cells in accordance with the instructions contained in the FastTrack mRNA kit from Invitrogen Corp. (Carlsbad, CA). Briefly, poly(A)+ RNA was selected from the total RNA obtained from the SCC-CA cells using the FastTrack mRNA kit according to the manufacturer's instructions. Five micrograms of poly(A)+ RNA was used to synthesize first-strand cDNA, using oligo(dT) primers and Superscript reverse transcriptase from GIBCO/BRL (Gaithersburg, MD). Then the BSTXI cloning linkers, 5′-GAATTCCACCACA and 5′-GTGGAATTC were added to both ends of oligo(dT)-primed cDNA. The cDNA with linkers was then purified using a cDNA spin column from Pharmacia (Bridgewater, NJ) and ligated to the BSTXI site of the eukaryotic expression vector pRC/RSV from Invitrogen. The ligation mixture was used to transform *Escherichia coli* strain DH10B. The organisms were plated out on Luria-Bertani plates containing ampicillin at 50 μg/ml as described in Radloff et al. (22) and the cDNA library was used to transfect the nontumorigenic squamous cell carcinoma cell line SCC-83-01-82. The transfection procedure is briefly described here: cultured cells at approximately 60% confluency were washed twice with 20 ml of phosphate-buffered saline (PBS) and then 40 μl of LipofectAce (GIBCO/BRL) and 30 μg of *Sal*I-linearized plasmid in 5 ml

of serum-free medium was added to each plate. After overnight incubation, 5 ml of fresh medium containing 20% FBS was added. The plates were incubated for another 24 h and the medium then replaced with fresh MEM containing 10% FBS and 250 μg/ml of G418 (GIBCO/BRL) 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 $>10^7$ cells for tumorigenic evaluation.

Cloning and RACE Expansion of the ML-1 *Gene*

Either total RNA or polyadenylated mRNA was isolated from tumors induced by cells transfected with the cDNA library. An 0.5-μg sample of total RNA from the SCC-CA cells and the vector downstream primer RS2 were used in the reverse transcription reaction to produce the first strand cDNA on a standard thermal cycler according to the methods described in the GeneAmp RNA PCR kit obtained from Perkin-Elmer/Cetus (Norwalk, CT). One fourth of the reverse transcription reaction mixture was then used for PCR amplification with the cloning linker specific primer, 5′-GCCAGTGTGGTGGAATTC. The amplification cycle protocol was as follows: 95°C for 2 min, 95°C for 1 min, 56°C for 1 min, and 72°C for 2 min, through 35 cycles. One 0.6-kb reverse transcriptase RT-PCR product, designated 4J21 (*ML-1*), was subcloned into the BSTXI-digested eukaryotic expression vector pRC/RSV. Several clones were selected for the purpose of sequencing.

Sequencing of the ML-1 *Gene*

Double-stranded plasmid bearing *ML-1* cDNA was sequenced by using USB Sequenase from United States Biochemical (Cleveland, OH). An additional upstream cDNA sequence was cloned by 5′ RACE using Clontech Marathon RACE Kit (Clontech, Palo Alto, CA). The primers used in the RACE reactions were as follows: a) adaptor primer 1: 5′-CCATCC TAATACGACTCACTATAGGGC, and nested adaptor primer 2: 5′-ACTCACTATAGGGCTCGAGC GGC; b) first RACE gene-specific primer: 5′-GCTGCCCCGCAACAGCGT, and first RACE nested gene-specific primer: 5′-GAACAGCGTCGGGG GCGTCTTC; c) second RACE gene-specific primer: 5′-CTTGGTCACCCGGTTGTAGCTC, and second RACE nested gene-specific primer: 5′-GCTCCAGG CTCCATGAACCAAC.

Preparation of ML-1 *Expression Construct*

The *ML-1* cDNA was produced in the sense and antisense orientation as described above. The construction of vectors was accomplished by using an eukaryotic expression vector and ligating to the BstX1 site of the pRC/RSV promoter. Individual clones transfected with either the sense or antisense construct were verified for the orientation of the insert by restriction enzyme digestion patterns. The insert for this construct was amplified from 1 μg of total RNA using primer sets specific for human *ML-1* cDNA: forward: 5′-GACTCTCTGTGTGGCTTATGC, and reverse: 5′-GAGACCTCACAGAGCACAGTTG by RT-PCR. This amplified fragment was digested by Bsp120I and Not I and cloned into Bsp120I digested pcDNA3 (Invitrogen). Orientation was checked by restriction digestion and the insertional region was sequenced for correct in-frame expression.

ML-1 *Transfection of Cells and Tumorigenicity in Nude Mice*

Nontumorigenic SCC 83-01-82 cells were transfected with plasmids containing inserts in either the sense or antisense orientation. The sense strand construct is orientated in the direction in which the messages are transcribed and the antisense strand construct is in the opposite direction of the transcriptionally active sense strand. The transfection procedures of the *ML-1* constructs were carried out using Lipofectin according to the methodology described by GIBCO/BRL and in compliance with the manufacturer's directions. Two 10-cm plates of the nontumorigenic cells were grown to a 60% confluent density and used for the transfection experiments with each of the sense and antisense constructs. For each dish, 20 μl of Lipofectin and 10 μg of supercoiled plasmid were incubated in the selection medium overnight. The reactants were removed at that time and the cultures rinsed with 2 ml of complete MEM to gently rinse the cell sheet. The G418-resistant colonies, still attached to the surface of the dishes, were removed and pooled. *ML-1* transfected cells were harvested by trypsinization, washed, and resuspended in MEM at 2×10^7 cells per ml. Three- to 4-week-old male genotobiotic nude mice were splenectomized and then 10^7 cells were injected SC into their flanks as described (12,14). For each batch of cells, one to four sites on each mouse were injected. Tumors that were 1–2 cm in diameter were harvested for histological analysis and cell culture.

Southern Analysis

The integration of the *ML-1* pcDNA expression vector construct into tumor cell DNA was determined by Southern analysis. Genomic DNA from tumors, formed by injection of chemically transformed or SCC 83-01-82 cells transfected with *ML-1* constructs,

was isolated according to the procedures described in Maniatis (13). The DNA was digested to completion with appropriate restriction enzymes at an activity dilution of 5 units of enzyme per microgram of DNA. The recovered samples were electrophoresed on 0.8% agarose gels, transferred onto nylon membranes, and hybridized for Southern analysis (27). The multiprimer-labeled vector/insert (i.e., *pcDNA-ML-1* probe) was synthesized with a random primer labeling kit purchased from USB (Cleveland, OH). Labeled probe was added to the hybridization medium at a final concentration of 10^6 cpm/ml.

Northern Analysis and RT In Situ PCR of ML-1 *in Normal and Tumor Tissues*

The blot (MTC™) used in these studies was purchased from Clontech Inc. (Palo Alto, CA). Each lane of the blot contained approximately 2 μg of purified poly(A)+ RNA from normal human tissues. The probes were labeled by PCR using a pair of gene-specific primers (i.e., N045PE1: 5′-CCCCAGCTC TACAAAATC, and 4J21R2: 5′-CTTGAGAAAA AGCATACCCATGCG), and a probe at 1×10^6 cpm/ml was used in the RT in situ PCR hybridization protocol according to the method described by Nuovo et. al. (18). The tissue sections of the mouse tumors and/or tumors from human patients were prepared as 5-μm sections from paraffin-embedded tissue. The paraffin section was placed on Silane-coated glass slides and the paraffin removed by soaking in 60 ml of xylene for 5 min and then serially passaged into 60 ml of 100% ethyl alcohol (EtOH) for 5 min. The slides were air dried and prepared for protease digestion in the following manner in 20×5-mm boats. Each boat can handle four slides. The pepsin solution (4 mg of pepsin at pH 1.0 in 0.1 ml of 2N HCl and 1.9 ml of 3× water) was used to digest the tissue slices in the boats. After 4–5 min of digestion for the head and neck tumors, the digestion mixture was poured off and the tissue slices rinsed gently with 2 volumes of 3× water and the slides immersed in 3 ml of 100% EtOH and then air dried. These slides were then digested with 200 μl of DNase to remove any interfering cellular and genomic DNA. The DNase digestion solution was composed of 200 μl of DNase in 20 μl of 10× buffer (35 μl of 3 M sodium acetate, 5 μl of 1 M $MgSO_4$). Three volumes of 3× water were used to rinse the slides. The slides were dehydrated under 100% alcohol and then air dried for 1 h before use. The reverse transcription assay was carried out in the following manner: 80 μl of the digestion mixture was split up between four slides with digested tissue on the slide. This volume of reaction mixture contained: 8 μ of 5 mM $MgCl_2$, RT buffer at pH 7.0, dNTP mix with 4 μl of Hexamer primer diluted to 20 μl, 4 μl of RNase inhibitor, and 44 μl of 3× water. These slides were incubated on a cycler block and run for 1 cycle at 42°C for 45 min, then 25°C for 10 min, and lastly at 4°C for 24 h. The expression of the gene transcripts was detected in the digested tissue slices by first placing the prepared slides in 60 ml of 0.2% bovine serum albumin at 50°C for 10 min to block the digestion of the tissue. Antidigoxigenin alkaline phosphatase conjugate (5 μl) was then added and the slides incubated at 37°C for 30 min. This step was followed by adding to the reaction mixture 25 μl of NBT/BCIP (Boehringer Genie kit) to the wells. The slides were incubated in a humidified chamber at 37°C for 5 min, rinsed well with several volumes of 12°C tap water for 2–3 min, and the fluorescent tissues were counterstained with Contrast Red dye at full strength of the stain for 3–6 s. The slides were then incubated in 3× water for 1 min, dipped into 95% EtOH, 100% EtOH, Histoclear added to the slides, and coverslip mounted with Permount.

Cytogenetic Analysis

Metaphase preparations of human cells were made as described in Molecular Cloning (13) with and without colcemide as a mitotic inhibitor. Cells were harvested in hypotonic KCl (0.075 M), centrifuged at $850 \times g$ for 10 min, the pellet fixed in methanol/glacial acetic acid (3:1), an aliquot of fixed cells dropped onto a microscope slide, and the slides stored at 20°C until used. Localization of the *ML-1* gene was carried out by fluorescent in situ hybridization (FISH) in accordance with the manufacturer's recommendations (Oncor Inc., Gaithersburg, MD). The fixed cells were counterstained with propidium iodide and FITC-conjugated sheep antidigoxigenin and propidium iodide. The metaphases were visualized and photographed through a Zeiss Axioskop with an epifluorescent excitation using a pass band filter at 490–520 μm. Slides were restained with DAPI to determine the location of the probe signal on the banded chromosomes.

RESULTS

Tumorigenicity in Nude Mice

Sense and antisense constructs to the newly identified gene, *ML-1*, were prepared and transfected into a nontumorigenic cell line, SCC 83-01-82. The cell line was converted into a tumorigenic phenotype by the antisense, but not by the sense, cDNA construct. The cDNA construct appears to be part of the *ML-1*

FIG. 1. Nude mouse 6 weeks after receiving 10^7 cells in 0.01 ml of medium. The cells were transfected with an *ML-1* antisense cDNA construct and injected into a 6-week-old mouse as described in Materials and Methods.

gene that consists of 2177 bp. In three mice, multiple injections of 10^7 cells at four sites resulted in the development of 10 progressively growing tumors >2.0 cm in diameter within 2–3 months after injection (Fig. 1).

When the tumors were histologically evaluated, they were interpreted as squamous cell carcinomas (Fig. 2) and presented a histopathology consistent with that of the original patient tumor (i.e., a squamous cell carcinoma).

The sense transfected cells produced no tumors in the nude mice over the same 2–3-month time frame. An initial bleb of 0.4 cm formed within 48 h following the injection of these cells into mice, but no mass was visible after 5 days.

Sequencing of the ML-1 *Gene*

As presented in Fig. 3, the *ML-1* cDNA is 2177 bp in length. This cDNA encodes 617 amino acids and contains a polyadenylation site: ATTAAA-sequence and poly(A) at its 3′ untranslated region. A homology search in the GenBank revealed that this sequence is a previously unidentified cDNA in humans.

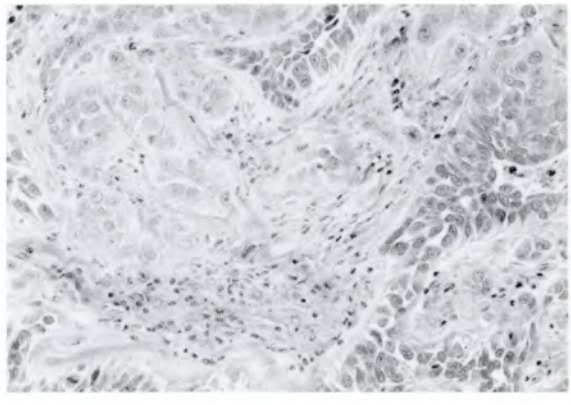

FIG. 2. Histopathology of a malignant tumor harvested 8 weeks after injection of transfected cells into the subscapular area of the nude mouse. The tumor measured over 2.0 cm in diameter. Notice that the tumor still exhibits its SCC origin histopathologically. H & E stain at an on-plate magnification of 250×.

ML-1 *Expression in Normal and Tumor Tissues*

Several different normal human tissue mRNAs (contained on blots from Clontech) were monitored for levels of expression of the *ML-1* gene mRNA using cDNA probes (Fig. 4). Northern analysis revealed detectable levels of expression of the sense transcripts in human brain and placenta (Fig. 4), whereas other tissues, such as heart, had no expression (Fig. 4), which suggests that *ML-1* expression may be tissue specific.

Restriction enzyme digests of the genomic DNA from tumors produced by *ML-1* transfected cells revealed multiple bands (Fig. 5) on the Southern plate that contained vector sequences, thereby indicating that the *ML-1* cDNA was truly integrated into the genomic DNA of the different tumors that were induced by *ML-1* transfected cells.

In all cases, the nontumorigenic cellular counterparts contained no *ML-1* construct vectors in the restriction enzyme digests. Examination of 8-μm sections of surgically excised SCC tumor tissue from patients indicated that the *ML-1* gene sense transcripts were severely depressed (Fig. 6A). Normal tissue from other sites of the same patients (Fig. 6D) showed normal distribution of the sense transcripts in lower layers in the skin. Detectable levels of the *ML-1* sense transcripts were not found in 10 human cell tumors produced in nude mice when evaluated by RT-PCR (data not shown).

Localization of the ML-1 *Gene*

Examination of 16 metaphase spreads using a digoxigenin-fluorescent stain revealed a consistent probe signal on the long arm of chromosome 13, localized at bands q12-14 (Fig. 7, upper and lower panels). As identified in the metaphase spreads, the lower panel of Fig. 7 presents the location of the *ML-1* gene on an ideogram.

DISCUSSION

Recently, we demonstrated that the transfection of antisense cDNA to a putative human tumor suppressor gene, *CATR1*, into the nontumorigenic SCC 83-01-82 cell line converted these cells into a tumorigenic phenotype (10). In addition, normal human cells that were transformed to an intermediate, non-

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  Total Sequence Length:         2177 bases
  Top Strand:                       1 sequences
  Bottom Strand:                    0 sequences
  Total:                            1 sequences
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 1..2177       /Note="Only_once"
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With Standard Genetic Code

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        65 Strongly Acidic(-) Amino Acids (D,E)
        245 Hydrophobic Amino Acids (A,I,L,F,W,V)
        158 Polar Amino Acids (N,C,Q,S,T,Y)

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        4.778 Charge at PH 7.0

        Total number of bases translated is 1842
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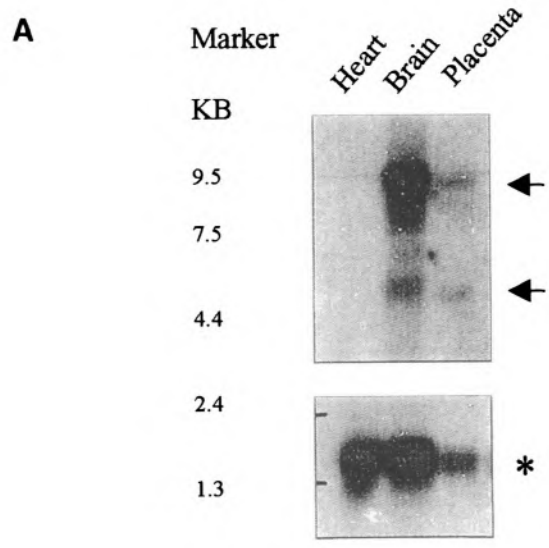

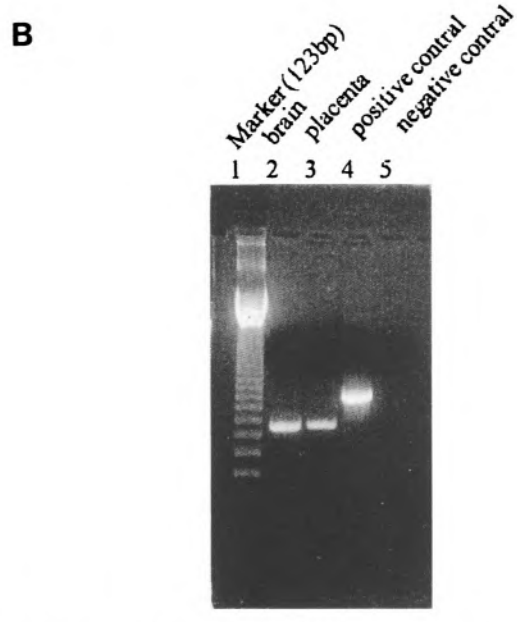

FIG. 4. Northern analysis of *ML-1* mRNA expression in normal human tissue. The blot (MTC™) used here was purchased from Clontech, Inc. Each lane of the blot contains approximately 2 μg of purified poly(A)+ mRNA from normal human brain and placenta. The probes were labeled by PCR using a pair of gene-specific primers and probed at 1×10^6 cpm/ml. Arrows indicate the location of the *ML-1* targets and asterisk (*) indicates the presence of *HPRT* that was used as a positive control (A). The data presented here were supported and confirmed by RT-PCR reaction and analysis of the products using G3PDH as a positive control (B). The primer mix was supplied by Clontech, Inc.

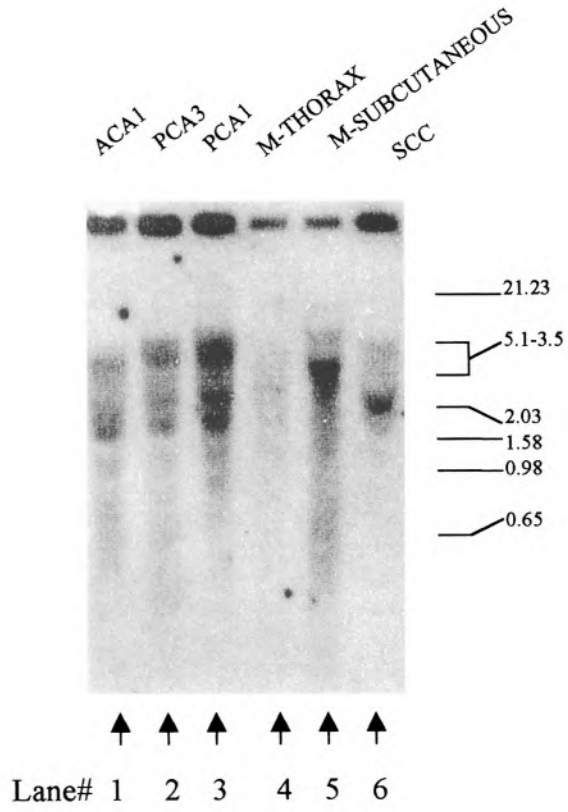

FIG. 5. Southern analysis of the genomic DNA from different *ML-1* transfected tumors excised from the nude mice. Ten micrograms of genomic DNA, previously digested with *Xho*I, was used in each lane and the membrane probed with the multiprimer labeled vector/insert (i.e., *pcDNA-ML-1*). The origin of the tumor cell DNA is indicated at the top of each lane (left to right: lane 1, aflatoxin B1 transformed cells transfected with antisense *ML-1*; lane 2, propane sultone transformed cells transfected with antisense *ML-1*; lane 3, second tumor from propane sultone transformed cells transfected with antisense *ML-1*; lane 4, thorax tumor induced by SCC cells transfected with antisense *ML-1* and injected into the tail vein; lane 5, subcutaneous tumor induced by SCC cells transfected with antisense *ML-1*; lane 6, SCC cells transfected with antisense *ML-1*). Markers for the analysis were *Eco*RI- and *Hin*dIII-digested lambda DNA.

malignant, AIG phenotype with chemical carcinogens were also converted to a tumorigenic phenotype when these cells were transfected with an antisense cDNA construct of the *CATR1* gene (12).

Analysis of diverse phenotypes isolated from human malignant tumors shows a lack of a consistent and causal relationship between the presence of mutations in several tumor suppressor genes and proto-

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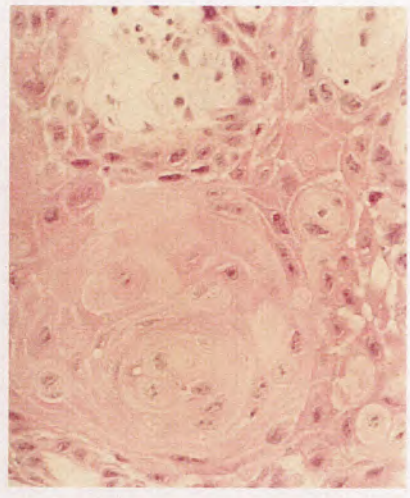
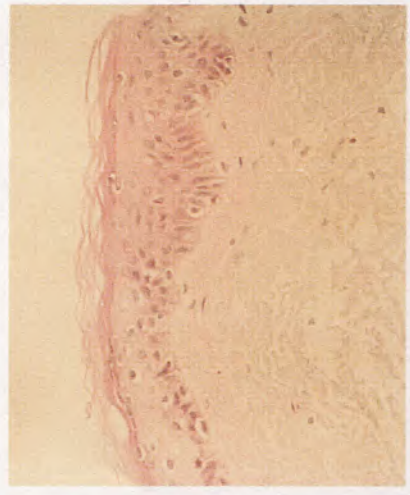
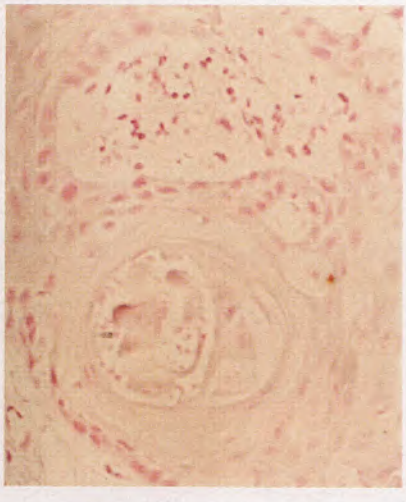
FIG. 3. Coding sequence of the nucleotide region on chromosome 13 site 12-13 and the peptide sequence. This human protein has previously not been identified in human cells. Both nucleotide and peptide sequences were used to search the GenBank data base, using the BLAST program. No homology with human cDNA was found except for partial homology with *BRAC2*.



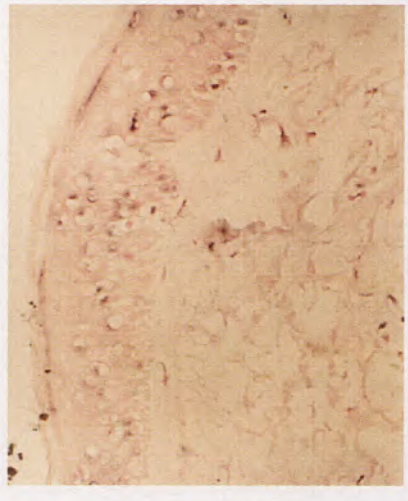
C



F

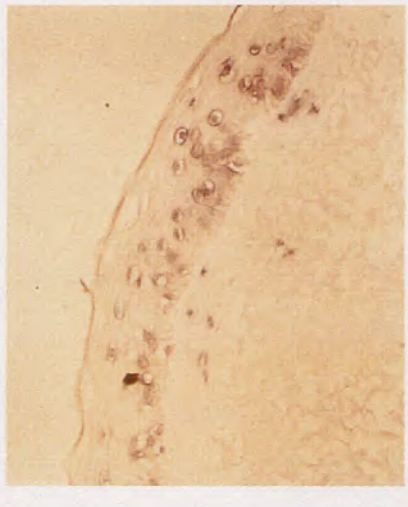


B

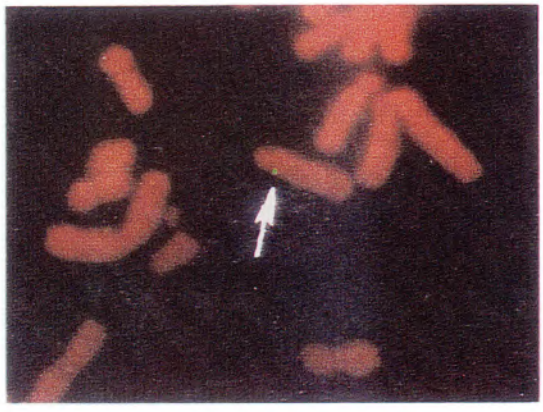


E

A



D



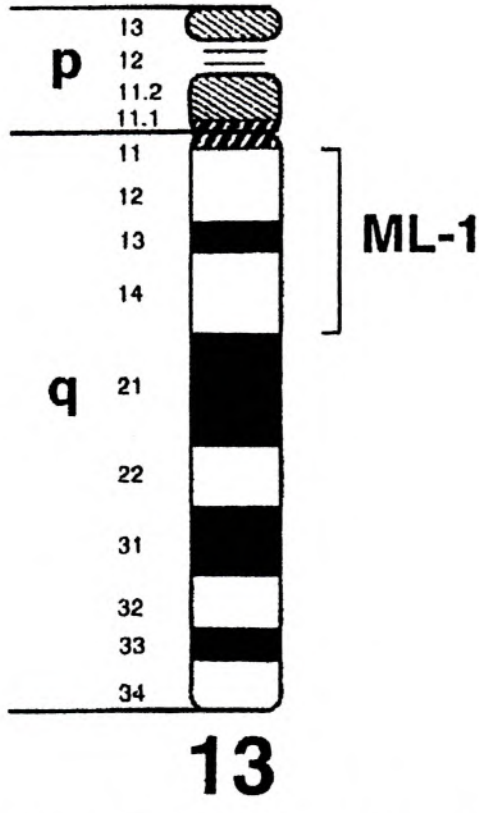


FIG. 7. The upper panel shows the location of the *ML-1* gene on human chromosome 13q by FISH. The identification of the *ML-1* gene was confirmed by hybridization of the alpha-satellite probe to the same chromosome at the centromere. The probe was localized to 13q12-13 following DAPI staining of the same metaphase (not shown here) to produce a banding pattern. The lower panel is an ideogram of chromosome 13 showing the approximate location of the *ML-1* gene (between the two arrows) on the chromosome.

oncogenes and the cells' ability to produce tumors in a surrogate host (9,15). Mutated H-*ras* oncogene is found inconsistently in many head and neck tumors and in chemically transformed normal human cells, which leads us to believe that both the frequency and occurrence of mutated H-*ras* is not correlated with the presence of SCC head and neck malignant phenotypes (9). The variability in the spectrum of mutations in tumor suppressor genes and oncogenes in nontumorigenic and tumorigenic SCC phenotypes strongly suggests that there may be more than one pathway leading to the development of SCC and that this cascade in progression may involve different loci on different human chromosomes. Our findings that both the *CATR1* gene and the *ML-1* gene (as described in this manuscript) are located on different chromosomes, in similar oral tumors with a similar pathology, strongly support this contention. Further, we have shown herein that a decrease in the expression of normal sense transcripts of the *ML-1* gene, following transfection of nontumorigenic cells with antisense *ML-1* cDNA constructs, was consistently associated with the establishment of a tumorigenic phenotype in nude mice. In confirmation, we have found results similar to those reported here in another human cell model system. In this model, EBV growth-transformed human cells were converted to B-cell lymphomas by transfection with *CATR1* cDNA antisense constructs (11), and it was found that the level of *CATR1* sense strand transcripts was greatly reduced in the B-cell tumors in contrast to that in the EBV growth-transformed lymphocytes (11). Naylor et al. (17) have shown that a loss of tumor suppressor loci on chromosome 9, due to a partial or complete loss of chromosome 9 or deletion at p21-22, is also associated with expression of the malignant stage of progression of head and neck tumors. Also, in some head and neck cancers a loss of heterozygosity of the *Rb* gene has been observed (30) and lesions in the *p53* gene were found in some head and neck tumors (26).

An interesting feature of the *ML-1* gene is that an antisense cDNA construct could convert nontumorigenic cells to a tumorigenic phenotype, whether the nontumorigenic phenotype was isolated from a heterogenous human tumor or from normal human cells transformed to a nontumorigenic, anchorage-indepen-

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FIG. 6. Photographs of RT in situ PCR showing depressed expression of the *ML-1* gene transcripts in tumor tissue. (A, B, C) Tumor tissue. (D, E, F) Normal tissue analyzed for *ML-1* expression. There is an absence of expression of the *ML-1* normal sense transcripts in tumor tissue (A) compared to normal tissue (D). (B) and (C) represent negative and positive controls for tumor tissue, respectively. (E) and (F) are of negative and postivie controls for normal tissue, respectively.

dent growth phenotype by chemical carcinogens. Lastly, the location of *ML-1* at the 13q12-14 site is intriguing, as this region of chromosome 13 has been shown to be deleted in a number of diverse human neoplasms. These include: chronic lymphocytic leukemia, which has frequently shown a loss of a region encompassing *BRCA2* at 13q12,3 (6), and familial and sporadic breast cancer at site 13q12-13 (31). There are also reports that describe structural rearrangements of 13q12-13 in atypical myeloproliferative disorder with T-cell leukemia and eosinophilia (28). Interestingly, LOH at 13q has been described in head and neck squamous cell carcinomas, which appears to involve *BRAC2* (8), but other unidentified candidate tumor suppressor gene(s) may also be present in this region. *ML-1* appears to be one of those candidate genes that exhibits a pivotal role in oral squamous cell tumor progression.

ACKNOWLEDGMENT

This work was supported in part by NIH/NIDR Grant 1PO1 DE12704.

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