Molecular Cloning and Characterization of an Antigen Associated with Early Stages of Melanoma Tumor Progression

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ABSTRACT

The melanoma-associated antigen ME491 is expressed strongly during the early stages of tumor progression. The ME491 gene was molecularly cloned by means of DNA-mediated gene transfer followed by screening a λ genomic library with repetitive Alu sequences as a probe. The cloned DNA, after transformation into mouse L-cells, generated a protein with characteristics that were indistinguishable in Western blot analysis from the ME491 antigen expressed by human melanoma cells. Repeat-free subfragments of the cloned DNA were used for further studies. By Northern blot analysis, the subfragments detected a single 1.2-kilobase mRNA in the transformants and various human melanoma cell lines. ME491 complementary DNA clones were then obtained by probing a melanoma complementary DNA library with the genomic subfragments. Nucleotide sequence analysis of the cloned complementary DNA indicated that the ME491 antigen consists of 237 amino acids (M, 25,475) with four transmembrane regions and three putative N-glycosylation sites. No significant structural homology was observed with other proteins thus far reported. We observed that the amounts of mRNA varied greatly with different melanoma cell lines. Southern blot analysis revealed no amplification or rearrangement of the ME491 gene in the human melanoma cell lines tested, including both high and low expressers of this antigen. The ME491 gene has been mapped to chromosome region 12p12→12q13 by somatic cell hybrid analysis and more narrowly localized to 12q12→12q14 by in situ hybridization.

INTRODUCTION

Because melanoma is a pigmented, cutaneous lesion, it has been possible to observe the development of the disease and to develop a detailed model of melanocytic tumor progression (1). A large number of MABs against human melanomas have been prepared to analyze the stages of progression (2–4). Most of these MABs bind to both melanoma and the benign melanocytic lesion, the nevus, but not to normal tissue melanocytes. There are also a few MABs that bind only to melanoma but not to dysplastic nevi (3). Although the tissue distributions and molecular weights have been determined for many of these MAAs, the biological functions and relevance to tumor progression are little understood. Among the human MAAs, only two antigens, M, 97,000 protein (4) and NGF receptor (5, 6), have been characterized thus far by means of gene cloning.

ME491 antigen is a MAA which is a marker for the early stages of tumor progression of human melanoma (7, 8). It is not detected on normal tissue melanocytes but is strongly expressed in dysplastic nevi and radial growth phase primary melanomas. The antigen expression, however, is weaker or sometimes even negative in more advanced stages of melanoma such as vertical growth phase primary melanoma and metastatic melanoma. Because of its stage-specific expression, this antigen has been considered as one of the interesting MAAs. ME491 antigen is also strongly expressed in adenocarcinomas of the colon and prostate, and to a lesser degree in some other human tumors (8, 9). It is detected even on undifferentiated colorectal carcinomas which lack most other gastrointestinal tumor-associated antigens (9).

Biochemical analyses have shown that ME491 antigen is a membrane-bound glycoprotein present both inside the cell and at the cell surface the molecular weight of which ranges from 30,000 to 60,000 with a single core protein of about 20,000 (10). This heterogeneity is apparently due to N-linked glycosylation since tunicamycin eliminates this heterogeneity. The NH₂-terminal amino acid sequence of ME491 antigen has been determined for the first 20 amino acids and shows no homology with known proteins (10). Other investigators have independently obtained MABs which detect very similar or identical antigens and have characterized them in pathological and clinical studies (11–13).

As a step toward understanding the significance of ME491 antigen for tumor progression, we have molecularly cloned the human gene and cDNA-encoding ME491 antigen. In the present study, we describe the molecular cloning, expression, and chromosomal localization as well as nucleotide sequence analysis of this antigen.

MATERIALS AND METHODS

DNA-mediated Gene Transfer to Mouse L-Cells

High molecular weight DNA was isolated from human melanoma cell line A875 and from human cervical carcinoma cell line HeLa, both of which express ME491 antigen. The DNA was cotransfected with purified herpes simplex virus thymidine kinase gene into Ltk" cells by the calcium phosphate precipitate method as described (5, 14). After selection in a 15 μg/ml-hypoxanthine-1 μg/ml aminopterin-5-Mg/ml thymidine medium, resulting tk" colonies were screened with an immunological rosette assay (5) in which cells are incubated with MAB to ME491 antigen and then a second antibody (rabbit anti-mouse IgG) coupled to erythrocytes. Positive colonies were cloned and grown in bulk to extract high molecular weight DNA. To reduce the amount of extraneous human DNA, a second round of transfection was performed using DNA extracted from one of the primary transformants (ME8).

Genomic DNA Library

High molecular weight DNA from one of the secondary transformants (ST2-3) was partially digested with Sau3A restriction enzyme and fractionated by 10 to 40% sucrose gradient ultracentrifugation (15). Fragments from 15–23 kilobases were ligated with both arms of EMBL 3 DNA (Promega Biotech, Madison, WI), packaged into phage particles using a commercial packaging extract (Giga-Pack Gold; Stratagene, Unpublished observations.)
San Diego, CA), and then inoculated onto Escherichia coli NM539 to make a genomic DNA library. This library was screened for human DNA sequences using human repetitive Alu sequences (16) as a probe.

Southern and Northern Blot Analyses

Total cellular DNA isolated from cultured cells and tissues was digested with appropriate restriction enzymes, fractionated by agarose gel (0.8 or 1%) electrophoresis, and transferred to nitrocellulose filter paper (15). To identify human DNA sequences present in the transformed mouse cells, the transferred DNA was hybridized with nick-translated 32P-labeled human repetitive Alu sequences (16) in 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, and 100 µg of salmon sperm DNA per ml at 42°C for 20 h. To identify ME491 sequences in human and mouse-hybrid DNA sequences, filters were hybridized to radiolabeled pMe1-17 DNA in the same hybridization mix without formamide at 65°C. The blots were washed with 0.2× SSC-0.1% SDS at 65°C unless otherwise stated, dried, and exposed to Kodak XAR-5 film at -70°C.

Polyadenylated RNA isolated from cultured cells was denatured, fractionated by electrophoresis through 1.5% agarose/formaldehyde gel, and transferred to nitrocellulose paper (15). Filters were hybridized with 32P-labeled probes under the same conditions used for Southern blot analysis.

Cell Extracts and Western Blot Analysis

Cultured cells were swollen in hypotonic buffer, lysed by freeze-thawing, and centrifuged at 100,000 × g for 30 min as described elsewhere (10, 17). The membrane-containing pellet fraction was extracted with 0.5% Nonidet P-40-140 mM NaCl-10 mM NaF-10 mM Tris-5 mM EDTA-100 U/ml aprotinin-1 mM phenylmethylsulfonyl fluoride, pH 7.5, for 30 min at 4°C. The sample was then centrifuged at 100,000 × g for 30 min, and the supernatant was analyzed by SDS-polyacrylamide gel (10%) electrophoresis. Fractionated proteins were transferred electrophoretically to nitrocellulose paper and incubated with mouse anti-ME491 MAb and then 125I-labeled goat anti-mouse IgG to visualize the antigen by autoradiography.

cDNA Library

Polyadenylated RNA was isolated from WM1158 melanoma cells by oligo-deoxymethylidate chromatography (18) and used to construct a cDNA library in λgt11 as described (19) with certain modifications. Briefly, 25 μg of polyadenylated RNA were used for the synthesis of the first strand using reverse transcriptase and oligodeoxymethylidate (chain length, 12–18 residues) as primer. Second strand synthesis was performed with E. coli DNA polymerase I and E. coli RNase H as described (20). The double strand cDNA was made blunt ended using T4 DNA polymerase and methylated with EcoRI methylase. Phosphorylated EcoRI linkers were ligated to the ends of the double strand cDNA, and excess linkers were removed by EcoRI endonuclease. The cDNA was ligated into the EcoRI site of λgt11 vector DNA, packaged in vitro, and inoculated onto E. coli Y1088 to make a cDNA library.

DNA Sequence Analysis

Cloned cDNAs were digested with appropriate restriction endonuclease to generate small fragments. The fragments were subcloned into M13mp18 and M13mp19 vectors (Bethesda Research Laboratory, Inc., Gaithersburg, MD). Single strand DNA was prepared, and the nucleotide sequences were determined by the dideoxynucleotide chain termination method (21). Both strands were then sequenced.

Chromosome Mapping of the ME491 Gene

Somatic Cell Hybrid Hybrid Analysis. Isolation, propagation, and characterization of parental cells and somatic cell hybrids used in this study have been described previously (22–24). DNAs extracted from the hybrids and the parental cells were subjected to Southern blot analysis using a subcloned DNA fragment (pMe1-17; 1.1-kilobase BamHI-EcoRI fragment) as probe. This probe has been shown to hybridize to ME491 mRNA.

For regional localization of the ME491 gene on chromosome 12, three mouse-human hybrids which had previously been observed to retain partial chromosomes 12 (22, 24) were used. The presence of a group of chromosome 12 DNA markers, including the ME491 gene, was determined in order to determine which parts of chromosome 12 were present or absent in the three hybrids. The chromosome 12-linked probes for T4, K-ras-2, homeobox gene 8, HPV 18 integration site flanking region 7.161, and insulin-like growth factor I have been described (22, 24–27).

In Situ Hybridization. Metaphase spreads were prepared from normal human male lymphocyte cultures stimulated with phytohemagglutinin for 72 h. The 1.1-kilobase subcloned DNA fragment (pMe1-17) was 3H-labeled by nick-translation and hybridized to the metaphase spreads as described elsewhere (26, 28, 29).

RESULTS

DNA-mediated Gene Transfer to Mouse Ltk" Cells. After transfection with high molecular weight DNA from human cell lines HeLa and A875, positive colonies expressing ME491 antigen were obtained at a frequency of 1 per 10,000 tk" colonies. A stable primary transformant, ME8, was derived from HeLa DNA and was used for further experiments. In the second round of transfection using genomic DNA from the ME8 primary transformant, positive colonies were obtained at practically the same frequency as that determined in the first round. Southern blot analysis using human repetitive Alu sequences as a probe revealed that secondary transformants contained much smaller amounts of human DNA than did primary transformants and that two independent secondary transformants (ST1-1 and ST2-3) appeared to share some Alu-positive fragments (6.8- and 3.2-kilobase BamHI fragments) (Fig. 1). The human repetitive Alu sequences did not hybridize to Ltk" DNA. These observations indicated that the gene encoding ME491 antigen was very close to or even included in the 6.8- and/or 3.2-kilobase fragments.

Molecular Cloning of Genomic DNA Sequences Encoding ME491 Antigen. An EMBL 3 genomic library was prepared with DNA from one of the secondary transformants (ST2-3) and probed with human repetitive Alu sequences. Two Alu-positive recombinant clones, designated λR31 and λR33, were obtained from 500,000 recombinant clones. Restriction enzyme map analysis revealed that λR31 and λR33 partially overlapped and that the overlapping regions contained the Alu-positive 6.8- and 3.2-kilobase BamHI fragments detected by Southern blot analysis of the secondary transformants (Fig. 2). Another family of human repetitive sequences was also mapped to the overlapping regions. For further studies, fragments free of human repetitive sequences (pMe1-29, pMe1-17, and pMe2-13) were subcloned into pBR322.

To determine whether these genomic DNA fragments contained genetic information necessary for ME491 antigen expression, λR31 and λR33 DNA as well as a cloned mouse DNA (λR47) as a negative control were transfected into Ltk" cells and expression of ME491 antigen was determined by the immunological rosette assay. Both λR31 and λR33 DNA could transform the cells to express the antigen (Table 1), suggesting that the gene necessary for ME491 antigen expression is located on the overlapping region. Digestion of λR31 DNA with BamHI or EcoRI prior to transfection completely abolished ME491 antigen expression, whereas HindIII digestion did not affect antigen expression (Table 1).

To determine which subfragment(s) contains exon(s) for ME491 antigen, Northern blot analysis was done using the subcloned fragments as a probe. pMe1-17 (1.1-kilobase BamHI-
MOLECULAR CLONING OF ME491 ANTIGEN

Fig. 1. Detection of human DNA sequences in primary and secondary transformants using Alu repetitive sequences as a probe. Total cellular DNA was digested with BamHI and separated on a 1% agarose gel (20 μg/lane). After transfer to a nitrocellulose filter, the DNA was probed with 300 base pair Alu human repetitive sequence. (Washing conditions were 0.5x SSC-0.1% SDS at 65°C.) (Lane 1) N2C, a primary transformant which was generated by transfection of Ltk− cells with total human DNA; (Lane 2) mouse Ltk−; (Lane 3) a primary transformant MES the DNA of which was used to generate secondary transformants; (Lane 4) a secondary transformant ST2-3; (Lane 5) an independently obtained secondary transformant ST1-1-2; (Lane 6) Sill, a partially cloned culture with 30% antigen-positive cells; the ST1-1-2 clone was derived from this culture.

Table 1 Frequency of positive colonies after transfection with the recombinant DNA clones

<table>
<thead>
<tr>
<th>Recombinant DNA clones</th>
<th>% of positive colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>XR31</td>
<td>50 (1000)*</td>
</tr>
<tr>
<td>XR31, BamHI treated</td>
<td>0 (500)</td>
</tr>
<tr>
<td>XR31, EcoRI treated</td>
<td>0 (200)</td>
</tr>
<tr>
<td>XR31, HindIII treated</td>
<td>45 (1000)</td>
</tr>
<tr>
<td>XR33</td>
<td>70 (1000)</td>
</tr>
<tr>
<td>XR47 (mouse DNA)</td>
<td>0 (2000)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, approximate number of colonies tested.

Fig. 2. Restriction maps of ME491 genomic clones. XR31 and XR33 were cloned from a λ genomic library prepared with secondary transformant ST2-3 DNA using Alu human repetitive sequences as a probe. □, mouse cellular sequences which were identified by Southern blotting using Ltk− DNA as a probe; □, Alu human sequences; □, human repetitive sequences other than the Alu family. B, H, and E, restriction sites for BamHI, EcoRI, and HindIII, respectively. pMe1-29, pMe1-17, and pMe2-13 are fragments subcloned into pBR322. kb, kilobase.

Fig. 3. Detection of ME491 mRNA in L-λR33 and A875 human melanoma cells. Polyadenylated RNA was separated on a 1.5% agarose/formaldehyde gel (3 M g/lane). After transfer to a nitrocellulose filter, the RNA was probed with pMe1-17 (Lanes 1, 2, and 3), pMe1-29 (Lane 4) or pMe2-13 (Lane 5). (Lane 1) Mouse Ltk−; (Lane 2) A875; (Lanes 3, 4, and 5) L-λR33.

Identity of the Antigen Expression in L-λR33 and Human Melanoma Cells. The antigen expressed in the L-λR33 transformant was biochemically characterized and compared with that expressed in human melanoma cells. Western blot analysis of the membrane fractions from L-λR33 and A875 melanoma cells showed practically the same pattern, i.e., a broad band with a molecular weight ranging from 30,000 to 60,000 (Fig. 4). This broad band has been observed in all melanoma cells thus far tested and may be the result of extensive and heterogeneous glycosylation (8, 10). The cytosol fraction of L-λR33 cells contained almost no ME491 antigen (Fig. 4), in agreement with previous reports with melanoma cells (7).

Nucleotide Sequences and Deduced Amino Acid Sequences of ME491 Antigen. A λgt11 cDNA library prepared with mRNA from melanoma cell line WM1158 was probed with the repeat-free genomic subfragments (pMe1-17 or pMe1-29), and several overlapping clones were obtained. Fig. 5 shows the nucleotide sequences and deduced amino acid sequences. The predicted amino acid sequence following the first ATG codon coincides perfectly with the NH2-terminal amino acid sequence (Nos. 1 to 20) of purified ME491 antigen except for No. 8 (Cys) which was not previously assigned (10). The ME491 antigen consists...
Molecular Cloning of ME491 Antigen

Fig. 4. Detection of ME491 antigen in L-AR33 and human melanoma cell lines by Western blot analysis. Cells were separated to a membrane fraction and a cytosol fraction. The membrane fractions, unless otherwise stated, were subjected to SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter. The filter was incubated with ME491 MAb and then with 125I-labeled anti-mouse IgG to visualize the antigen by autoradiography. (Lane 1) L-XR33; (Lane 2) cytosol fraction of L-AR33; (Lane 3) Litt; (Lane 4) A875 melanoma; (Lane 5) WM49 melanoma; (Lane 6) human neurofibroma. KDa, molecular weight in thousands.

Fig. 5. Complete nucleotide sequence of ME491 antigen and its predicted primary amino acid sequence. NH2-terminal amino acid sequence of purified ME491 antigen (32) is underlined. Putative recognition sites for asparagine-linked N-glycosylation are double-underlined. A polyadenylation signal (ATTAAA) (23) is dot-underlined. *, nucleotide position of +1. Right ordinate, nucleotide positions and amino acid coordinates of mature ME491 antigen. of 237 amino acids (M, 25,475) with three putative sites for N-linked glycosylation. Computer searches (NBRF Protein Data Bank) revealed no significant homology with known proteins. The apparent difference in molecular size between the estimated molecular weight (25,475) and observed value of nonglycosylated core protein (about 20,000) (8, 10) might rest in the faster migration of hydrophobic membrane protein in SDS-polyacrylamide gel than marker proteins with the same molecular sizes (30). Hydrophobicity plot analysis (31) suggests that there are four transmembrane regions: three consecutive ones near the amino terminus; and the remaining one near the carboxyl terminus (Fig. 6). All three putative N-glycosylation sites are located in a region between the third and the fourth transmembrane domains. Since the region with the putative N-glycosylation sites should be located on the extracellular surface of the plasma membrane, it is most likely that the amino terminus of this protein lies inside the cytoplasm.

The nucleotide sequence from -3 to +4 (GCCATGG) is a consensus sequence for initiation of translation (32). Hence, this ATG codon is most probably the initiation codon and the terminal methionine is removed following translation. The ME491 antigen sequence does not include an amino terminal signal sequence. It has been reported, however, that some membrane proteins with multiple transmembrane domains lack signal sequences, since the hydrophobic domains act as an internal signal sequence allowing integration into the plasma membrane (33).

The following experiments show how the cDNA sequence corresponds to the genomic DNA. A CDNA clone (nucleotide +30 to +790) was fragmented into two portions by Psfl digestions and the resulting fragments were used as probes for Southern blot analysis. The fragment closer to the 5' end (nucleotides +30 to +622) hybridized to the 1.5- and the 1.1-kilobase BamHI-EcoRI fragments (corresponding to pMel-29 and pMel-17, respectively; see Fig. 2), but not the intervening 0.4-kilobase EcoRI-EcoRI fragment of XR31 and XR33 genomic DNAs, while the fragment closer to the 3'-end (nucleotides +623 to +790) hybridized only to the 1.1-kilobase fragment (data not shown). These results, together with the results shown in Table 1, suggest that the 5' end of the ME491 gene is located either in the 1.5-kilobase fragments or in an adjoining small fragment at the very end of XR31 DNA and that the intervening 0.4-kilobase fragment is located in an intron.

ME491 Gene Is Not Amplified in High Expressor Melanomas. ME491 antigen expression in cultured human melanoma cells differs greatly with different cell lines. WM98, WM35, WM1158, and WM983-A cell lines are high expressors for ME491 antigen as judged by the immunological rosette assay, whereas A875 and HS294 are low expressors (data not shown). Polyadenylated RNAs from some of the cell lines were analyzed by Northern blot analysis using the cloned DNA subfragment as a probe (Fig. 7). Consistent with results in the immunological

Fig. 6. Hydrophobicity plot of predicted amino acid sequences of ME491 antigen. Hydrophobicity indices were determined as described by Kyte and Doolittle (31). I to IV, putative transmembrane regions; arrows, positions of asparagine for putative N-glycosylation sites.
rosette assay, ME491 mRNA was much more abundant in WM98 and WM35 than in HS294 or A875 cells. The presence of equal amounts of polyadenylated RNAs was verified by probing with a housekeeping gene (phosphoglycerate kinase gene) (data not shown). To determine whether any significant difference in DNA levels might be responsible for altering gene expression, high molecular weight DNAs extracted from the above cell lines were subjected to Southern blot analysis using the 1.1-kilobase fragment of pMel-17 (Fig. 8). No significant difference was observed between high expressors and low expressors in terms of intensity and size of DNA fragments corresponding to the ME491 gene. These results indicate that there is no amplification or apparent rearrangement of the ME491 gene. The additional weaker band(s) observed in every human DNA tested may indicate the presence of a ME491-related gene. Further studies including molecular cloning of the related gene will clarify this matter. The different pattern of the weaker band for WM9 DNA appears to be due to restriction fragment length polymorphism, because DNA from peripheral blood lymphocytes of the same patient showed a pattern identical with that of WM9 melanoma DNA (data not shown).

Cross-Species Sequence Homology of the ME491 Gene. Monkey DNA was obtained from the COS cell line (34) and mouse DNA from Ltk− and NIH/3T3 cells (35, 36). Rat and squirrel DNA were obtained from tissue spleen cells, and chicken DNA was obtained from chick embryo. Southern blot analysis of these DNA samples using the 1.1-kilobase fragment of pMel-17 was performed to detect any cross-species sequence homology. Significant hybridization was observed to DNA from monkey, rat, mouse, and squirrel, but only negligible hybridization was detected with chick embryo under low stringency conditions (2× SSC-0.1% SDS at 65°C) (Fig. 9). Under high stringency conditions (0.2× SSC-0.1% SDS at 65°C), significant hybridization was observed only in human, monkey, and squirrel DNA (data not shown).

Chromosomal Localization of ME491 Gene. To determine which human chromosome bears the gene for the ME491 antigen, a panel of 18 DNAs derived from mouse-human hybrids retaining defined overlapping subsets of human chromosomes was tested for the presence of ME491 sequences by analysis of hybrid DNA-containing Southern blots after hybridization with the pMel-17 subclone. Screening of hybrid DNAs by this method showed that ME491 sequences were present in mouse-human hybrids retaining chromosome 12 and absent in hybrids which had lost chromosome 12. Fig. 10 summarizes these results.

In mapping genes to chromosome 12 in earlier experiments, we had observed that some of the hybrid cells retained some chromosome 12-linked genes but not others. Thus, we used hybrid cells M44c12SS, M44c12S9, and GL-3a to determine the order of some of the chromosome 12-linked genes, including ME491. As shown in Table 2, all three hybrids retain ME491 sequences, localizing the ME491 gene between 12p12, where K-ras-2 maps (27), and the SW756 papillomavirus integration site 7.16.1 at 12q12-q13 (24).

The assignment of the ME491 locus to human chromosome 12 was confirmed and refined by in situ hybridization of 3H-labeled pMel-17 plasmid DNA to metaphase chromosomes from peripheral blood lymphocytes of a normal male. After autoradiography, metaphase spreads were analyzed for grain localization. About 23% of all grains were located on the long arm of chromosome 12. Over 82% of the 12q grains were between 12q12 and 12q14 with most grains at 12q13. Fig. 11 shows a histogram depicting the silver grain distribution among the human chromosomes. The long arm of chromosome 12 represents approximately 3.3% of the haploid genome, and our observations that more than 19% of the pMel-17 probe hybridization was localized to this region are highly significant (P < 0.001). Thus cytological hybridization localizes the ME491 gene to the region between 12q12 and 12q14.

5 N. Popescu and J. DiPaolo, personal communication.
DISCUSSION

DNA-mediated gene transfer was used to introduce the human gene encoding the ME491 antigen (7–9) to mouse fibroblast Ltk" cells and to obtain primary and secondary transformants. The gene was then molecularly cloned, using Alu human repetitive sequences (16) as a probe, from a λ genomic library prepared with DNA from one of the secondary transformants. The identity of the cloned gene as the ME491 gene is suggested by the following observations: (a) the cloned gene, after transfection to Ltk" cells, mediates expression of an antigen indistinguishable by Western blot analysis from the ME491 antigen present in melanoma cells; (b) subfragments of the cloned gene, which are free of human repetitive sequences, detect a single probe perfectly match the INII C-terminus amino acid chromosomal region. We therefore conclude that the cloned gene is the ME491 gene.

Our previous reports have indicated that expression of ME491 antigen is tumor stage specific in melanocytic cell lineages in human tissues (7, 8); the antigen is strongly expressed in dysplastic nevi and radial growth phase primary melanoma both of which represent the early stages of tumor progression, whereas it is completely negative in normal tissue melanocytes and is weaker or sometimes even negative in advanced stages of melanoma. It is also to be noted that when melanocytic cells are placed in culture, the stage specificity of the antigen expression is considerably reduced (3); normal melanocytes rapidly growing in culture express the ME491 antigen (data not shown), suggesting that the cellular environment influences antigen expression. The regulation of activation and suppression of the ME491 gene, and its role, if any, in melanoma tumor progression remain unknown. To obtain tools to answer these questions, we cloned the ME491 gene.

We found a large variation in ME491 antigen expression among human melanoma cell lines; i.e., some are high expressors, and others are low expressors. The difference in the degree of the antigen expression is not associated with gene amplification or obvious gene rearrangement, unlike the findings for some oncogenes and growth factor receptors in malignant cells (37–39). Interestingly, NIH/3T3 cells transfected with the cloned ME491 gene do not express the antigen despite the stable incorporation of the gene into the NIH/3T3 genome (data not shown), while another mouse fibroblast cell line Ltk" can express the antigen when transfected with the same gene (see Table 1). It has been observed that Ltk" cells express trans-
abscissa, chromosomes in their relative size proportion; ordinate, number of silver

It will also be of interest to determine any relationship

cluster Hox-3 (22), and the SW756 HPV 18 integration site

ACKNOWLEDGMENTS

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