incubation temperature to 37°C or up to 50°C. Under such conditions, the ladders shown here could be obtained within 3 hours rather than within days.
8. C. Gaillard and F. Strauss, data not shown.
9. Cell culture (CV1 line), nuclei purification, protein extraction, gel retardation, as well as phosphocellulose, DNA-sephacryl, and hydroxyapatite chromatography were performed as described by F. Strauss and A. Varshavsky [*Cell* **37**, 889 (1984)]. Because HMG1 and HMG2 copurified on these columns, a fourth chromatographic step on carboxymethyl-cellulose was performed as described by G. H. Goodwin and E. W. Johns [in *Methods in Cell Biology*, D. M. Prescott, Ed. (Academic Press, New York, 1977), vol. 16, chap. 15]. In appropriate salt conditions, HMG1 does not bind to this column, whereas HMG2 does.
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19. A 120-bp Cla I–Eco RI restriction fragment that contained a 60-bp tract of poly(CA)·poly(TG) was used. The nucleotide sequence of the poly(CA)-containing strand of the fragment was as follows: CGATAAGCTTCTAGAGATCCC-$(CA)_{30}$-GGGATCCGTCGACTCTAGAGGATCCCCGGGCGAGCTCG.
20. Four-percent polyacrylamide gels were used. Two different electrophoresis buffers were used, with identical results [either 40 mM tris-acetate, 20 mM sodium acetate, 1 mM EDTA (pH 7.8), or 6.7 mM tris-acetate, 3.3 mM sodium acetate, 1 mM EDTA (pH 7.8) with buffer recirculation]. Identical results were obtained with gels run at 4°C or 25°C.
21. DNA spreading was done with cytochrome c on an aqueous hypophase, followed by rotary shadowing with platinum. A control, in which the same DNA was spread at a 1000-fold higher concentration without preincubation at high ionic strength to form complexes, failed to show X-shaped structures, confirming that these are not due to random crossing of DNA molecules on the electron microscope grid.
22. We thank L. Jonk, who participated in an early stage of this work, C. Antony for help with the electron microscopy facility, and S. Elsevier for critical reading of the manuscript. Supported by grants from the Association Française des Myopathes, the Association pour la Recherche contre le Cancer, and the Ligue Nationale Française Contre le Cancer.

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A Cell Cycle Regulator Potentially Involved in Genesis of Many Tumor Types

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A putative tumor suppressor locus on the short arm of human chromosome 9 has been localized to a region of less than 40 kilobases by means of homozygous deletions in melanoma cell lines. This region contained a gene, Multiple Tumor Suppressor 1 (*MTS1*), that encodes a previously identified inhibitor (p16) of cyclin-dependent kinase 4. *MTS1* was homozygously deleted at high frequency in cell lines derived from tumors of lung, breast, brain, bone, skin, bladder, kidney, ovary, and lymphocyte. Melanoma cell lines that carried at least one copy of *MTS1* frequently carried nonsense, missense, or frameshift mutations in the gene. These findings suggest that *MTS1* mutations are involved in tumor formation in a wide range of tissues.

The genetics of cancer involves positive regulators of the transformed state (oncogenes) as well as negative regulators (tumor suppressor genes). More than 100 oncogenes have been characterized, and although less than a dozen tumor suppressor genes have been identified to date, the number is expected to increase beyond 50 (*1*). The involvement of so many genes underscores the complexity of the growth control mechanisms that maintain the integrity of normal tissue. This complexity is manifested in another way. So far no single gene has been shown to participate in the development of all or even the majority of human cancers. The most common oncogenic mutations are in *HRAS*, found in 10 to 15% of solid tumors (*2*). The most frequently mutated tumor suppressor gene is in the p53 gene, mutated in roughly 50% of all tumors (*3*). Without a target that is common to all transformed cells, the dream of a "magic bullet" that can destroy or revert cancer cells while leaving normal tissue unharmed is improbable. The hope for a new generation of specifically targeted antitumor drugs may rest on the ability to identify tumor suppressor genes or oncogenes that play general roles in control of cell division.

One of the mechanisms for controlling tumor growth might involve direct regulation of the cell cycle. Genes that control the decision to initiate DNA replication are attractive candidates for oncogenes or tumor suppressor genes depending on whether they have a stimulatory or inhibitory role in the process. Indeed, several oncogenes and tumor suppressor genes have been found to participate directly in the cell cycle. For instance, one of the cyclins, a class of proteins that promotes DNA replication

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Table 1. Deletions in tumor cells and primary tumors.

Tumor type	Lines (*n*)	Deletions (*n*)	Deletions (%)
Astrocytoma	17	14	82
Bladder	15	5	33
Breast	10	6	60
Colon	20	0	0
Glioma	35	25	71
Leukemia	4	1	25
Lung	59	15	25
Melanoma	99	57	58
Neuroblastoma	10	0	0
Osteosarcoma	5	3	60
Ovary	7	2	29
Renal	9	5	56
Total	290	133	46

and mitosis, has been implicated as an oncogene (*4*), and the retinoblastoma tumor suppressor is a substrate of the cyclin-dependent protein kinases (cdks) (*5*).

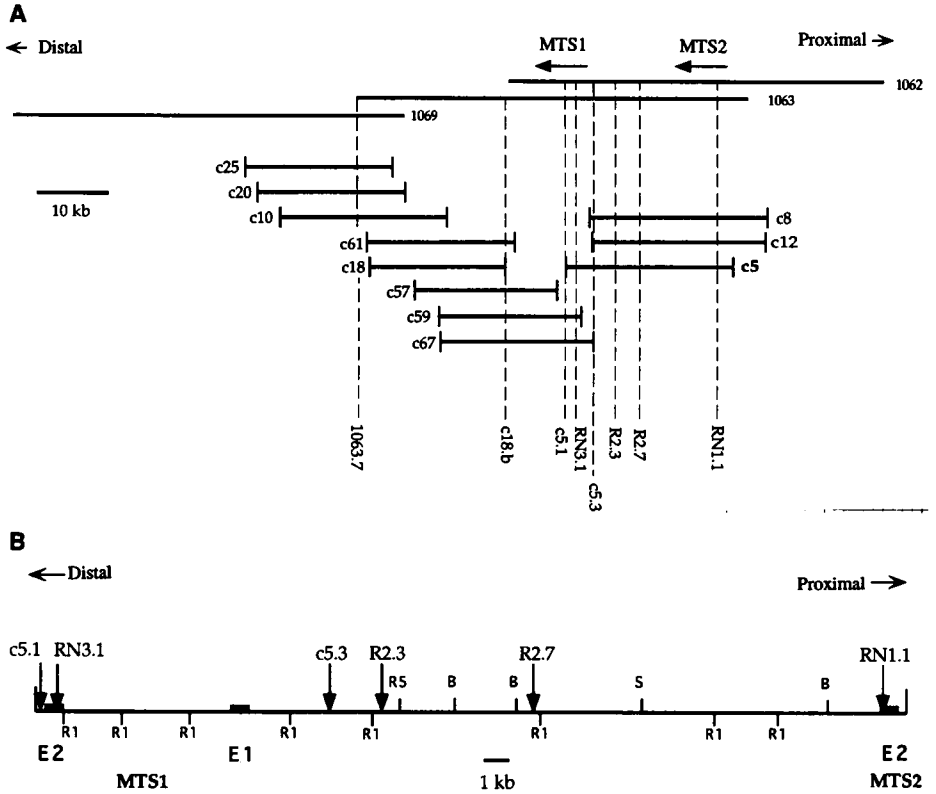
A hallmark of tumor suppressor genes is that they are deleted at high frequency in certain tumor types. The deletions often involve loss of a single allele, so-called loss of heterozygosity (LOH), but may also involve homozygous deletion of both alleles. For LOH, the remaining allele is presumed to be nonfunctional, either because of a preexisting inherited mutation or because of a secondary somatic mutation.

The human 9p21 region contains chromosomal inversions, translocations, heterozygous deletions, and homozygous deletions in glioma cell lines, non–small cell lung cancer lines, leukemia lines, and melanoma lines (*6–8*). Certain 9p21 markers are deleted in more than half of all melanoma lines (*9*). These findings suggest that 9p21 contains a tumor suppressor locus that may be involved in genesis of several tumor types. In a previous study, we reported the results of a YAC and P1 chromosomal walk in a region of 9p21 (*9*). This work produced a physical map and a set of sequence tagged sites (STSs) that were used to analyze nearly 100 melanoma cell lines for homozygous deletions. More than half of these cell lines contained homozygous deletions that clustered around a single cosmid, c5 (Fig. 1A). Fine structure mapping experiments with STSs derived from c5 revealed the presence of small, nonoverlapping, homozygous deletions of c5 sequences in six melanoma cell lines. On the basis of this result, it was probable that a tumor suppressor gene lay at least partly within cosmid c5.

To search for candidate tumor suppressor genes, the DNA sequence of parts of cosmid c5 was determined (*10*). When this sequence was compared with sequences in GenBank, two distinct regions of c5 were identified that were similar to a region of a previously defined gene encoding human cdk4 inhibitor, or p16 (*11*). These two sequences were named Multiple Tumor Suppressor 1 (*MTS1*) and *MTS2* (Fig. 1B).

Detailed comparison of genomic sequence from c5 with the p16 mRNA sequence revealed that *MTS1* contained a stretch of 307 bp that was identical to a portion of the p16 coding sequence. This stretch of nucleotides in *MTS1* was flanked by recognizable splice junction sequences. Further characterization of *MTS1* showed that it included the entire coding sequence of p16 plus two introns. The two introns divided the coding sequence of p16 into three regions: a 5′ region of 126 bp (coding exon 1), a middle region of 307 bp (coding exon 2), and a 3′ region of 11 bp (coding exon 3).

MTS2 contained a region of DNA sequence 93% identical to p16 sequence



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Class	# lines	1063.7	c18.B	c5.1	RN3.1	c5.3	R2.3	R2.7	RN1.1	c1.B
		Sts								
1	57	-	-	-	-	-	-	-	-	-
2	5	+	-	-	-	-	-	-	-	-
3	1	+	+	-	-	-	-	-	-	-
4	3	+	+	+	+	-	-	-	-	-
5	23	-	-	-	-	-	-	-	-	+
6	8	+	-	-	-	-	-	-	-	+
7	4	+	+	-	-	-	-	-	-	+
8	2	+	+	+	+	-	-	-	-	+
9	7	-	-	-	-	-	-	-	+	+
10	1	+	+	-	-	-	-	-	+	+
11	1	+	+	+	-	-	-	-	+	+
12	1	+	+	+	+	-	-	-	+	+
13	2	-	-	-	-	-	-	+	+	+
14	4	-	-	-	-	-	+	+	+	+
15	6	+	-	-	-	-	+	+	+	+
16	1	+	+	-	-	-	+	+	+	+
17	1	-	-	-	-	+	+	+	+	+
18	4	+	+	-	-	+	+	+	+	+*
19	1	+	-	-	+	+	+	+	+	+
20	1	+	+	-	+	+	+	+	+	+
21	157	+	+	+	+	+	+	+	+	+
Deletions	Total 133	94	114	126	125	126	115	113	103	66

Fig. 1. Maps of cosmid c5 region. These maps are part of a larger physical map derived from a chromosomal walk in the region (*1*). (**A**) Relevant STSs used for the deletion analysis are shown, as are cosmids and P1s mentioned in the text. The c1.b marker lies proximal to P1 1062 and is not shown. The transcriptional orientations of *MTS1* and *MTS2* are shown by arrows. (**B**) Restriction map and STS map of cosmid c5. Positions of coding exons for *MTS1* and *MTS2* are shown as thick bars. E1 and E2, coding exon 1 and coding exon 2, respectively; B, Bam HI; S, Sal I; R1, Eco RI; and R5, Eco RV. (**C**) Deletions in tumor cell lines of STSs. Positive controls and negative controls were included in every polymerase chain reaction (PCR) experiment and cell lines in which only one or two of the STSs were deleted (such as class 20) were retested at least twice. The cell lines used for this study and their class designations are available from the authors upon request. +, presence of DNA; −, absence (homozygous deletion).

which extended from the 5′ end of coding exon 2 roughly 255 bp toward intron 2. By analogy with *MTS1*, we refer to this region in *MTS2* as coding exon 2. The coding exon 2 sequences of *MTS1* and *MTS2* diverged abruptly at a point 50 bp upstream of intron 2 in *MTS1* (Fig. 2). No obvious 5′ splice sequence was found at the position equivalent to the 5′ splice junction of intron 2 in the *MTS1* gene. A stop codon occurred in the open reading frame of coding exon 2 at the codon immediately following the divergence point. Thus, if the proteins encoded by *MTS1* and *MTS2* were identical in size upstream of the divergence point, and if an alternative splice site upstream of the divergence point were not used, the *MTS2* product would be 20 residues shorter than p16. The sequence similarity between *MTS1* and *MTS2* also extended nearly 40 nucleotides upstream from the 3′ splice junction of intron 1. Thus, portions of presumptive noncoding DNA were as conserved as some areas of presumptive coding DNA (*12*).

The occurrence of two closely related genes on cosmid c5 suggested that other related genes might exist in the region. To test this possibility, Southern blots were prepared from restriction enzyme digests of cosmids c5, c12, c59, P1s 1063 and 1062, and human genomic DNA (Fig. 1A). These blots were probed with a fragment containing most of exon 2 from *MTS1*, including the region shared with *MTS2*. Two Eco RI fragments and two Hind III fragments were detected with the probe in both cloned DNA and genomic DNA (Fig. 3). Because coding exon 2 sequences from *MTS1* and *MTS2* do not contain Eco RI or Hind III sites, this result was consistent with the presence of only two p16-like genes in the genome, *MTS1* and *MTS2*. A second Southern blot identical to the first was probed with coding exon 1 from *MTS1*. Only a single hybridizing fragment was seen in genomic DNA and in cloned DNA (*13*). This suggested that *MTS2* does not contain an exon that closely resembles coding exon 1 of *MTS1*.

Because of the high frequency of deletions at 9p21 in multiple tumor types, we analyzed cell lines derived from 12 different types of tumor for deletions of *MTS1*, *MTS2*, or both. A set of STSs located in or around *MTS1* and *MTS2* was used to test genomic DNA from tumor cell lines for the presence or absence of the expected fragment (Fig. 1) (*14*). Lack of amplification of the predicted STS fragment from cell line genomic DNA was interpreted as indicative of homozygous deletion of the specific STS in that cell line. Homozygous deletions of at least one marker were detected in all tumor types tested other than neuroblastoma lines and colon tumor lines (Table 1). Excluding these types, the percentage of deletions varied from 25% in lung cancer and leukemia lines to 82% in astrocytomas. Collectively, 133 of 290 tumor lines contained deletions of at least one marker in the region tested. This number represented a minimum estimate of the percentage of tumor lines that harbor homozygous deletions, because the STSs used for the analysis did not completely cover the two genes. Thus, certain small deletions could escape detection. In addition, lesions such as insertions or deletions of a few nucleotides, and nucleotide substitutions, would be missed by this approach.

To improve the estimate of the total number of cell lines containing *MTS1* or *MTS2* mutations, a set of melanoma cell lines that did not contain obvious homozygous deletions of *MTS1* or *MTS2* sequences were examined more closely for genetic lesions. Genomic DNA sequences from coding exons 1 and 2 of *MTS1*, comprising 97% of the coding sequence, were amplified and screened for polymorphisms (*15*). Eighteen mutations, distributed in 14 of 34 melanoma lines, were observed (Table 2). Three of these mutations were frameshifts, seven were nonsense mutations, four were missense mutations, and four were silent. Three of the four lines that contained silent mutations also contained additional mutations and 16 of 18 mutations were located in coding exon 2. All but one line contained exclusively hemi- or homozygous polymorphisms, suggesting that the other homologous chromosomes had incurred de-

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MTS1     0 TGTGTGGGGGTCTGCTTGGCGGTGAGGGGGCTCTACACAAGCTTCCTTTCCGTCATGCCG
MTS2     0 AATTAGGTGTTTCTTTAAATGGCTCCACCTGCCTTGCCCCGGCCGGCATCTCCCATACCT
           |---------|---------|---------|---------|---------|---------
                                          ∇
MTS1    60 GCCCCCACCCTGGCTCTGACCATTCTGTTCTCTCTGGCAGGTCATGATGATGGGCAGCGC
MTS2    60 GCCCCCACCCTGGCTCTGACCACTCTGCTCTCTCTGGCAGGTCATGATGATGGGCAGCGC
           |---------|---------|---------|---------|---------|---------

MTS1   120 CCGAGTGGCGGAGCTGCTGCTGCTCCACGGCGCGGAGCCCAACTGCGCCGACCCCGCCAC
MTS2   120 CCGCGTGGCGGAGCTGCTGCTGCTCCACGGCGCGGAGCCCAACTGCGCAGACCCTGCCAC
           |---------|---------|---------|---------|---------|---------

MTS1   180 TCTCACCCGACCCGTGCACGACGCTGCCCGGGAGGGCTTCCTGGACACGCTGGTGGTGCT
MTS2   180 TCTCACCCGACCGGTGCATGATGCTGCCCGGGAGGGCTTCCTGGACACGCTGGTGGTGCT
           |---------|---------|---------|---------|---------|---------

MTS1   240 GCACCGGGCCGGGGCGCGGCTGGACGTGCGCGATGCCTGGGGCCGTCTGCCCGTGGACCT
MTS2   240 GCACCGGGCCGGGGCGCGGCTGGACGTGCGCGATGCCTGGGGTCGTCTGCCCGTGGACTT
           |---------|---------|---------|---------|---------|---------
                                                              ↓
MTS1   300 GGCTGAGGAGCTGGGCCATCGCGATGTCGCACGGTACCTGCGCGCGGCTGCGGGGGGCAC
MTS2   300 GGCCGAGGAGCGGGGCCACCGCGACGTTGCAGGGTACCTGCGCACAGCCACGGGGGACTG
           |---------|---------|---------|---------|---------|---------
                                                    ∇
MTS1   360 CAGAGGCAGTAACCATGCCCGCATAGATGCCGCGGAAGGTCCCTCAGGTGAGGACTGATG
MTS2   360 ACGCCAGGTTCCCCAGCCGCCCACAACGACTTTATTTTCTTACCCAATTTCCCACCCCCA
           |---------|---------|---------|---------|---------|---------

MTS1   420 ATCTGAGAATTTGTACYCTGAGAGCTTCCAAAGCTCA
MTS2   420 CCCACCTAATTCGATGAAGGCTGCCAACGGGGAGCGG
           |---------|---------|---------|---------
```

Fig. 2. DNA sequence alignment of *MTS1* and *MTS2*. Regions of sequence identity between *MTS1* and *MTS2* are shown in boldface. The positions of the 3′ splice junction of intron 1 and 5′ splice junction of intron 2 for *MTS1* are shown by triangles. The divergence point near the 3′ end of coding exon 2 is indicated by an arrow.

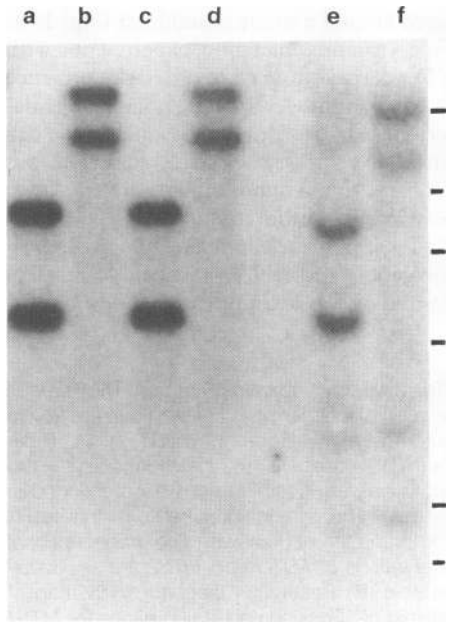

Fig. 3. Southern blot of P1 1062 (a and b), P1 1063 (c and d), and genomic DNA (e and f) digested with Eco RI (a, c, and e) and Hind III (b, d, and f). P1 DNA (500 pg) and 5 μg of human genomic DNA were blotted from a 0.7% agarose gel. Size markers in kilobases from top to bottom of the autoradiogram are: 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0. The probe was a 142-bp PCR product that contained nucleotides 98 to 240 of *MTS1* coding exon 2 (Fig. 2). Hybridization was performed with standard procedures (*19*). The final wash was in 0.1× SSPE, 0.1% SDS at 50°C for 15 min.

letions. The single line that was heterozygous contained two nonsilent mutations, a finding consistent with the view that each homolog had undergone independent mutational events. Based on this DNA sequence and deletion analysis of *MTS1*, a minimum of 75% melanoma lines contained mutant *MTS1* or had lost the gene from both homologs.

A similar analysis of coding exon 2 from *MTS2* did not reveal any polymorphisms. In addition, no deletions unequivocally involved *MTS2* alone (Fig. 1C). When RN1.1, the marker within *MTS2*, was deleted, markers within *MTS1* were also lost with five exceptions (classes 4, 8, and 12 in Fig. 1C). However, two of these exceptional lines were shown to have *MTS1* mutations or deletions: melanoma line SK-MEL-26 (class 8) was missing coding exon 1 from *MTS1* (*13*) and melanoma line SK-MEL-12 (class 8) contained a hemizygous missense mutation in coding exon 2 of *MTS1* (Table 2) (*13*). The remaining three lines apparently did contain coding exon 1 of *MTS1*; however, it is possible that the regulatory region of *MTS1* was affected by the deletion breakpoints located upstream. Thus, the function of *MTS2* has not been resolved. The striking sequence similarity between *MTS1* and *MTS2* implies that *MTS2* may also inhibit one or more cdks, perhaps including cdk4. The majority of homozygous deletions removed both *MTS1* and *MTS2*. Thus, *MTS2* may have some role in tumorigenesis. Alternatively, *MTS2* may be a nonfunctional gene or may have a role entirely different from *MTS1*.

It is possible that mutation or loss of *MTS1* is a product of cell growth in culture. However, a high percentage of primary leukemia cells also contain homozygous deletions of the α-interferon gene cluster, a gene family located less than 500 kb from *MTS1* (*8*). Studies of melanoma cell lines suggest that deletions of α-interferon genes invariably involve markers that extend beyond *MTS1* toward the centromere (*13*). In addition, LOH studies of human primary, non–small cell lung cancer and primary head and neck carcinoma have demonstrated that the minimal area of heterozygous deletion in these tumors encompasses 9p21-22 (*8*). Finally, nonsense and splice-junction mutations in *MTS1* have been observed in primary melanomas and bladder tumors (*16*). Because deletions of the 9p21 region and nonsense mutations of *MTS1* occur in primary tumor cells as well as cultured cell lines, the deletions observed in tumor cell lines are unlikely to be purely a result of cell growth in culture.

Dividing eukaryotic cells must pass through two critical decision points: the G_1-S transition where DNA synthesis commences and the G_2-M transition where mitosis begins. The machinery that controls cell division has multiple components, many of which are related (*16*). The cdks may be at the heart of the control apparatus in that they regulate by phosphorylation a number of key substrates that in turn trigger the transition from G_1 to S and from G_2 to M. So far, four types of cdk have been defined (cdk2-5) that may participate in G_1-S control as well as a set of positive regulators of these cdks (for example, cyclins C, D1-3, E). Recently several negative regulators have also been identified, including p21 (p20 in mouse), and the *MTS1* gene product p16 (*11*, *17*, *18*). On the basis of in vitro studies and on its interaction with p53, p21 has been proposed as a general inhibitor of all cdks (*18*). Thus, in vitro, p16 appears more specific than p21. Both of these inhibitors are expected to antagonize entry into S phase.

If p16 inhibits cdk4 in vivo, cdk4 and its cyclin partners are strong candidates for oncogenes that could be major factors in tumorigenesis. The prevalence of mutations in the p16 gene suggests the possibility that cdk4 may serve as a general activator of cell division in most, if not all, cells. Further biochemical studies of the effects of p16 on different cdks may help clarify the hierarchy of cdk activity in both normal cells and transformed cells. Proof of our model awaits direct demonstration of the tumor suppressor function of p16 and of the interaction between p16 and cdk4 in vivo.

Table 2. Mutations in melanoma lines determined by DNA sequence analysis.

Cell line	Mutation	Coding effect	Location†
SK-MEL-61	G→A	None	258
	G→A	Gly→Ser	259
SK-MEL-21	C→T	Arg→stop	166
SK-MEL-17	G→A	Ala→Thr	436
SK-MEL-112	5-base deletion	Frameshift	284–288
SK-MEL-178	C→T	Arg→stop	232
SK-MEL-124	G→A	Trp→stop	324
SK-MEL-86	C→T	Arg→stop	232
SK-MEL-131	8-base deletion	Frameshift	166–173
	C→A	None	165
SK-MEL-150(het)*	C→T	Pro→Leu	335
	C→T	None	231
	C→T	Arg→stop	232
SK-MEL-12	C→T	Pro→Leu	335
SK-MEL-156	C→T	None	372
SK-MEL-101	G→A	Trp→stop	323
SK-MEL-158	C→T	Gln→stop	142
SK-MEL-145	2-base deletion	Frameshift	122–123

*Het stands for heterozygote and refers to the presence in the sample of both the wild-type and mutant sequence. †The nucleotide positions of the base changes (location) derive from the numbering scheme used in Serrano *et al.* (*11*).

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10. DNA sequence determination of c5 was carried out by subcloning the internal Hind III fragments and Eco RI fragments and determining the DNA sequences of these subclones independently. Primer sequence walking steps were carried out sequentially on each subcloned template. DNA sequencing reactions were performed with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing kit (ABI), and the products were analyzed on an ABI 373 sequencer.
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12. To exclude the possibility that the sequence divergence in coding DNA might be a cloning artifact, we designed PCR primers to amplify specifically across the sequence divergence point of *MTS2*. These primers amplified a fragment of the predicted size from cosmid, P1, and genomic DNA (*15*). Therefore the divergent sequence located near the 3′end of exon 2 in *MTS2* is bona fide genomic sequence.
13. A. Kamb *et al.*, unpublished data.
14. These STSs included two markers on the distal side of *MTS1* (1063.7 and c18.b), two markers within *MTS1* (c5.1, located between coding exons 2 and 3, and RN3.1, located within coding exon 2), three markers between *MTS1* and *MTS2* (c5.3, R2.3, and R2.7), one marker within *MTS2* (RN1.1, located at the 3′ end of coding exon 2), and one marker on the proximal side of *MTS2* (c1.b). The method for preparation of cell line DNA has been described (*9*). The PCR conditions used for STS amplification were one cycle at 95°C (5 min); four cycles at 95°C (10 s) with

the annealing temperature (T_{ann}) = 68°C (10 s) and 72°C (10 s); four cycles with T_{ann} = 66°C; four cycles with T_{ann} = 64°C; four cycles with T_{ann} = 62°C; and 30 cycles with T_{ann} = 60°C. The DNA sequences of primers used for the STS analysis were 1063.7F, CCGTTTCAGCTTCTCATCAC; 1063.7R, CCGACTGTCCCATTGTGATT; c18.bF, CAAAGACTTTATGGATGGGG; c18.bR, TCCATTTCTCTGCTTGCTC; c5.1F, GAAGTCTTGGTCCTGATGTC; c5.1R, CTCTTCTGCACAACTCAACT; RN3.1F, GGATAGAGAACTCAAGAAGG; RN3.1R, TCTGAGCTTTGGAAGCTCT; c5.3F, GTGGTAGAACTAGGACAGGG; c5.3R, CTGTGTTAAGCCTTCATAGA; R2.3F, GAAAATGAAACTGTACCCATTG; R2.3R, GGGACACACATTAAATACACT; R2.7F, GAGAACAGGTTTTGGGCAG; R2.7R, AACTAGACCTAGGGATAAGG; c1.bF, AAGCTTTCCCACAAACTGGC; c1.bR, AATGCCTTGGCATAAGGGAC.

15. Fragments for DNA sequence determination were amplified as described (*14*), except that 5% dimethyl sulfoxide was added to the reaction. The products were purified from a 1.2% agarose gel with Qiaex beads (QIAGEN). Genomic fragments corresponding to coding exons 1 or 2 from *MTS1* or coding exon 2 from *MTS2* were amplified with specific primers and analyzed by cycle sequencing with [α-P^{32}]deoxyadenosine triphosphate (*19*). Products were run on 6% polyacrylamide gels. All A reactions were loaded side by side, followed by the C reactions, and so on. Detection of polymorphisms was by eye with confirmation on the other strand. The DNA sequences of the primers used for amplification and sequence determination were
Coding exon 1, *MTS1*. Amplification: 2F, GAAGAAAGAGGAGGGGCTG; 1108R, GCGCTACCTGATTCCAATTC. Sequencing: 1108R.
Coding exon 2, *MTS1*. Amplification: 42F, GGAAATTGGAAACTGGAAGC; 551R, TCTGAGCTTTGGAAGCTCT. Sequencing: 42F and 551R.
Coding exon 2, *MTS2*. Amplification: 89F, TGAGTTTAACCTGAAGGTGG; 50R, GGGTGGGAAATTGGGTAAG. Sequencing: 89F and 50R.
16. N. A. Gruis *et al.*, in preparation.
17. J. W. Harper, G. R. Adami, N. Wei, K. Keyomarsi, S. J. Elledge, *Cell* **75**, 805 (1993); Y. Gu, C. W. Turck, D. O. Morgan, *Nature* **366**, 707 (1993); W. S. el-Deiry, T. Tokino, V. E. Velculescu, *Cell* **75**, 817 (1993).
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19. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).
20. We thank D. Ballinger, S. Bayer, J. Collet, P. Dayananth, W. Ding, C. Frye, C. Hussey, M. McClure, D. Shattuck-Eidens, S. Stone, and T. Tran for suggestions and assistance. Supported in part by NIH grant CA-48711.

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