Alterations of INK4A and INK4B Genes in Adult Soft Tissue Sarcomas: Effect on Survival

Irene Orlow, Marija Drobnjak, Zuo-Feng Zhang, Jonathan Lewis, James M. Woodruff, Murray F. Brennan, Carlos Cordon-Cardo

Background: The INK4A and INK4B genes map to chromosome 9p21, with the INK4A gene encoding two protein products, p16 and p19ARF. Alterations of the INK4A and INK4B genes occur frequently in certain primary malignant neoplasms. This study was undertaken to evaluate the frequency of INK4A and INK4B gene alterations in a cohort of adult soft tissue sarcomas. Methods: The status of the INK4A and INK4B genes was determined in 46 soft tissue sarcomas by use of the following methods: Southern blotting, polymerase chain reaction (PCR), single-strand conformation polymorphism analysis, comparative multiplex PCR, and a methylation assay focusing on the p16 promoter. Associations between alterations of the INK4A and INK4B genes and clinicopathologic variables, as well as with p53 and pRB (retinoblastoma protein) status, were evaluated by use of the two-tailed Fisher’s exact test. Disease-specific survival was evaluated by use of the Kaplan–Meier method and the logrank test. Proportional hazards analysis was used to obtain estimates of relative risks. All P values are two-sided. Results: Homozygous and hemizygous deletions, but no point mutations, were observed in these two genes. The overall frequency of gene alteration (deletion or rearrangement) was approximately 15% for the INK4A and INK4B genes, with changes restricted to high-grade sarcomas. Statistically significant associations were observed between INK4A/INK4B deletions (P = .036) or alterations (P = .005) and poor survival. Alteration of the INK4A and INK4B genes was the only statistically significant predictor for poor survival when controlling for tumor grade and size (P = .03). Conclusion/Implications: Coincident homozygous deletion of the INK4A and INK4B genes occurs frequently in adult soft tissue sarcomas. Loss of p16 and p19ARF function in primary tumors, although not equivalent to alterations in p53 and pRB function, appears to be associated with cancers that have an aggressive biologic behavior. [J Natl Cancer Inst 1999;91:73–9]

A new group of negative cell cycle regulators has been described whose members act as cyclin-dependent kinase inhibitors (CKIs) (1–3). CKI family members have been subdivided into two groups on the basis of sequence homology. The first CKI member to be characterized was p21 (also known as WAF1, Cip1, or Sdi1) (4–6). The other members of this group are p27/Kip1 (7–9) and p15/kip2 (10,11). The other CKI subgroup includes four members: p16/INK4A/MTS1/CDKN2A (12,13), p15/INK4B/MTS2/CDKN2B (14), p18/INK4C (15), and p19/INK4D (16). The INK4A and INK4B genes map to the short arm of chromosome 9q (9p21), with the INK4A gene encoding two protein (p16) products that have molecular masses (M_r) of approximately 65 295 and 60 000, whereas the INK4B gene encodes a protein of 70 000. The INK4A and INK4B genes are both expressed in a wide variety of normal tissues and cell lines and several primary tumors (13,25–28). Deletions in 9p12–p22 occur non-randomly in some soft tissue tumors (24). The INK4A and INK4B genes have been found to be mutated in many tumor cell lines and several primary tumors (13,25–29). In addition, methylation of the 5’ CpG island located in the promoter region of the p16 gene has been reported to be a frequent mechanism of that gene’s inactivation in certain neoplasms (30,31). More recently, independent studies reported the targeted deletion of the Ink4a and Ink4b loci within p19ARF exon 1B in murine models (32,33). Both Ink4a- and p19ARF-deficient mice were viable, but they developed spontaneous tumors at an early age. Soft tissue sarcomas were one of the most common tumor types observed in these knockout mice. The evidence that INK4A encodes two products that have an impact on different tumor suppressors—p16 acting through pRB and p19ARF preventing Mdm2 neutralization of p53—positions this gene at the nexus of the two most critical tumor suppressor pathways controlling neoplasia (21–33).

The present study was undertaken to better delineate the frequency and potential clinical relevance of INK4A and INK4B gene alterations in a well-characterized cohort of adult soft tissue sarcomas. We have also evaluated alterations of pRB, p53, and Mdm2 in the same cohort of sarcomas and analyzed their association with INK4A and INK4B mutations.
Materials and Methods

Tissue and Patient Characteristics

Tumors from 46 patients with adult soft tissue sarcomas were analyzed. The tumor specimens corresponded to 12 liposarcomas, 11 leiomyosarcomas, seven malignant fibrous histiocytomas, five malignant peripheral nerve sheath tumors, four fibrosarcomas, four synovial sarcomas, one rhabdomyosarcoma, one desmoid tumor, and one unclassified sarcoma. Twenty-six tumors were confined to the extremities (19 primary, four recurrent, and three metastatic), four tumors were retroperitoneal (one primary and three recurrent), and 16 tumors were localized in other sites including the lung, liver, stomach, rectum, small bowel, prostate, and pelvis (seven primary, one recurrent, and eight metastatic). Ten tumors were classified as low grade; 36 were classified as high grade. In nine cases, the tumor size was less than or equal to 5 cm; in 37 cases, it was greater than 5 cm. Samples were embedded in cryopreservation compound O.C.T. (i.e., optimal cutting temperature compound; Miles Laboratories, Elkhart, IN), snap-frozen in isopentane that had been precooled in liquid nitrogen, and stored at −70 °C. Representative hematoxylin–eosin-stained sections of each frozen block were examined microscopically to confirm the presence of tumor, and only lesions with more than 50% neoplastic cells were included in the study. Normal tissues were obtained from all patients, either from a tumor-free area, such as skeletal muscle, or from peripheral blood.

Southern Blotting Analysis

A 0.5-kb complementary DNA (cDNA) fragment containing human p16 sequences and a 2-kb cDNA fragment containing human p15 sequences were used as probes to assess deletion and rearrangement of the INK4A and INK4B genes, respectively. A cDNA fragment containing glyceraldehyde phosphate dehydrogenase (GAPDH) sequences was used as a control. In general, Southern blot analysis was performed in a subset of the tumors following protocols described previously (26) (see below for details). The primers used to amplify the INK4A and INK4B genes can be summarized as follows: 1) p16, exon 1a–5′GGG AGC AGC ATG GAG CCG 3′ (F) and 5′ AGT CGC CCG CCA TCC TTC 3′ (R); 2) p16, exon 2–5′GGA ATT AAA TGG AAA CTG GAA GC 3′ (F) and 5′ GTC TAA GGT GCC GTA ACC CG 3′ (R); 3) p15, exon 1b (fragment 2, 160–280 bp)–5′GGG AAC GTG TTG AAG GTG TGC TAA GTC GTC TTG AAG TGC 3′ (F) and 5′ AGT ATC AGC AGC AGG 3′ (R); 4) p19arf, exon 1b (fragment 2, 160–280 bp)–5′GGG AAC GTG TTG AAG GTG TGC TAA GTC GTC TTG AAG TGC 3′ (F) and 5′ AGT ATC AGC AGC AGG 3′ (R); 5) p15, exon 1a–5′GGG AAC GTG TTG AAG GTG TGC TAA GTC GTC TTG AAG TGC 3′ (F) and 5′ AGT ATC AGC AGC AGG 3′ (R).

Polymerase Chain Reaction–Single-Strand Conformation Polymorphism and Multiplex Polymerase Chain Reaction Analyses

Polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP) assays were performed in a subset of the tumors following protocols described previously (26) (see below for details). The primers used to amplify the INK4A and INK4B genes can be summarized as follows: 1) p16, exon 1a–5′GGG AGC AGC ATG GAG CCG 3′ (F) and 5′ AGT CGC CCG CCA TCC TTC 3′ (R); 2) p16, exon 2–5′GGA ATT AAA TGG AAA CTG GAA GC 3′ (F) and 5′ GTC TAA GGT GCC GTA ACC CG 3′ (R); 3) p15, exon 1b (fragment 2, 160–280 bp)–5′GGG AAC GTG TTG AAG GTG TGC TAA GTC GTC TTG AAG TGC 3′ (F) and 5′ AGT ATC AGC AGC AGG 3′ (R); 4) p19arf, exon 1b (fragment 2, 160–280 bp)–5′GGG AAC GTG TTG AAG GTG TGC TAA GTC GTC TTG AAG TGC 3′ (F) and 5′ AGT ATC AGC AGC AGG 3′ (R); 5) p15, exon 1a–5′GGG AAC GTG TTG AAG GTG TGC TAA GTC GTC TTG AAG TGC 3′ (F) and 5′ AGT ATC AGC AGC AGG 3′ (R).

Analysis of Methylation

The methylation status of the 5′ CpG island in the promoter region of the p16 gene was determined with the CpG Wiz™ p16 Methylation Kit (Oncor, Inc.). Briefly, 0.5–1 μg of DNA was denatured with 3 M NaOH at 50 °C for 10 minutes and treated with sodium bisulfite following the manufacturer’s protocol. After completion of the DNA modification, the DNA was purified by precipitation. The dissolved DNA was amplified by PCR, utilizing primers specific for the methylated (M) or unmethylated (U) sequences. A 2- to 3-μL aliquot of template (corresponding to treated DNA, positive control for methylated DNA, negative control for unmethylated sequences, and distilled water as negative control) was amplified in the presence of 10 μL of a PCR product was digested with the restriction enzyme BamHI. For the SSCP analysis of exon 2 of the INK4A, 4 μL of a PCR product was digested with the restriction enzyme BstNI. For the SSCP analysis of exon 3 of the INK4A, 4 μL of a PCR product was digested with the restriction enzyme BstNI. For the SSCP analysis of exon 3 of the INK4A, 4 μL of a PCR product was digested with the restriction enzyme BstNI. For the SSCP analysis of exon 3 of the INK4A, 4 μL of a PCR product was digested with the restriction enzyme BstNI. For the SSCP analysis of exon 3 of the INK4A, 4 μL of a PCR product was digested with the restriction enzyme BstNI.
determined by the identification of a 145-bp fragment in those samples amplified with the M primers. All the cases were evaluated for the presence of an unmethylated specific fragment (154 bp), which served as internal control for the quality of the treated DNA.

**Monoclonal Antibodies and Immunohistochemistry**

Well-characterized mouse monoclonal antibodies to p53, Mdm2, and pRB proteins were used for this study. Antibody PAb801 reacts with both wild-type and mutant p53 products, recognizing a denaturation-resistant epitope that expands between amino acids 32 to 70 (34). A mouse monoclonal antibody identifying an epitope in the central portion of Mdm2, clone 2A10, was also used in the study (34). Antibody Rb-PMG3-245 was generated by immunizing mice with the TrpE/Rb fusion protein, and it has been shown to specifically recognize the 110-kd RB gene product (35). A class-matched mouse monoclonal antibody (MIgS-Kp-1; Pharmingen, San Diego, CA) was used at the same working concentrations as the primary antibodies, serving as a negative control.

Immunohistochemistry was performed on 5-μm frozen sections by use of the avidin–biotin complex immunoperoxidase technique (34,35). Sections were incubated with appropriately diluted primary antibodies (PAb810—200 ng/mL; 2A10—1:1000 dilution from tissue culture supernantant; Rb-PMG3-245—10 μg/mL) for 1 hour at room temperature. Secondary, biotinylated horse anti-mouse antibodies (Vector Laboratories, Inc., Burlingame, CA) were used at 1:100 dilution, and avidin–biotin peroxidase complexes (Vector Laboratories, Inc.) were used at a dilution of 1:25. Diaminobenzidine was used as the final chromogen (0.06%), and hematoxylin was used as the nuclear counterstain.

Immunohistochemical evaluation was performed by scoring the estimated percentage of tumor cells that showed nuclear staining. Nuclear immunoreactivities of p53 and Mdm2 were considered positive when at least 20% of the tumor cells showed nuclear staining (34). pRB was considered undetectable when nuclear staining was observed in normal endothelial cell and/or inflammatory elements but not in tumor cells (35). The immunohistochemical analysis was done in a blinded fashion (i.e., without knowledge of the molecular data or clinical information).

**Statistical Analyses**

Patients were categorized according to the stage, grade, histologic type, site, and presentation of their tumors as defined in Table 1. INK4A/INK4B deletions included those patients with homozygous or hemizygous deletions of the INK4A and the INK4B genes. INK4A/INK4B alterations included patients with gene deletions and rearrangements. A cut point of 20% of tumor cells stained (<20% staining as negative and ⩾20% staining as positive) was used for immunohistochemical variables, including altered patterns of p53 and Mdm2. TP53 mutations were coded as “yes” or “no” in the data analysis. A two-tailed Fisher’s exact test (36) was used to assess the associations between INK4A and INK4B deletions or alterations and clinicopathologic parameters (including tumor grade, size, site, and presentation) and to explore the association between INK4A and INK4B alterations and other variables, including TP53 mutations and immunophenotypic patterns. The FREQ procedure in SAS was used (37). In the analysis of disease-specific survival, patients who died of soft tissue sarcomas were classified as dead of disease, whereas patients who were still alive, had died of unrelated causes, or were lost to follow-up during the study period were coded as censored. Survival time was defined as the time from date of surgery to the endpoint (death or censoring). Disease-specific survivals were evaluated with the use of the Kaplan–Meier method (38) and logrank test (39). The LIFETEST procedure in SAS was used for analysis of these data (37). Proportional hazards analysis was used to obtain maximum likelihood estimates of relative risks (RRs) and their 95% confidence intervals (CIs) in a multivariate analysis (40,41). The data were consistent with the assumptions of Cox proportional hazards analysis.

**RESULTS**

Forty-six paired samples of normal and tumor DNA were available for Southern blot analysis of the INK4A and INK4B genes. Fig. 1 illustrates normal and altered patterns of bands obtained through

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Table 1. Association between deletions and alterations of the INK4A/INK4B genes and clinicopathological variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>INK4A/INK4B deletions</th>
<th>INK4A/INK4B alterations</th>
</tr>
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<tr>
<td></td>
<td>No. (%), Yes. (%), P*</td>
<td>No. (%), Yes. (%), P*</td>
</tr>
<tr>
<td>Dead of disease</td>
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</tr>
<tr>
<td>No</td>
<td>24</td>
<td>23 (95.8), 1 (4.2), .70</td>
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<tr>
<td>Yes</td>
<td>22</td>
<td>17 (77.3), 5 (22.7), .09</td>
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<tr>
<td>Low</td>
<td>10</td>
<td>10 (100.0), 0 (0.0), .0</td>
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<tr>
<td>High</td>
<td>36</td>
<td>30 (83.3), 6 (16.7), .32</td>
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<td>Tumor size, cm</td>
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<tr>
<td>⩽5</td>
<td>9</td>
<td>8 (88.9), 1 (11.1), .10</td>
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<tr>
<td>&gt;5</td>
<td>37</td>
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<tr>
<td>Tumor site</td>
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<td></td>
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<tr>
<td>Extremity</td>
<td>26</td>
<td>22 (84.6), 4 (15.4), .0</td>
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<tr>
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</tr>
<tr>
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<tr>
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<td>9</td>
<td>8 (88.9), 1 (11.1), .10</td>
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<td>p53 overexpression</td>
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</tr>
<tr>
<td>&lt;20%</td>
<td>33</td>
<td>29 (87.9), 4 (12.1), .0</td>
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<tr>
<td>⩾20%</td>
<td>13</td>
<td>11 (84.6), 2 (15.4), .0</td>
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<td>MDM2 amplification</td>
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<tr>
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<td>40</td>
<td>35 (87.5), 5 (12.5), .0</td>
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<tr>
<td>Yes</td>
<td>6</td>
<td>5 (83.3), 1 (16.7), .0</td>
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<td>MDM2 overexpression</td>
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<tr>
<td>&lt;20%</td>
<td>27</td>
<td>24 (88.9), 3 (11.1), .0</td>
</tr>
<tr>
<td>⩾20%</td>
<td>18</td>
<td>16 (88.9), 2 (11.1), .0</td>
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<td>pRB</td>
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<tr>
<td>Positive</td>
<td>25</td>
<td>21 (84.0), 4 (16.0), .0</td>
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</table>

*P values by Fisher’s exact test (two-tailed).
†Statistically significant (*P<.05).
§Malignant fibrous histiocytoma.
$Malignant peripheral nerve sheath tumor.
Southern blot analysis of 46 sarcomas, we found that seven (15%) had abnormalities in the INK4A and INK4B genes, including total or partial allelic deletions of both genes and a rearrangement of INK4A. Tumor types exhibiting these abnormalities were represented by three malignant peripheral nerve sheath tumors, two leiomyosarcomas, one malignant fibrous histiocytoma, and one liposarcoma. Homozygous deletion (i.e., loss of both alleles) of the INK4A gene was detected in five tumors (Fig. 1, D), and hemizygous deletion (i.e., loss of one allele) was observed in one tumor (Fig. 1, C). An abnormal pattern of INK4A bands was observed in one tumor (Fig. 1, B); the control probe did not show any abnormality in this DNA, suggesting a possible gene rearrangement. All of the tumors that exhibited homozygous deletions of the INK4A gene also presented with homozygous deletion of the INK4B gene (Fig. 1, D). The single case exhibiting partial loss or loss of heterozygosity (LOH) of the INK4A gene also displayed LOH of the INK4B gene (Fig. 1, C). The single case that displayed an abnormal pattern of bands for INK4A appeared to be normal for the INK4B gene (Fig. 1, B).

The analysis of PCR–SSCP data for all of the exons screened—including the exons 1α, 1β, and 2 of the INK4A gene, as well as the exons 1 and 2 of the INK4B gene—did not reveal altered fragment mobility shifts (data not shown). In cases where no signal was obtained after amplification by PCR, homozygous deletion of the INK4A and INK4B genes was confirmed by Southern blot analysis. The comparative multiplex-PCR data for exon 1β of INK4A, corresponding to p19ARF, revealed that losses of this locus occurred in six cases. The five tumors displaying homozygous deletion of p19ARF (i.e., INK4A exon 1β) showed concomitant homozygous deletions of the remaining exons of the INK4A gene, as well as of the INK4B gene (Fig. 2). Similarly, the single tumor that exhibited partial loss of p19ARF exon 1β also showed LOH of the INK4A gene and of the INK4B gene.

Samples that retained one or more alleles of the INK4A gene were analyzed for the methylation status of the 5’ CpG island. Methylated and unmethylated control DNAs showed the corresponding methylated- and unmethylated-specific fragments, respectively. All of the 33 tumors analyzed revealed the absence of the methylation-specific fragment.

The overall frequency of alterations in this cohort of soft tissue sarcomas was approximately 15% for the INK4A and INK4B genes, with these changes affecting high-grade sarcomas exclusively. Statistically significant associations were observed between INK4A and INK4B gene deletions and poor survival (P = .036; Fig. 3, A) and between INK4A/INK4B gene alterations and poor survival (P = .005; Fig. 3, B). In a multivariate analysis that used the Cox proportional hazards model, the calculated RRs after controlling for tumor grade and size were 2.29 (95% CI = 0.80–6.58; P =...
for INK4A/INK4B deletions and 2.98 (95% CI = 1.09–8.18; \( P = .03 \)) for INK4A/B alterations. None of the RR, calculated from the proportional hazards model, for either tumor grade or size were statistically significant (data not shown).

The tumors were also analyzed for the presence of TP53 mutations (exons 4–8) and MDM2 amplifications. In addition, altered patterns of p53, Mdm2, and pRB protein expression were further characterized. Table 1 summarizes the results obtained from the analyses of the INK4A and INK4B status with regard to TP53 and MDM2 alterations, as well as with regard to p53, Mdm2, and pRB protein expression phenotype. Sixteen (35%) of the 46 sarcomas showed TP53 mutations and/or p53 nuclear overexpression (data not shown). Shifts in mobility corresponding to point mutations affecting TP53 exon 5 (three tumors), exon 6 (one tumor), exon 7 (three tumors), and exon 8 (two tumors) were identified. Thirteen (28%) tumors showed p53 overexpression. Two tumors that displayed TP53/p53 alterations presented with a homozygous INK4A and INK4B deletion, while another tumor with abnormal p53 expression showed a partial deletion of the INK4A and INK4B genes. MDM2 gene amplification was detected in six tumors, while Mdm2 protein overexpression in the nucleus was observed in 18 (40%) of 45 sarcomas (data not shown). One sample could not be evaluated because of limited tissue availability. It is interesting that only three of the six tumors that showed MDM2 amplification also displayed Mdm2 protein overexpression. Overall, MDM2/Mdm2 alterations were identified in 21 (46%) of 46 sarcomas. Only one tumor that displayed TP53/p53 and MDM2/Mdm2 alterations had heterozygous deletions of INK4A and INK4B; the remaining INK4A/INK4B alleles were wild type. Sixteen (39%) of 41 assessable tumors showed negative staining for the pRB protein. Only in one tumor was the negative pRB phenotype associated with an alteration of the INK4A gene, corresponding to the tumor in which an abnormal pattern of INK4A bands was detected. There were no significant associations between INK4A/INK4B status and TP53 or MDM2 mutations or between INK4A/INK4B status and alterations affecting either p53, Mdm2, or pRB protein expression.

### DISCUSSION

We report an overall frequency of deletions and rearrangements for the INK4A and INK4B genes in adult soft tissue sarcomas of 15%. We did not detect any point mutations either in exons 1 and 2 of the p16 gene (97.5% of the total coding sequence) or in exons 1 and 2 of the p15 gene (75% of the total coding sequence). Similarly, we did not identify a case in which the p16 promoter underwent de novo methylation. The additional complexity that results from the presence of a second INK4A-encoded gene product, p19\(^{ARF}\), was also taken into consideration in this study. The analysis of the INK4A-alternative reading frame (exons 1B and 2, 100% of the total coding sequence) revealed the absence of tumor-specific point mutations. However, five homozy-
gous deletions and a partial allelic loss were identified as affecting exon 1β, which specifically encodes the amino-terminal domain of p19ARF. All tumors displaying homozygous or heterozygous p19ARF exon 1β deletions had concomitant deletions of the p16 and p15 genes. Genotypic or phenotypic alterations of TP53 were detected in 16 cases, only two of which simultaneously displayed a homozygous deletion affecting both INK4A and INK4B genes. Alterations in the MDM2 gene and/or in Mdm2 protein expression were identified in 21 tumors. Of interest, a tumor in which both TP53 and MDM2 were altered also showed LOH at the INK4A/INK4B region, but the contralateral alleles, including INK4A exon 1β, were found to be of the wild type. Similarly, only one tumor in which concomitant pRB and INK4A alterations were identified possessed an abnormal pattern of INK4A bands, suggestive of a rearrangement. Six tumors had p53 and pRB alterations; however, none of these cases showed mutations in either the INK4A or the INK4B genes. These results suggest that additional alterations in the p53 pathway (through changes in p19ARF) and the pRB pathway (through changes in p16 and p15) as part of tumorigenesis of sarcomas would be unnecessary and redundant. Therefore, p16/CDK4/cyclin D1/RB pathway (through changes in p16 and p19ARF) as part of tumorigenesis of sarcomas would be unnecessary and redundant. Alterations in the MDM2 gene and/or in Mdm2 protein expression were identified in 21 tumors. Of interest, a tumor in which both TP53 and MDM2 were altered also showed LOH at the INK4A/INK4B region, but the contralateral alleles, including INK4A exon 1β, were found to be of the wild type. Similarly, only one tumor in which concomitant pRB and INK4A alterations were identified possessed an abnormal pattern of INK4A bands, suggestive of a rearrangement. Six tumors had p53 and pRB alterations; however, none of these cases showed mutations in either the INK4A or the INK4B genes. These results suggest that additional alterations in the p53 pathway (through changes in p19ARF) and the pRB pathway (through changes in p16 and p15) as part of tumorigenesis of sarcomas would be unnecessary and redundant. Coordinate inactivation of p53 and pRB appears to be an essential requirement for the genesis of most human cancers (42–44). It has also been postulated that the loss of function of certain CKIs, mainly p16, might also lead to tumor development (32). Even though, at the protein sequence level, p16 and p15 share 82% homology, their activities are regulated differently. p15 is an effector of transforming growth factor-β (TGF-β)-induced cell cycle arrest (44). The regulatory pathway involving p16 has not yet been well characterized; however, membrane-signaling pathways, including those mediated by TGF-β, do not appear to be involved. A report (45) has shown that p16 accumulates in cell lines devoid of pRB, which suggests that its expression may be influenced by a transcription factor modulated by pRB. Evidence suggests that the p16/CDK4/cyclin D1/RB pathway behaves as a single mutagenic target during tumorigenesis (1–3). Cell cycle arrest produced by p19ARF has been shown to be dependent on p53, because cells that lack p53 do not respond to the action of p19ARF (33). More recently, it has been reported that p19ARF interacts with Mdm2 protein and blocks the Mdm2-induced degradation and transactivation silencing of p53 (21,22). Several studies (44,46,47) have reported that alterations of p53 and pRB are potentially synergistic on the proliferative activity of tumors and that they exert a cooperative negative effect on both progression and survival in certain human primary tumors. It is postulated that aberrant p53 and pRB expression deregulates cell cycle control at the G1 checkpoint, resulting in tumor cells with reduced response to programmed cell death. The imbalance produced by enhanced proliferative activity combined with decreased apoptosis may explain the aggressive clinical course of tumors harboring both p53 and pRB alterations. The recent evidence that INK4A encodes two products that have an impact on these two suppressor pathways, p16 through the pRB pathway and p19ARF through the p53 pathway, positions the INK4A gene at the nexus of the two most critical tumor-suppressor pathways governing neoplasia (21–33). Inactivation of p16 and p19ARF in primary tumors may not be functionally equivalent to alterations in p53 and pRB expression but still might produce aggressive biologic behavior. Data from our study confirm this prediction, because adult patients affected with high-grade soft tissue sarcomas that harbor homozygous deletions of the INK4A gene had a poor clinical outcome. It is of interest that, in the cohort of sarcomas analyzed by us, there were no point mutations or methylation changes that could silence p16 alone and consequently affect the pRB, but not the p53, pathway.

In summary, recent data (21,22,33) provide for a “one gene/two products/two pathways” hypothesis that can explain the high rate of INK4A alterations in human primary cancers, as well as in a wide variety of tumor cell lines. These data also offer an answer for the tumor-prone phenotype observed in the Ink4a (32) and p19ARF (33) knockout models, as compared with other CKIs such as p21 (48) and p27 (49–51). Our study shows that the alteration of the INK4A and INK4B genes, particularly a coincident homozygous deletion that eliminates three important cell-growth inhibitors (p16, p19ARF, and p15), appears to be a frequent event in adult soft tissue sarcomas. Inactivation of p16 and p19ARF in primary tumors may not be functionally equivalent to alterations of p53 and pRB, yet it appears to produce cancers with aggressive biologic behavior. The statistically significant reduction in survival of patients bearing tumors with INK4A and INK4B gene alterations suggests that these genes may provide prognostic molecular parameters for the evaluation of patients affected by soft tissue sarcomas.

REFERENCES


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**NOTES**

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