Infrequency of MDM2 Gene Amplification in Pediatric Solid Tumors and Lack of Association with p53 Mutations in Adult Squamous Cell Carcinomas

Pamela G. Waber, Jun Chen, and Perry D. Nisen

ABSTRACT

Loss of function of the p53 tumor suppressor gene by point mutation is the most commonly detected genetic alteration in human cancer. There is growing evidence that amplification and overexpression of the MDM2 gene are alternative mechanisms that also lead to functional inactivation of p53. While p53 mutations and MDM2 amplification have been reported to occur in rhabdomyosarcoma and osteogenic sarcoma, the incidence of MDM2 in other pediatric solid tumors is not known. We therefore tested a series of other pediatric solid tumors for MDM2 gene amplification. MDM2 amplification could not be detected in specimens from 40 Wilms' tumors, 15 neuroblastomas, 12 sarcomas, or 4 hepatoblastomas tested. To determine whether MDM2 amplification was an alternative mechanism of p53 inactivation in adult carcinomas that frequently possess p53 mutations, 68 samples of squamous cell carcinomas of the upper aerodigestive tract, 24% of which were previously shown to contain p53 mutations, were also tested for MDM2 amplification. MDM2 amplification did not occur in any of the tumor specimens tested. These findings suggest that MDM2 amplification may only occur in a limited subset of human tumors. Loss of function of p53 may be an essential event in human tumorigenesis. If so, then other mechanisms of p53 inactivation must occur in those tumors that exhibit neither p53 mutation nor MDM2 amplification.

INTRODUCTION

The MDM2 gene was originally identified as a component of highly amplified double minute chromosomes in the mouse BALB/c 3T3 cell line (1). More recently, the human homologue of MDM2, located on chromosome band 12q13-14, has been cloned and characterized (2). MDM2 can complex with the p53 tumor suppressor gene and inhibit p53-mediated transcriptional transactivation by binding to a region that coincides with the p53 acidic activation domain (3, 4). In addition, overexpression of MDM2 increases the tumorigenicity of NIH3T3 cells (1) and overcomes wild-type p53 suppression of transformed cell growth (5).

Initial studies to determine whether MDM2 plays a role in human cancer focused on sarcomas since the chromosomal region where MDM2 is located is frequently altered in these tumors. MDM2 was found to be amplified in 17 of 47 sarcomas tested (2). Subsequently, MDM2 amplification was also observed in 3 of 11 cases of metastatic osteosarcoma and 1 case of local recurrence, but not in 16 cases of primary osteosarcoma (6). MDM2 amplification was also detected in 8 of 24 cases of other human soft tissue sarcomas (malignant fibrous histiocytoma and liposarcoma); while the p53 gene was mutated in 8 of these tumors, none of them exhibited alteration of both genes (7). Similarly, amplification of the MDM2 gene was detected in 8–10% of glioblastomas (8) and inhibit either p53 mutations (9–11) or MDM2 amplification (2, 6), the incidence of MDM2 amplification in other pediatric solid tumors including neuroblastoma and Wilms' tumor, which do not tend to have p53 mutations (12), is not known. In addition, neuroblastoma frequently exhibits double minute chromosomes and homogeneously staining regions indicative of gene amplification (13, 14). We therefore sought to determine the incidence of MDM2 amplification in a series of Wilms' tumors, neuroblastomas and other pediatric solid tumors. In addition, we also determined the incidence of MDM2 amplification as an alternative mechanism of p53 inactivation in a series of adult squamous cell carcinomas which were previously shown to frequently contain p53 mutations (15).

MATERIALS AND METHODS

Clinical Specimens. Tumor tissue was obtained as part of a protocol approved by the University of Texas Southwestern Medical Center Human Subjects Review Board. Tumor specimens were obtained from multiple institutions. Tumors were immediately frozen in liquid nitrogen and stored at −70°C.

Isolation and Characterization of Human MDM2 Probes. Two sequences corresponding to human MDM2 were isolated by reverse transcriptase-PCR. A 5′ sequence was obtained using the following primers: 5′:CCGAGCTTTCGGAACAAGAGAC and 3′:TGAAGGTTTCTCTTCTCCCTG-CAA. These sequences were chosen from the published sequence of the human MDM2 gene (2). A reverse transcriptase-PCR kit was used (PerkinElmer, Norwalk, CT) under conditions recommended by the manufacturer, and RNA extracted from the human CaCo2 cell line. The CaCo2 cell line was derived from a human colon carcinoma and was the source of the original human MDM2 cDNA clone (2). The 386-base pair PCR product was characterized by direct nucleotide sequence analysis using Sequenase Version 2.0 (United States Biologicals, Cleveland, OH). A second sequence from the middle of the coding region of human MDM2 was similarly obtained and characterized using commercially available primers (human mdm-2 amplier sequences; Clontech, Palo Alto, CA).

Southern Blot Analysis. High molecular weight chromosomal DNA was extracted from tumor samples, digested to completion with EcoRI restriction endonuclease (Boehringer Mannheim, Indianapolis, IN) using conditions recommended by the manufacturer, separated by 1% agarose gel electrophoresis, and transferred to nylon membranes (Nytran; Schleicher and Schuell, Keene, NH) as described previously (16). MDM2 sequences were labeled with 32P by PCR and hybridized to the nylon membranes using conditions described elsewhere (16). The extent of hybridization was quantitated using a Molecular Dynamics Phosphorimager and normalized to a single copy gene control (Ig heavy chain, JH) (16).

p53 Mutational Analysis. Tumor samples were initially screened for p53 mutations by single strand conformational polymorphism analysis. Exons 5–8 of the human p53 gene were separately amplified by PCR using conditions described elsewhere (17). Digested PCR products were denatured in formamide, heated to 95°C, and loaded onto a 6% acrylamide nondenaturing gel containing 10% glycerol. Gels were run at 4 W for 16–18 h at room temperature with a cooling fan. Gels were dried at 80°C for 1.5 h, and autoradiography was performed with an intensifying screen for 10–48 h at −70°C. Fragments exhibiting altered mobility were subjected to direct DNA sequence analysis using Sequenase Version 2.0 (United States Biologicals) (17).

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2 To whom requests for reprints should be addressed, at Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235–9063.
RESULTS

One hundred thirty-nine different tumor specimens were tested for MDM2 gene amplification by Southern blot analysis (Table 1; Fig. 1). While MDM2 gene amplification could be readily detected using chromosomal DNA from the OsA human osteogenic sarcoma cell line (positive control), no amplification of MDM2 could be detected in any of the tumor specimens tested. Five of the 15 neuroblastoma specimens tested exhibited n-myc gene amplification and cytogenetic evidence of double minute chromosomes or homogeneously staining regions. Many of the tumor specimens were also screened for p53 mutations: while none of the Wilms' tumor specimens (12) or congenital mesoblastic nephromas (this report) had detectable p53 alterations, 24% of the squamous cell carcinomas exhibited point mutations of the p53 gene (15).

DISCUSSION

Inactivation of the p53 tumor suppressor gene is the most commonly detected genetic alteration in human cancer (18). While loss of function of p53 is most frequently accomplished by point mutation of the gene, there is growing evidence that amplification and resultant overexpression of the MDM2 gene can also lead to functional inactivation of p53 (2, 6–8). MDM2 protein regulates p53 protein by forming oligomers with p53 that inhibit transcriptional transactivation of other genes (3). To date, amplification and overexpression of MDM2 has been detected in rhabdomyosarcomas, other soft tissue sarcomas, osteogenic sarcoma, and malignant gliomas (2, 6–8). Since other pediatric solid tumors, particularly neuroblastomas, often contain double minute chromosomes and homogeneously staining regions indicative of gene amplification, it was plausible that MDM2 amplification would occur in these tumors as well. However, this could not be detected in the series of neuroblastomas, Wilms' tumors, and other pediatric solid tumors that we tested. It should be noted that our series did not include other pediatric solid tumors such as neuroepithelioma or Ewing's sarcoma. Our inability to detect MDM2 amplification in sarcomas is probably due to the relatively small number of tumors that were tested.

In adult neoplasms, it has been suggested that MDM2 amplification can be an alternative mechanism for p53 inactivation in tumors that do not exhibit mutations of the p53 gene. We therefore similarly studied a series of 68 samples of squamous cell carcinoma of the upper aerodigestive tract. We previously determined that p53 mutations occurred in 24% of these tumors (15). We were unable to detect MDM2 amplification in any of these neoplasms. These findings suggest that MDM2 amplification may only occur in a limited subset of human tumors. Inactivation of p53 may be an essential event in human tumorigenesis. If so, then additional mechanisms, perhaps overexpression of other p53-binding proteins, may occur in those tumors that exhibit neither p53 mutations nor MDM2 amplification.

Table 1. Tumor samples tested for MDM2 amplification

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number Tested</th>
<th>Incidence of p53 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilms Tumor</td>
<td>40</td>
<td>ND&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neuroblastoma&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15</td>
<td>ND&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sarcoma&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>Hemangiendothelioma</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>Congenital Mesoblastic Nephroma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Renal Cell Carcinoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Squamous Cell Carcinoma</td>
<td>68</td>
<td>24%&lt;sup&gt;15&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Five n-myc amplified tumors.
<sup>2</sup> Seven rhabdomyosarcomas, 3 undifferentiated sarcomas, 2 osteogenic sarcomas
<sup>3</sup> ND, not done.


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