Expression of Mutant p53 in Melanoma

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ABSTRACT

Mutant p53 has been noted in a variety of human malignancies including carcinomas of lung, breast, and colon, which have also been reported to have frequent karyotype anomalies involving the locus of the p53 gene (17p13). Whereas chromosomal abnormalities of chromosomes 1, 6, and 7 have been noted previously in melanoma, frequent aberrations in chromosome 17 have not been reported previously. Due to the common mutation of this locus in so many types of neoplasms, a range of melanomas from different stages of tumor progression were examined immunohistochemically for expression of mutant p53, in order to assess its prevalence and consider the role of this oncogene in the biological progression of melanoma. Forty-five of 53 (85%) specimens from a range of primary and metastatic melanomas were found to have detectable evidence of p53 gene mutation, by virtue of the immunohistochemical detection of mutant p53 protein. Significantly increased prevalence of mutant p53 was found in metastatic melanoma, compared with primary tumors (P < 0.05). These findings represent one of the highest incidences of this oncogenic mutation yet recorded in a human malignancy and support the concept that p53 may have a functional role in development of the metastatic tumor phenotype.

INTRODUCTION

The p53 phosphoprotein was initially detected as a host cell protein bound to large T antigen (1, 2), which is the dominant transforming oncogene of the SV40 DNA tumor virus. Experiments in murine systems indicate that only a few thousand molecules of wild-type p53 exist in a normal cell and that they may have a function regulating the replication of DNA (3, 4). However, when point mutations occur within the coding region of the gene, it is converted functionally to an oncogene that can interfere with the function of wild-type p53 (3, 5, 6). Furthermore, it is recognized that mutation of p53 facilitates ras oncogene transformations (7).

Mutant p53 expression has been identified as a frequent genetic change in a variety of human tumor types (8). Primary malignancies of breast (9), lung (10, 11), esophagus (12), and colon (13) and osteosarcoma (14) are frequently associated with mutations of the p53 gene. The chromosome locus of p53 is 17p13 (15, 16), and deletions involving this locus are common in the aforementioned neoplasms (8, 17-19). Mutation of p53 is strongly associated with loss of heterozygosity at this locus, suggesting that the normal allele is lost, with only the mutant remaining.

Although aberrations of chromosomes 1, 6, and 7 are well documented in melanoma, and others involving chromosomes 2, 3, 9, and 10 have been described (20-23), those of chromosome 17 have not been reported. Furthermore, the chromosome alterations reported have most commonly involved translocations, deletions, and isochromosomes.

Wild-type p53 has a short intracellular half-life of only 15 min, whereas mutant p53 proteins, which form complexes with heat shock protein 70 in the cytoplasm and also bind wild-type p53 (24), have a longer half-life, of several hours (25-27). The extension of the half-life of p53 results in a sufficient increase in the amount of intracellular protein for it to become immunohistochemically detectable. Thus, higher cellular levels of the proteins and their distribution in the cytoplasm provide parameters by which the mutations of the gene can be assessed. This approach has been used to detect evidence of the mutant oncogene in primary breast cancers (28) and 82% of squamous cell carcinomas of the lung (10). The aim of this study was to ascertain the frequency of mutant p53 expression in human melanoma and to explore any correlation between such mutations and the stage of tumor progression, as described by Clark et al. (29).

MATERIALS AND METHODS

Primary and metastatic melanomas, together with a range of other melanocytic lesions, were collected from fresh surgical material at the Department of Plastic Surgery, Radcliffe Infirmary, and the Department of Pathology, Rigshospitalet, Copenhagen. Representative samples of tumor were snap-frozen in liquid nitrogen and stored at −70°C. Conventional diagnostic histopathology was performed on sections prepared by standard techniques from paraffin-embedded material. Twenty primary cutaneous melanomas, 20 regional lymph node metastases, and 13 distant (non-nodal) metastases were examined (Table 1). The primary tumors were considered in subgroups, according to the Breslow thickness assigned to the tumor at the diagnostic assessment.

Immunohistochemical examination of the tumor library was performed with a murine monoclonal antibody PAb 240 (30), that detects an evolutionarily conserved epitope on p53. The mutant protein-antibody complexes were detected using an alkaline phosphatase-antialkaline phosphatase technique (31). A squamous lung cancer, which had been shown by nucleotide sequencing to contain p53 mutations, and omission of the primary antibody were used, respectively, as positive and negative immunocytochemistry controls. Briefly, 7-µm-thick cryostat sections were incubated in undiluted hybridoma supernatant at room temperature for 30 min. The sections were then washed in Tris-buffered saline (0.05 M Tris–HCl in isotonic saline, pH 7.6) and incubated for 30 min sequentially with rabbit anti-mouse antibody (Dakopatts Z259 at 1/25 dilution) and then preformed alkaline phosphatase/antialkaline phosphatase complex (32). A squamous lung cancer, which had been shown by nucleotide sequencing to contain p53 mutations, and omission of the primary antibody were used, respectively, as positive and negative immunocytochemistry controls. Briefly, 7-µm-thick cryostat sections were incubated in undiluted hybridoma supernatant at room temperature for 30 min. The sections were then washed in Tris-buffered saline (0.05 M Tris–HCl in isotonic saline, pH 7.6) and incubated for 30 min sequentially with rabbit anti-mouse antibody (Dakopatts Z259 at 1/25 dilution) and then preformed alkaline phosphatase/antialkaline phosphatase complex (Dakopatts D651 at 1/50 dilution), following intervening rinses in Tris-buffered saline. The latter two incubations were repeated for 10 min each before development with chromogen, in the form of naphthol AS–BI phosphate (Sigma no. N2250), with new fuchsin (Merck no. 4041).

RESULTS

Overall, 85% of tumor specimens examined were found to express detectable amounts of p53, using monoclonal antibody 240. More than 70% of positive tumors displayed a homogeneous staining pattern, with more than half the tumor cells in the section exhibiting positive staining. In the remaining tu-
Mutant p53 expression

Table 1 Expression of mutant p53 in melanoma detected with monoclonal antibody (MAb) PAb 240

<table>
<thead>
<tr>
<th>Melanoma</th>
<th>No. of lesions examined</th>
<th>No. of lesions expressing p53</th>
<th>Frequency of nuclear staining with MAb 240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplastic naevi</td>
<td>3</td>
<td>0 (0%)</td>
<td>+</td>
</tr>
<tr>
<td>&lt;1.5 mm</td>
<td>5</td>
<td>3 (60%)</td>
<td>++</td>
</tr>
<tr>
<td>&gt;1.5 mm</td>
<td>15</td>
<td>11 (73%)</td>
<td>+++;</td>
</tr>
<tr>
<td>Metastases</td>
<td>33</td>
<td>31 (93%)</td>
<td>+++;</td>
</tr>
<tr>
<td>Total no. of melanomas</td>
<td>53</td>
<td>45 (85%)</td>
<td>+++;</td>
</tr>
</tbody>
</table>

*+, ++, and +++ are visual estimates of the approximate percentages of labeled tumor cells; +, <20%; ++, 20-50%; +++, >50%.

DISCUSSION

This study adds further data confirming that mutation of the p53 gene, as assessed by sequencing and usually also indicated by the abnormal expression of p53 protein, is a widespread abnormality in cancer (8, 32). Furthermore, it demonstrates that the frequency of this mutation in human cutaneous melanoma is higher than has been detected previously in many other human malignancies. Although it is not readily apparent why the prevalence of p53 mutations should be so high in melanoma, it is relevant to consider the capacity of UV light to induce point mutations in proto-oncogenes. In vitro studies have established that UV irradiation can induce mutations in N-ras (33), and subsequently this effect has been confirmed in clinical melanomas from sun-exposed body sites (34). There are no data describing UV-induced mutations of the p53 proto-oncogene, although it would appear to be a relevant topic for investigation.

The increased prevalence of the mutation in metastatic tumors is of particular significance because it provides the first clinical evidence that p53 may have a functional role in development of the metastatic tumor phenotype. Pohl et al. (35) have demonstrated that elevated p53 levels resulting from the transfection of bladder cancer cells result in an increased metastatic capacity. Again, the ras oncogenes are more frequently detected in melanoma metastases (36, 37). Since progression in cancer is considered a multistep process, it is interesting to note that activation of both the ras and p53 proto-oncogenes are more commonly detected in advanced forms of melanoma. The potential significance of these oncogenes in tumor pro-

Fig. 1. Representative examples of the cytological patterns of staining seen in this series of melanomas with the anti-p53 monoclonal antibody PAb 240. In A, approximately 50% of the tumor cells in a primary melanoma are p53 positive, with a virtually exclusive nuclear pattern of staining. Note the reactive lymphocytes above the melanoma, which are completely negative. B is a case of metastatic melanoma in a regional lymph node. The tumor cells are practically all p53 positive and, although the location is predominantly nuclear, some definite cytoplasmic staining can be seen. This is highlighted at higher magnification (inset). C is an additional case of primary melanoma, in which most of the tumor cells are p53 positive. Although some nuclear staining can be discerned, the great majority of the labeling is clearly cytoplasmic.
gression is highlighted by the finding that mutated p53 has the capacity to cooperate with ras in transformation assays (7). It has been suggested previously that the nonrandom alterations of chromosomes 1, 6, and 7 noted in human melanoma cell karyotypes (21, 22) may have a role in the carcinogenesis of this tumor, and linkage analysis has suggested that a susceptibility gene for cutaneous melanoma may be located on the short arm of chromosome 1, near the Rh locus (38). Although deletions affecting chromosome 17 are not reported for melanoma, point mutations and small deletions would not have been detected by most forms of karyotype assessment. Furthermore, although a single normal allele may still be present in some of these cases, mutant p53 is able to complex the wild-type p53 and thereby act as a dominant negative oncogene (6).

It is notable that the cellular localization of the mutant protein is particularly variable in melanoma. The immunocytochemistry staining patterns included discrete nuclear staining, combined nuclear and cytoplasmic staining, and staining of the cytoplasm alone. Cytoplasmic staining has been previously reported in melanoma, point mutations and small deletions would not have been detected by most forms of karyotype assessment. Furthermore, although a single normal allele may still be present in some of these cases, mutant p53 is able to complex the wild-type p53 and thereby act as a dominant negative oncogene (6).


phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes).


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