Expression of mdm2 and p53 in epithelial neoplasms of the colorectum

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Abstract

Aims—To evaluate the respective roles of mdm2 (murine double minute 2) and p53 in the development of colorectal carcinoma.

Methods—Formalin fixed, paraffin wax embedded tissues from 72 sporadic adenomas and 55 carcinomas were investigated by means of immunohistochemistry for mdm2 and p53.

Results—mdm2 was expressed weakly in 17 of 72 (23.6%) adenomas and in 14 of 55 (25.4%) carcinomas. p53 was expressed in 19 of 72 (26.4%) adenomas and in 23 of 55 (41.8%) carcinomas. Four adenomas and five carcinomas showed positive staining for both proteins. Overexpression of p53 in adenomas was associated with moderate and severe dysplasia but not with tumour size. No associations were found between the expression of mdm2 and either the degree of dysplasia or tumour size. In carcinomas, neither the expression of p53 nor mdm2 correlated with Dukes’s stage, metastasis, or differentiation. No associations were found between the expression of p53 and mdm2 in either adenomas or carcinomas.

Conclusions—Although mdm2 has been reported to be an oncogene, it does not appear to play a major role in the development of colorectal carcinoma.

Keywords: mdm2; p53; colorectal carcinoma
Elite ABC kit (1/50 dilution for 30 minutes; Vector Laboratories). 3–3′diaminobenzidine was used as the chromogen. The same procedure was used for p53 using a 1/100 dilution of the monoclonal antibody Do-7 (Dako, High Wycombe, Bucks, UK). Sections from a uterine leiomyosarcoma and a breast carcinoma were used as positive controls for mdm2 and p53, respectively. Negative controls were obtained by replacing the primary antibody with PBS.

**SCORING METHODS**

The immunohistochemistry slides were evaluated independently by two observers (XPH and TG) who were blind to the categorisation of the tumours. The whole slide was examined and the percentage of tumour cells that showed nuclear staining was scored. Twenty per cent was taken as the cut off point to define positivity for mdm2, and 10% as the threshold of positivity for p53. The two sets of results were correlated and in the event of disagreement the slides were reviewed together and a consensus reached.

**STATISTICS**

The χ² test was used to examine the associations between either mdm2 or p53 expression and clinicopathological factors (such as tumour size, degree of dysplasia, differentiation). Fisher’s exact test was used to assess the correlations between mdm2 and p53 in both adenomas and carcinomas. Statistical significance was defined as p < 0.05.

**Results**

**NORMAL TISSUES**

Normal mucosa from non-inflamed diverticular disease in 3 of 16 (18.8%) cases, and adjacent to an adenoma or carcinoma in 15 of 85 (17.6%) cases showed positive staining for mdm2 (fig 1A). There was no statistical difference between mdm2 expression in adenomas or carcinomas and adjacent normal epithelial cells (both p > 0.1; table 1). Furthermore, 31 of 127 (24.4%) adenomas and carcinomas also contained positively stained lymphocytes and stromal cells, including some smooth muscle cells. In some cases, smooth muscle cells showed nuclear accumulation...
Table 1  mdm2 expression in normal, adenomatous, and carcinomatous tissues

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>+ve</th>
<th>−ve</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mucosa</td>
<td>15</td>
<td>85</td>
<td>&gt;0.1*</td>
</tr>
<tr>
<td>Adenoma</td>
<td>17</td>
<td>55</td>
<td>&gt;0.1†</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>14</td>
<td>41</td>
<td>&gt;0.75‡</td>
</tr>
</tbody>
</table>

*Normal mucosa v adenoma; †Normal mucosa v carcinoma; ‡Adenoma v carcinoma.

Table 2  Expression of mdm2 and p53 in colorectal adenomas

<table>
<thead>
<tr>
<th>mdm2</th>
<th>p53</th>
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<tbody>
<tr>
<td>+ve</td>
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</tr>
<tr>
<td>+ve</td>
<td>4</td>
</tr>
<tr>
<td>−ve</td>
<td>15</td>
</tr>
</tbody>
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Table 3  Correlation between mdm2 and p53 in colorectal adenomas and carcinomas

<table>
<thead>
<tr>
<th>mdm2</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>−ve</td>
</tr>
<tr>
<td>+ve</td>
<td>4</td>
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<tr>
<td>−ve</td>
<td>15</td>
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<tr>
<td>p</td>
<td>&gt;0.75</td>
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Table 4  Expression of mdm2 and p53 in colorectal carcinomas

<table>
<thead>
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<th>p53</th>
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</thead>
<tbody>
<tr>
<td>+ve</td>
<td>−ve</td>
</tr>
<tr>
<td>+ve</td>
<td>8</td>
</tr>
<tr>
<td>−ve</td>
<td>3</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.50</td>
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Discussion

Using immunohistochemistry, the mdm2 protein was detected in 23.6% of adenomas and 26.4% of carcinomas. This is less frequent than is found in breast (40.9%) and bronchogenic carcinomas (63%).13 14 As is seen in breast carcinoma,15 no associations were detected between overexpression of mdm2 and any clinicopathological parameters; however, in bronchial carcinoma,16 soft tissue sarcoma,17 and osteosarcoma,18 mdm2 overexpression is related to reduced differentiation and greater metastatic potential. In the present study, the positive cases showed only weak staining and a low percentage of positive cells. No significant differences in mdm2 expression were detected between adenomas and carcinomas or between adenomas or carcinomas and adjacent normal epithelial cells. In addition, normal mucosa from non-inflamed diverticular disease, and lymphocytes, stromal cells, and smooth muscle cells in tumour tissues also showed some positive staining. Therefore, as is the situation in cervical cancer,19 mdm2 does not seem to play an important role in colorectal carcinogenesis.

The tumour suppressor gene p53 is a transcriptional activator that regulates cell growth and differentiation. Mutations of the p53 gene are common in human malignancies, including colorectal carcinoma, in which such mutations are thought to be late events.17 p53 immunoreactivity was not found in any normal epithelia adjacent to the adenomas or carcinomas or in tissue from non-inflamed diverticular disease. p53 staining was detected in 26.4% of adenomas and 41.8% of carcinomas. This is in line with previous studies.18 19 p53 nuclear accumulation begins to appear at the stage of mild dysplasia, but it is increased significantly in moderate and severe dysplasia. No difference was found between carcinomas and adenomas with moderate and severe dysplasia. This suggests that p53 mutations may occur at the moderately and severely dysplastic stages in adenoma development.

The consequence of the interaction between mdm2 and p53 remains obscure. Previous studies have shown that overexpression of the mdm2 gene can abolish the transactivating capability of the p53 protein, leading to the overexpression of p53.20 On the other hand, overexpression of p53 can induce mdm2 mRNA and protein expression. A reciprocal relation between p53 and mdm2 has been proposed as a mechanism for regulating the cell

while adenomatous or carcinomatous epithelium was negative (fig 1B). All non-neoplastic tissues were negative for p53.

ADENOMAS

The immunoreactivities of mdm2 and p53 are shown in tables 2 and 3. mdm2 immunoreactive cells were seen in 17 of 72 (23.6%) adenomas. All positive cells showed nuclear staining but this was generally weak. No cytoplasmic staining was seen (fig 1C). There were no associations between mdm2 expression and either the grade of dysplasia or tumour size. p53 immunoreactivity was detected in 19 of 72 (26.4%) adenomas (fig 1D). p53 overexpression was associated with moderate and severe dysplasia (p = 0.005) but not with tumour size. Four cases (5.6%) showed positivity for both mdm2 and p53 (table 3). There was no correlation between expression of mdm2 and p53 (table 3). There was also no significant difference between mdm2 expression in adenomas and carcinomas (p > 0.75; table 1).

CARCINOMAS

Fourteen of the 55 carcinomas (25.4%) expressed mdm2 (fig 1E), while 23 (41.8%) were positive for p53 (fig 1F) (table 4). Five cases (9.1%) were immunoreactive for both proteins (table 3). Neither mdm2 nor p53 were associated with clinicopathological factors such as Dukes’s stage, differentiation, or metastasis. No differences in p53 overexpression were found between carcinomas and adenomas with respect to moderate and severe dysplasia (table 5). There was also no correlation between the expression of mdm2 and p53 in carcinomas (table 3).
cycle. Recent research has suggested that mdm2 expression can also lead to the rapid degradation of both wild-type and mutant p53.\textsuperscript{11, 12} These conflicting results may be related to different isoforms of mdm2\textsuperscript{14} interacting with p53 or to different interacting domains in mdm2 or the p53 protein.\textsuperscript{21} Hence, the relation between mdm2 and p53 is complex and is not understood fully at the moment. In this study, we did not find any correlation between the expression of mdm2 or p53 in either adenomas or carcinomas. It is possible that the interaction between p53 and mdm2 depends on cell type,\textsuperscript{22} so that the results of this interaction might also vary in different types of tissues. Further investigations are under way to clarify the molecular relation between p53 and mdm2 in colorectal tumours.