Immunohistochemically detectable p53 and mdm-2 oncoprotein expression in colorectal carcinoma: prognostic significance

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Abstract

Aims—To investigate the correlation between the expression of the p53 and mdm-2 oncoproteins and to assess their prognostic value in colorectal cancer.

Methods—Using a polyclonal (CM1) and a monoclonal antibody directed against p53 and mdm-2, respectively, these oncoproteins were stained immunohistochemically in 109 colorectal adenocarcinomas.

Results—p53 was detected in less than 10% of tumour cells in 11 of 109 adenocarcinomas, in 10–50% of tumour cells, in 17 of 109 adenocarcinomas, and in more than 50% of tumour cells in 32 of 109 adenocarcinomas. Expression of mdm-2 was detected in 22 of 109 (20%) cases investigated, of which 19 showed concomitant p53 expression. In most cases mdm-2 immunoreactivity was strongly associated with a small proportion of p53 positive tumour cells. Both p53 and mdm-2 expression lacked statistical significance when correlated with common staging and grading parameters.

Conclusions—Detection of p53 and mdm-2 oncoprotein expression, detected using immunohistochemistry, is of no prognostic value in colorectal cancer. However, the close correlation between mdm-2 immunoreactivity and the proportion of p53 positive cells provides further evidence that the mdm-2 gene product interacts with p53 protein.

Keywords: Immunohistochemistry, p53, mdm-2, colorectal cancer.

Somatic mutations of the tumour suppressor gene p53 have attracted much attention as the most common single lesions in human tumours including colorectal cancer.5–8 p53 gene mutations are found in 50–70% of colorectal cancers. The mutant p53 protein has a different conformation to wild-type p53, is more stable and can be detected by immunohistochemistry. In colorectal,9,10 bladder,11 and lung cancer11 good agreement was found between the frequency of gene mutations and immunohistochemically detectable p53 protein. However, the value of detecting p53 overexpression for the determination of prognosis in colorectal adenocarcinoma is still a matter of debate.12–16 Recently, a cellular protein, the murine double-minute-2 (mdm-2) gene product, was shown to complex with wild-type and mutant p53 protein bringing about functional inactivation of the p53 gene.17–19 Amplification of this proto-oncogene was detected in sarcomas not carrying p53 mutations19 but has not been detected in colorectal carcinoma as yet.

In the present study an immunohistochemical analysis of 109 colorectal adenocarcinomas was performed using a polyclonal p53 antibody (CM1) and a monoclonal mdm-2 antibody. The study design was based on recent data suggesting that wild-type p53 protein may be detectable by immunohistochemistry in the presence of the mdm-2 gene product. The immunohistochemical results were compared with the clinical outcome of patients to evaluate the possible prognostic value of detecting one or both oncoproteins.

Methods

Tumour tissue from 109 consecutive cases of colorectal adenocarcinoma (43 rectal carcinomas and 45 carcinomas of the left and 21 of the right colon; 56 men, 53 women; mean age 67.8 years, range 35–90 years) was studied. All patients had undergone surgery between 1984 and 1986 at the Department of Surgery I, Innsbruck University Hospital, Austria, either with curative (n=92) or palliative intent (n=17). Patients who died within 30 days of surgery, with adjuvant chemotherapy and radiotherapy or both, or members of families with familial adenomatosis coli or hereditary nonpolyposis colorectal cancer were excluded from this study.

Tumour tissue was routinely processed, fixed in formalin, embedded in paraffin wax and classified according to Dukes’ classification,20 with an added D stage for those patients with
distant metastases, the TNM staging system, and the World Health Organisation (WHO) grading system. Lymphocytic infiltration at the advancing edge of the tumour was determined according to the criteria outlined by Jass et al. A detailed description of the staging and grading results is presented in Table 1.

Table 1 shows the frequencies of prognostic parameters investigated in 109 colorectal adenocarcinomas.

Table 2 shows the frequencies of p53 scores according to Dukes' stage.

Table 3 shows the frequencies of p53 scores according to tumour site.

Table 4 shows the p53 immunoreactivity with regard to mdm-2 scores.

Figure 1 shows the survival curve (Kaplan-Meier) for all 109 patients investigated in the study.

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Body (Oncogene Science, USA)), the enzyme colour reaction, light haematoxylin counterstaining, and section mounting were carried out as described elsewhere.

Semi-quantitative evaluation of p53 and mdm-2 immunoreactivity was performed by one of the authors (KWS). Immunoreactivity was scored as follows: no immunoreactivity (-); positive staining in less than 10% of tumour cells (+); positive staining in 10-50% of tumour cells with a mainly focal distribution (++); positive staining in more than 50% p53 positive tumour cells (+++).

All data were analysed using the SYSTAT statistical package including the SURVIVAL supplementary module. Patients were followed according to the oncological follow up scheme of the Department of Surgery I: patients underwent clinical and laboratory examinations every three months within the first three years, every six months four and five years after surgery, and once a year thereafter. Colonoscopy or barium enema and chest x ray were performed twice a year in the first three years and once a year until five years after surgery. The data concerning the date and cause of death were confirmed by the Österreichisches Statistisches Zentralamt, an Institute of the Austrian government. Cumulative patient survival was estimated using the Kaplan-Meier method; the log rank test was used to compare survival curves (Mantel–Haenszel). Descriptive statistics for continuous measures are given as the mean (SD); for discrete data, frequency counts and percentages are tabulated and groups were compared using χ² analysis with Yates' correction where appropriate.
Results

Positive p53 stained nuclei were detected in 60 of 109 (55%) colorectal carcinoma cases. Immunohistochemical staining results with respect to the Dukes' classification are summarised in Table 2. Pronounced p53 immunoreactivity (++) was found in advanced tumour stages (in two of 11 (18%) with Dukes' A; in 11 of 49 (22%) with Dukes' B; in 13 of 32 (41%) with Dukes' C; and in six of 17 (35%) with Dukes' D cancer) and was accompanied by a slight decrease in the number of totally negative cases. The frequency of p53 negative cases was higher in left sided compared with right sided tumours. None of these comparisons were statistically significant.

Expression mdm-2 was detected using immunohistochemistry in 22 of the 109 (20%) of cases investigated. The p53 and mdm-2 scores are presented in Table 4. In general, mdm-2 and p53 immunoreactivities were detected in serial sections from the same tumour areas. In three p53 negative cases mdm-2 was detected in less than 10% of tumour cells. Only one of 11 cases with less than 10% p53 positive cells lacked mdm-2 immunoreactivity. Of the 49 cases with p53 overexpression in more than 10% of tumour cells (+/++/+ +/+), nine showed mdm-2 staining. This association was highly statistically significant (total \( \chi^2 = 69.5, DF = 6, p = 0.0001 \)). Comparisons of p53 and mdm-2 scores with respect to various staging and grading parameters are presented in Table 5.

Results of univariate survival analysis of all parameters investigated are given in Table 6. Kaplan-Meier survival curves with regard to all p53 scores investigated (Mantel-Haenszel: \( \chi^2 = 3.7, DF = 2, NS \)) are shown in Fig. 2. The survival curve of mdm-2 positive cases was similar to the survival curve of cases with positive p53 immunoreactivity (Mantel-Haenszel: \( \chi^2 = 3.7, DF = 2, NS \)) (Fig 3).

Table 5 Correlation between p53, mdm-2 scores and the various prognostic factors investigated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p53</th>
<th>mdm-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \chi^2 )</td>
<td>DF</td>
</tr>
<tr>
<td>M stage</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>pN stage* (Pno vs Pn1–3)</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Dukes' classification</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td>pT stage</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>Tumour site</td>
<td>3.9</td>
<td>2</td>
</tr>
<tr>
<td>Lymphocytic infiltration</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>Histological tumour grade</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td>Age (( \leq 65 ) yrs)</td>
<td>4.0</td>
<td>2</td>
</tr>
<tr>
<td>Sex</td>
<td>1.0</td>
<td>1</td>
</tr>
</tbody>
</table>

*pN1 cases (n = 13; four with Dukes' A and nine with Dukes' D cancer) excluded.

Table 6 Prognostic factors examined in 109 colorectal carcinomas: a univariate approach to cancer-specific mortality

<table>
<thead>
<tr>
<th>Univariate ( \chi^2 ) for the log-rank test</th>
<th>DF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes' stage</td>
<td>88.1</td>
<td>3</td>
</tr>
<tr>
<td>pT stage</td>
<td>17.7</td>
<td>3</td>
</tr>
<tr>
<td>pN stage*</td>
<td>25.7</td>
<td>3</td>
</tr>
<tr>
<td>Lymphocytic infiltration</td>
<td>8.6</td>
<td>2</td>
</tr>
<tr>
<td>Histological tumour grade</td>
<td>2.7</td>
<td>2</td>
</tr>
<tr>
<td>p53 scores (( \leq 1 ) cell +/+/+/+)</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>mdm-2 scores (negative ( v +/++/+/+ ))</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Tumour type</td>
<td>4.0</td>
<td>2</td>
</tr>
<tr>
<td>Tumour site</td>
<td>2.2</td>
<td>2</td>
</tr>
<tr>
<td>Age (( \leq 65 ) yrs)</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>Sex</td>
<td>0.001</td>
<td>1</td>
</tr>
</tbody>
</table>

*pN1 cases (n = 13; four with Dukes' A and nine with Dukes' D cancer) excluded.
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Discussion

The extended palette of adjuvant chemotherapy and/or radiotherapy schedules in combination with biological response modifiers requires determination of groups of patients
with (morphological) prognostic features which are independent of common staging and grading classifications. Recent advances in the understanding of tumorigenesis of colorectal cancer suggest that p53 tumour suppressor gene dysfunction may play a central role. Therefore, promising results were expected when p53 abnormalities were evaluated on both the molecular and immunohistochemical level.30 Yet only one report dealing with p53 mutations in a large series of sporadic colorectal cancers demonstrated a statistical correlation between p53 mutation and patient survival.8 On the other hand, from the clearly demonstrated association between increased p53 protein stability and mutation,6931 several immunohistochemical investigations have been performed previously. In these studies, however, strict correlations between survival and p53 overexpression were not observed.

The results of the present study reveal a highly statistically significant (p = 0.0001) correlation between immunohistochemically detectable mdm-2 expression and low (+) or moderate (+ +) p53 overexpression (table 4). Only three of 109 cases (2.8%; two with Dukes' B and one with Dukes' C cancer) showed positive staining for mdm-2 with a concomitant lack of p53 expression. By contrast, two samples (1.8%; one with Dukes' A and one with Dukes' C cancer) classified as strongly (++ +) overexpressing p53 also showed mdm-2 positive immunoreactivity. Moreover, the survival curve of mdm-2 positive cases (irrespective of p53 expression) more closely resembled that of p53 positive than p53 negative patients (fig 2).

Concerning p53 frequency distribution and correlations with common prognostic parameters, the findings of the present study confirm those of other investigators.15-16 Although not statistically significant, a preponderance of left sided colon cancers with p53 positive immunoreactivity was observed. Furthermore, immunohistochemically detectable p53 was more frequently observed in advanced cancer stages and was associated with poor clinical outcome. All statistical comparisons, including survival analysis, lacked significance. These observations strongly suggest that p53 overexpression in less than 10% of nuclei probably occurs through complexing with the mdm-2 protein. We did not observe a correlation between immunohistochemically detectable p53 expression and clinical outcome.

Several molecular studies suggest that tumorigenesis in colorectal cancer occurs in a stepwise manner through a series of genetic alterations.123 This study demonstrates that a recently described oncoprotein, the mdm-2 gene product, is expressed in colorectal cancer. Its association with cases showing a small proportion of cells with immunohistochemically detectable p53 expression suggests that the mdm-2 gene may be involved in the auto-

regulation of p53 gene function. These multiple interactions may be responsible for the lack or only marginal value of proto-oncogenes as independent prognostic parameters. Thus, the Dukes' classification, which is easy to reproduce and relates well to survival, still remains the "gold standard" with regard to prognosis in colorectal cancer. Immunohistochemical or genetic, or both, investigations of proto-oncogenes, however, may be useful in helping to understand the molecular mechanism(s) underlying this simple and reliable "gold standard".

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