p73 Overexpression Is a Prognostic Factor in Patients with Colorectal Adenocarcinoma

Xiao-Feng Sun

Department of Oncology, Institute of Biomedicine and Surgery, Linköping University, S-581 85 Linköping, Sweden

ABSTRACT

Purpose: To examine the expression of p73 in the different stages of colorectal cancer development and the association of p73 expression with patient survival.

Experimental Design: Expression of p73 protein was evaluated by immunohistochemistry in 221 primary colorectal cancer patients, including 58 patients with matched normal mucosa and metastases in the regional lymph nodes.

Results: Frequency and intensity of p73 expression were markedly increased from the normal samples (19%) to primary tumors (67%) and to metastases (95%). Overexpression of p73 predicted poor outcome in the whole group of patients (P = 0.014) and the subgroups with left-sided (P = 0.002) or ras-positive tumors (P = 0.019). The prognostic significance remained in the whole group (P = 0.008) and the subgroup with left-sided tumors (P = 0.019) after adjustment for the patient’s sex, age, tumor stage, growth pattern, and differentiation. The p73 expression was positively correlated with ras expression (P = 0.006). The 5-year survival rates were 57, 53, 72, and 74% for the patients with p73+/ras+, p73+/ras−, p73−/ras+, and p73−/ras− tumors, respectively (P = 0.007).

Conclusion: Our data indicate that overexpression of p73 independently predicted poor prognosis in the patients with colorectal cancer.

INTRODUCTION

The p73 gene has been cloned and mapped at chromosome 1p36.2–3, a region that has highly frequent loss of heterozygosity in various human cancers (1). The p73 gene encodes a protein that contains two distinct α- and β-polypeptides. The structure of p73 protein is highly homogeneous to p53, and p73 also shares some common functions with p53 protein, such as activating the transcription of other genes, inhibiting cell growth, and inducing apoptosis, indicating that p73 is a p53-like tumor suppressor (2). However, the activation of a p73 allele may contribute to tumor progression, and the increased levels of p73 mRNA transcription are found in various tumors compared with the surrounding normal tissues (3–8). These factors suggest that p73 is unlikely to be a tumor suppressor gene. Moreover, several other studies show that either loss of heterozygosity or mutation of p73 is not a common genetic event in the development of human cancers (9–13), which suggests that p73 does not play an important role in tumor development. There has been no report concerning the analyses of p73 expression in different stages of colorectal cancer and its prognostic implication in patients with colorectal cancer.

In the present study, we examined the expression of p73 in primary colorectal tumor with matched normal mucosa and metastasis from 221 patients with colorectal cancer to determine whether p73 is involved in tumor development, whether p73 expression is related to prognosis, and whether the p73 expression is correlated with ras, p53, or DCC.

PATIENTS AND METHODS

Clinical and Pathological Data. Two hundred twenty-one patients with colorectal adenocarcinoma, diagnosed at the Department of Pathology, Linköping University, between 1972 and 1996 were collected. There were 58 cases with matched normal mucosa and primary and metastatic tumors. Normal samples were taken from the margin, corresponding to distant resection, and were histologically free from precancer and cancer. The patients were followed up until the end of June 1999, and 94 patients died because of cancer. The patient’s sex, age, tumor site, and Dukes’ stage were obtained from surgical and pathological records. Mean age was 70 years (range, 34 to 93 years). Right-sided tumors included tumors in the ascending and the transverse colon, and left-sided tumors were found in the descending and sigmoid colon and the rectum. Growth pattern and differentiation were determined by two pathologists. The numbers of unknown cases, by category, were: sex, 4; age, 11; location, 13; Dukes’ stage, 24; growth pattern, 20; and differentiation, 6. Expression of ras (14), p53 (15), and DCC (16) was estimated previously by immunohistochemistry on the serial sections at our laboratory. The ras protein was detected by using monoclonal antibody ras 11, which detects both normal and mutated forms of ras p21 (Oncogene Science, Inc., New York, NY). The expression was classified as a negative reaction if <5% of tumor cells were positive and otherwise as positive. NCL-p53-CM1 rabbit polyclonal antibody (Novocastra Laboratories Ltd., United Kingdom) was used to detect both wild-type and mutated forms of p53. The expression was considered positive when tumor cells were stained, irrespective of the

Received 4/5/01; revised 10/17/01; accepted 10/19/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the Swedish Cancer Foundation and FORSS.
2 To whom requests for reprints should be addressed, at Department of Oncology, Institute of Biomedicine and Surgery, Linköping University, S-581 85 Linköping, Sweden. Phone: 46-13-222066; Fax: 46-13-222846; E-mail: xiasu@onk.liu.se.

3 The abbreviation used is: DCC, deleted in colorectal cancer.
percentage of positive cells; otherwise, the expression was classified as negative. DCC protein expression was determined by using monoclonal antibody clone G97-449 (PharMingen, San Diego, CA). The expression was classified as positive if tumor cells were stained or negative if there were not any positive tumor cells. The numbers of available cases that matched with p73 expression were 131 for ras, 160 for p53, and 109 for DCC staining.

**p73 Antibody and Immunohistochemical Assay.** p73 is an affinity-purified goat polyclonal antibody raised against a peptide mapping at the COOH terminus of p73β of human origin (differs from corresponding p73α sequence by two amino acids), and p73 reacts with both p73α and p73β of human origin (Santa Cruz Biotechnology, Santa Cruz, CA). To demonstrate the specificity of the primary antibody, absorption of the primary antibody with a p73-blocking peptide (Santa Cruz Biotechnology) was performed, after which immunostaining was completely negative. Sections from paraffin-embedded tissue blocks were deparaffinized in xylene and rehydrated. Activity of endogenous peroxidase was blocked in 0.5% H$_2$O$_2$ in methanol. To expose the masked epitopes, the sections were incubated in citrate buffer (pH 6.0) at 80°C for 10 min and then kept at room temperature. After a short rinse in PBS-Tween 20 (PBS plus 0.05% Tween 20), the sections were treated with power block (BioGenex, San Ramon, CA) for 10 min to block nonspecific background staining. After rinsing, the primary antibody in a 1:150 dilution in antibody diluent (Dako, Glostrup, Denmark) was applied at 4°C overnight. Subsequently, the sections were incubated with biotinylated donkey antigoat IgG for 30 min, followed by goat avidin-biotin complex (Santa Cruz Biotechnology) for an additional 30 min. The peroxidase reaction was performed by an addition of 0.05% 3,3-diaminobenzidine tetrahydrochloride solution (Sigma Chemical Co., St. Louis, MO) and 0.02% H$_2$O$_2$ in PBS for 8 min. The sections then were counterstained. The matched normal mucosa, primary tumors, and metastases in the lymph nodes were stained in the same run of the immunostaining to avoid bias on the pattern and intensity of staining. Sections known to show strong immunostaining for p73 were included in each run receiving either primary antibody or PBS, as positive and negative controls. In all of the staining procedures, the positive controls showed staining clearly, and there was no staining in the negative controls.

The sections were examined microscopically and scored independently by two pathologists in a blinded fashion without knowledge of clinical and pathological information, including survival data. To avoid artifact effect, the cells on the margins of sections and areas of poorly presented morphology were not counted. The entire histological sections were scanned at low sections and areas of poorly presented morphology were not survival data. To avoid artifact effect, the cells on the margins of knowledge of clinical and pathological information, including independently by two pathologists in a blinded fashion without.

**Statistical Analysis.** χ$^2$ and McNemar’s methods (17) were used to test significance of the difference in frequency of p73 between normal samples and primary tumor and metastases and the association of p73 expression with clinical and pathological factors and expression of ras, p53, and DCC. Cox’s proportional hazards model (18) was used to estimate the relationship between the p73 expression and survival. The curves describing survival were computerized according to Kaplan and Meier (19). Tests were two-sided, and $P < 0.05$ was considered statistically significant.

**RESULTS**

**p73 Expression in Normal Mucosa and Primary and Metastatic Tumors.** There were no significant differences with respect to clinicopathological characteristics between negative and equivocal staining, nor with weak, moderate, or strong staining. Therefore, the former two groups were classified as negative, and the latter three groups were classified as positive in our following analyses. As shown in Table 1, 19% of normal samples exhibited p73-positive characteristics, 67% in primary tumors, and 95% in metastases. In matched samples, the frequency and the intensity of p73 immunostaining were increased markedly from normal tissue to primary tumor and to metastasis ($P < 0.05$; Fig. 1). In most of the negative primary tumors, the corresponding metastatic tumors were p73 positive. None of the normal samples revealed stronger p73 staining than that in the matched primary tumors.

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).

**Expression of p73 in Primary Tumors and Survival.** As shown in Fig. 2, the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors ($P = 0.014$). Furthermore, the prognostic significance of p73 expression was evaluated in the subgroups of patients with different-sided tumors. In the patients with left-sided tumors (Fig. 3A), the positive expression of p73 implicated shorter survival ($P = 0.002$). In the patients with right-sided tumors, p73 expression did not contribute prognostic significance ($P = 0.555$; Fig. 3B).
were unable to find the statistical significance of p73 expression in the ras-negative group ($P = 0.163$; Fig. 4B). The 5-year survival rates were 37, 53, 72, and 74% for the patients with p73+/ras+, p73+/ras−, p73−/ras+, and p73−/ras− tumors, respectively ($P = 0.007$).

In multivariate analyses, after adjustment for the clinical and pathological variables, the prognostic significance of p73 expression remained in the whole group of patients ($P = 0.008$; Table 4) and those with left-sided tumors ($P = 0.019$, Table 5) but not in the ras-positive group ($P = 0.269$).

**DISCUSSION**

In the present study, we found that the patients with p73-positive tumors had a shorter survival than those with p73-negative ones in the whole group of patients. The prognostic significance of p73 expression remained, even after adjusting for the clinical and pathological variables. Tanapfel et al. (20) have studied hepatocellular carcinomas and found that patients with p73-positive tumors have a poorer prognosis than those with p73-negative tumors. Furthermore, in subgroups of the patients, we realized that p73 was also an independent prognostic predictor in the patients with left-sided tumors. The patients with p73-positive tumors had a remarkably shorter survival period than those with p73-negative tumors. However, we failed to find the statistical significance of p73 expression in the ras-negative group ($P = 0.163$; Fig. 4B).

**Table 2** Expression of p73 in relation to sex, age, tumor site, Dukes’ stage, growth pattern, and differentiation in patients with colorectal adenocarcinoma

<table>
<thead>
<tr>
<th></th>
<th>p73 Positive (%)</th>
<th>p73 Negative (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>86 (72)</td>
<td>34 (28)</td>
<td>0.126</td>
</tr>
<tr>
<td>Female</td>
<td>60 (62)</td>
<td>37 (38)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>55 (57)</td>
<td>41 (43)</td>
<td>0.012</td>
</tr>
<tr>
<td>≥70</td>
<td>84 (74)</td>
<td>30 (26)</td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td>0.574</td>
</tr>
<tr>
<td>Right-sided</td>
<td>60 (69)</td>
<td>27 (31)</td>
<td></td>
</tr>
<tr>
<td>Left-sided</td>
<td>79 (65)</td>
<td>42 (35)</td>
<td></td>
</tr>
<tr>
<td>Dukes’ stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>14 (67)</td>
<td>7 (33)</td>
<td>0.369</td>
</tr>
<tr>
<td>B</td>
<td>40 (57)</td>
<td>30 (43)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>52 (71)</td>
<td>21 (29)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>22 (67)</td>
<td>11 (33)</td>
<td></td>
</tr>
<tr>
<td>Growth pattern</td>
<td></td>
<td></td>
<td>0.809</td>
</tr>
<tr>
<td>Expanding</td>
<td>52 (67)</td>
<td>26 (33)</td>
<td></td>
</tr>
<tr>
<td>Infiltrating</td>
<td>84 (68)</td>
<td>39 (32)</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td>0.312</td>
</tr>
<tr>
<td>Well/moderately</td>
<td>100 (65)</td>
<td>54 (35)</td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td>44 (72)</td>
<td>17 (28)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** Association of p73 expression with ras, p53, and DCC in patients with colorectal adenocarcinoma

<table>
<thead>
<tr>
<th></th>
<th>p73 Positive (%)</th>
<th>p73 Negative (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ras</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>58 (73)</td>
<td>22 (27)</td>
<td>0.006</td>
</tr>
<tr>
<td>Negative</td>
<td>25 (49)</td>
<td>26 (51)</td>
<td></td>
</tr>
<tr>
<td>p53 (CM1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>50 (59)</td>
<td>35 (41)</td>
<td>0.230</td>
</tr>
<tr>
<td>Negative</td>
<td>51 (68)</td>
<td>24 (32)</td>
<td></td>
</tr>
<tr>
<td>DCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>31 (70)</td>
<td>13 (30)</td>
<td>0.113</td>
</tr>
<tr>
<td>Negative</td>
<td>36 (58)</td>
<td>29 (45)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 The normal mucosa does not express p73 protein (A), the corresponding primary tumor shows p73-positive staining in the tumor cells (B), and corresponding metastasis reveals even stronger p73 staining throughout the tumor cells (C). The sections are counterstained with hematoxylin.

In multivariate analyses, after adjustment for the clinical and pathological variables, the prognostic significance of p73 expression remained in the whole group of patients ($P = 0.008$; Table 4) and those with left-sided tumors ($P = 0.019$, Table 5) but not in the ras-positive group ($P = 0.269$).
to find the prognostic value of p73 expression in the patients with right-sided tumors. Our results further support the theory that initiation and progression of left- and right-sided colorectal cancers may involve different mechanisms. There may be different pathways by which p73 is involved to develop right- and left-sided colorectal cancers. However, the mechanism by which p73 exerts its biological action, if it does, remains to be investigated. Overall, the evidence suggested that overexpression of p73 was an indicator of prognosis in certain types of cancer patients and a subgroup of the patients.

The analyses in the combinations of p73 and ras showed that the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors (P = 0.014). In the patients with right-sided tumors (B), there was no difference of survival between the patients with p73-positive and -negative tumors (P = 0.555).

**Fig. 2** Patients with p73-positive tumors had shorter survival than those with p73-negative tumors (P = 0.014).

**Fig. 3** In the group with left-sided tumors (A), the patients with p73-positive tumors had significantly shorter survival than those with p73-negative tumors (P = 0.002). In the patients with right-sided tumors (B), there was no difference of survival between the patients with p73-positive and -negative tumors (P = 0.555).
The studies on esophageal cancers (10), breast cancer (11), melanoma (13), and neuroblastoma (21) have shown that allelic loss, mRNA transcription, and mutations of p73 gene are not main genetic events in carcinogenesis and tumor development. To understand further the essential roles of p73 and provide an insight into the development of colorectal cancer, it is of interest to investigate the expression of p73 in the different stages of tumors. Therefore, in the present study, the expression of p73 protein was examined in the primary and metastatic tumors, together with the corresponding normal colorectal mucosa from patients with colorectal cancer. The results showed that both frequency and intensity of p73 expression were markedly increased from normal tissue to primary tumor and to metastasis, which indicated that p73 may be involved in the development of colorectal cancer, including metastasis. Up to now the data do not provide genetic evidence that the inactivation of p73 is required for tumorigenesis. The activation of a silent allele or overexpression of p73, rather than a tumor suppressor function, may contribute to tumor development.

In summary, the data suggest that p73 was up-regulated during tumor development from the normal mucosa to the primary and metastatic tumors. The overexpression of p73 was a valuable prognostic marker to predict poor outcome for the patients with colorectal cancer.

ACKNOWLEDGMENTS

I thank Dr. Hong Zhang for valuable discussion and Dr. D. Rogers for the linguistic revision.

REFERENCES


p73 Overexpression Is a Prognostic Factor in Patients with Colorectal Adenocarcinoma

Xiao-Feng Sun


Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/8/1/165

This article cites 20 articles, 6 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/8/1/165.full#ref-list-1

This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/8/1/165.full#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.