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Original Paper

Prognostic Significance of p53 Nuclear and Cytoplasmic Overexpression in Right and Left Colorectal Adenocarcinomas

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The prognostic significance of nuclear and cytoplasmic p53 protein, detected immunocytochemically using CM1 and PAb 1801 antibodies, was evaluated in right-sided and left-sided colorectal adenocarcinomas from 293 patients. CM1 nuclear and cytoplasmic p53 accumulation occurred in 38 and 25% of cases, respectively. PAb 1801 nuclear staining occurred in 18%, with no cytoplasmic staining. CM1 expression either in the nucleus or in the cytoplasm was positively related to PAb 1801 expression ($P < 0.001$ and $P = 0.009$, respectively). The incidence of CM1 nuclear and cytoplasmic expression was more frequent in right-sided tumours ($P = 0.023$ and $P = 0.034$, respectively), while PAb 1801 nuclear staining was more common in left-sided tumours ($P = 0.011$). In survival analyses, CM1 nuclear overexpression in the right-sided tumours ($P = 0.016$) and CM1 cytoplasmic overexpression in left-sided tumours ($P = 0.04$) were prognostic indicators, independent of Dukes' stage, DNA ploidy, PAb 1801 expression and each other. Further analysis showed that the prognostic value of CM1 nuclear expression was greater in right-sided tumours than in left-sided tumours ($P = 0.018$). The nuclear and cytoplasmic p53 protein detected with CM1 and PAb 1801 may play different roles in tumour progression and provide prognostic indicators for right- and left-sided colorectal tumours. Copyright © 1996 Published by Elsevier Science Ltd

Key words: p53, colorectal cancer, prognosis, tumour location, immunohistochemistry

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INTRODUCTION

THE *TP53* GENE, as a tumour suppressor gene, controls the activation of transcription by binding to DNA allowing the cells to progress from G_0/G_1 to S-phase. Thus, it regulates normal cell growth and division [1, 2]. The level of p53 protein in normal cells is undetectable by immunohistochemical staining due to the short-life of the protein [1–4]. The inactivated p53 protein, by mutation, deletion and complexing to other proteins, prolongs its half-life and often results in accumulation. The accumulated protein can be detected by immunohistochemistry [1–10]. Several studies have shown that p53 overexpression either in the nucleus or in the cytoplasm is related to unfavourable survival in patients with colorectal cancer [3–8], but other studies have not found this relationship [9, 10]. However, there is little information on the prognostic significance of p53 protein recognised by different antibodies according to the site of the tumours. For clinical and biological reasons, it is important to indicate whether antibodies with specificity to different regions of the protein vary in their prognostic value in the different tumour sites. In this large series, the aim was to investigate the prognostic significance of nuclear and cytoplasmic p53 protein detected by different antibodies in right and left colorectal adenocarcinomas, respectively.

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PATIENTS AND METHODS

Patients and pathological data

Tissue blocks of paraffin-embedded material were obtained from the files for 293 patients with primary colorectal adenocarcinomas diagnosed at the Department of Pathology, Linköping University between 1972 and 1986. None of the patients had received pre-operative radiotherapy or chemotherapy. Patients' sex, age, tumour site and Dukes' stage were obtained from surgical and patho-

Table 1. Patients' characteristics

Number of patients	Total ($n = 293$)
Male/Female	156/137
Mean age (years)	69
Range	(33–93)
Tumour site	
Right-sided tumour	105
Appendix	1
Ascending colon	83
Transverse colon	21
Left-sided	180
Descending colon	12
Sigmoid colon	53
Rectum	115
Unknown	8
Dukes' stage	
A	43
B	101
C	94
D	50
Unknown	5
Histology grade	
Well differentiated	17
Moderately differentiated	205
Poorly differentiated	39
Mucinous	29
Signet-ring cell carcinomas	3
Ploidy	
Diploid	135
Non-diploid	144
Unknown	14

logical records (Table 1). Histological grade were classified by two investigators as recommended by Morson [11] and Hermanek [12]. DNA ploidy was measured by flow cytometry described previously [5]. The patients were followed until the end of December 1990, and 123 deaths due to colorectal cancer had been registered.

In 19 cases, the presence of p53 in normal tissue, taken from the distant resection margin which was histologically free from tumour, was also examined.

Nineteen fresh tumours were used to determine the fixation and embedding effects on tissue p53 immunoreactivity with CM1 and PAb 1801. The tissue was flash frozen and stored in a −80°C freezer before sectioning. Corresponding specimens were fixed and paraffin-embedded in a routine manner.

Antibodies

NCL-p53-CM1 rabbit polyclonal antibody (Novocastra Laboratories Ltd, U.K.) detects both wild and mutant forms of p53 from amino acids 1–393. The monoclonal antibody PAb 1801 (Oncogene Science Inc., Manhasset, New York, U.S.A.) recognises an epitope located between amino acids 32 and 79 in both wild-type and mutant p53 protein.

Immunohistochemical assay

Serial sections from paraffin-embedded tissue were deparaffinised in xylene followed by rehydration. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in methanol for 20 min. Following a short rinse in phosphate-buffered saline (PBS), the sections were pre-incubated with 10% normal swine or rabbit serum to block non-specific immunostaining. After removing the blocking solution, CM1 or PAb 1801 was applied in 1:100, as recommended by the suppliers, for 30 min at room temperature. Subsequently, the sections were incubated with swine antirabbit or rabbit antimouse immunoglobulins (Dakopatts Co., Glostrup, Denmark), and rabbit or mouse PAP (Dakopatts Co.), for 30 min for each step. The slides were washed in PBS between each incubation step. The peroxidase reaction was performed for 8 min, using 0.05% 3,3-diaminobenzidine tetrahydrochloride solution (Sigma Chemical Co, St. Louis, Missouri, U.S.A.) in PBS containing 0.02% hydrogen peroxide. The sections were counterstained with light haematoxylin for 1 min, dehydrated in a series of ethanols, cleared in xylene and mounted under a coverslip. Sections known to stain positively were included in each run, receiving either a primary antibody as positive control, or the isotype MOPC-21 for IgG_1 (Sigma) or PBS was used as negative control.

Frozen sections were allowed to air-dry, fixed in acetone for 10 min and rehydrated in PBS for 15 min, followed by the procedure described above. A comparison of CM1 and PAb 1801 staining on the corresponding fixed, paraffin-embedded samples showed that identical findings were observed.

The slides were examined and scored independently by two investigators (X.-F. Sun and H. Zhang) without any clinical or pathological information. p53 expression was considered positive when tumour cells were stained, irrespective of the percentage of positive cells. However, we did not include faint staining cells or positive cells located on the margin of the section or in poor morphological areas.

Statistical analysis

The chi-square method for 2×2 tables was used to test the association between p53 expression detected by CM1 and PAb 1801, as well as the relationship between p53 expression in right- and left-sided tumours [13]. Cox's proportional hazards model was used to estimate and test the relation of p53 expression to prognosis [14]. The curves describing survival were computed according to the method of Kaplan and Meier [15]. All *P*-values cited are two-sided, and *P*-values less than 5% were judged as statistically significant.

RESULTS

The patterns of p53 expression with CM1 and PAb 1801 and their relationship

Among the 293 cases stained by CM1, nuclear p53 staining (Figure 1a) occurred in 112 cases (38%) and cytoplasmic staining (Figure 1b) in 72 cases (25%). In 289 available cases for PAb 1801 staining, 51 cases (18%) showed nuclear expression (Figure 1c), while no cytoplasmic expression was seen. The expression of p53, if present, was not detected in all tumour cells. Furthermore, variations of the staining intensity in the same microscopic

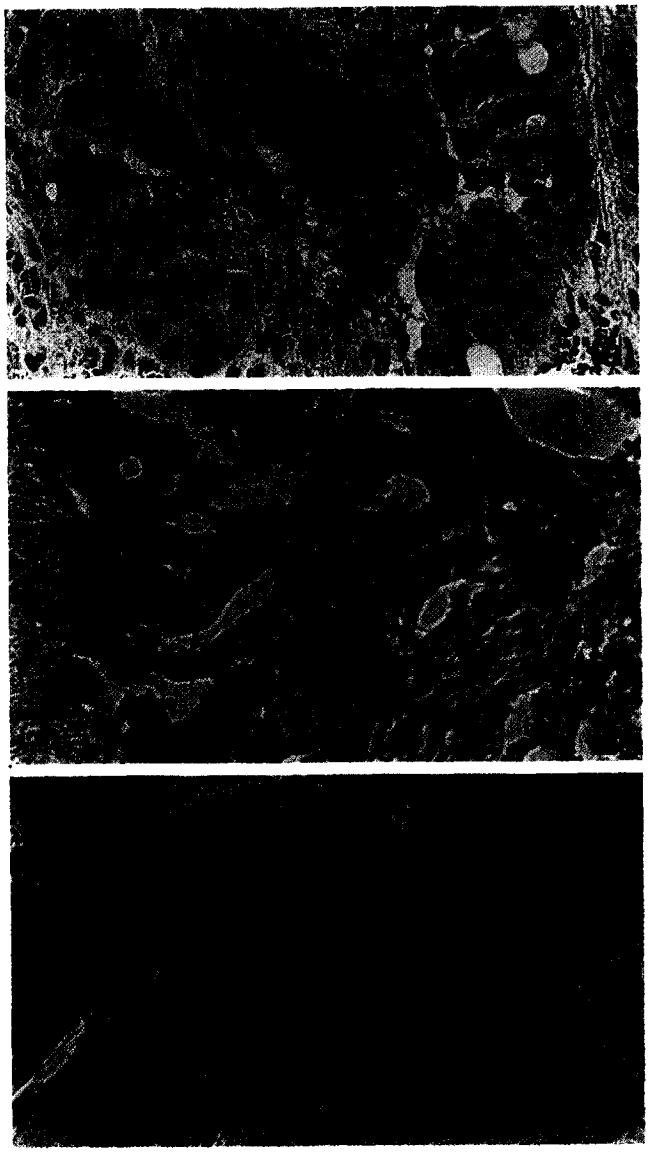

Figure 1. p53 overexpression of colorectal cancer (a) in the nuclei detected by CM1, (b) in the cytoplasm detected by CM1 and (c) in the nuclei detected by PAb 1801. The sections received a light haematoxylin counterstain. Magnification ×200.

area were noted. None of the 19 normal specimens had detectable p53 protein.

The relationships between p53 expression with CM1 and PAb 1801 are shown in Table 2 . Immunoreactivity in the nucleus with CM1 or PAb 1801 was concordant in 171 negative cases (59%) and 45 positive cases (16%); only 73 cases (25%) showed different expression with the two antibodies, i.e. 67 tumours (23%) showed positive nuclear expression with CM1 but were negative with PAb 1801, and 6 cases (2%) were positive with PAb 1801 but negative with CM1 ($P < 0.001$). For cytoplasmic p53 expression with CM1, 206 cases (186 negative and 20 positive cases, 71%) showed similar nuclear staining with PAb 1801, but 83 cases (52 and 31 cases, 29%) were not identical ($P = 0.009$).

Table 2. The relationship of p53 expression detected with CM1 and PAb 1801

	p53 expression with PAb 1801		
	Negative	Positive	*P*
p53 expression with CM1			
Nuclear expression			< 0.001
Negative	171 (59%)	6 (2%)	
Positive	67 (23%)	45 (16%)	
Cytoplasmic expression			0.009
Negative	186 (64%)	31 (11%)	
Positive	52 (18%)	20 (7%)	

p53 expression in relation to tumour location and prognosis

As Figure 2 shows, tumours with CM1 nuclear or CM1 cytoplasmic expression were more frequently right-sided ($P = 0.023$ and $P = 0.034$, respectively), whereas tumours with PAb 1801 nuclear staining were more often left-sided ($P = 0.011$).

In univariate Cox analysis of Dukes' stage A–C, right-sided tumours, CM1 nuclear expression was significantly related to survival ($P = 0.007$, Figure 3a); CM1 cytoplasmic overexpression ($P = 0.071$) and PAb 1801 nuclear overexpression ($P = 0.092$) tended to predict worse prognosis, although the differences did not reach statistical significance. Co-expression of CM1 nuclear and CM1 cytoplasmic staining ($P = 0.004$), as well as co-expression of CM1 and PAb 1801 ($P = 0.004$, Figure 3b) were significantly related to survival. In left-sided tumours, only CM1 cytoplasmic staining was significantly related to prognosis ($P = 0.047$, Figure 4), while CM1 nuclear ($P = 0.78$), PAb 1801 nuclear expression ($P = 0.88$), and any co-expression were not ($P > 0.05$).

In multivariate Cox analysis including Dukes' stage, DNA ploidy, CM1 nuclear expression, CM1 cytoplasmic expression and PAb 1801 nuclear expression, CM1 nuclear expression in right-sided tumours ($P = 0.016$), and CM1

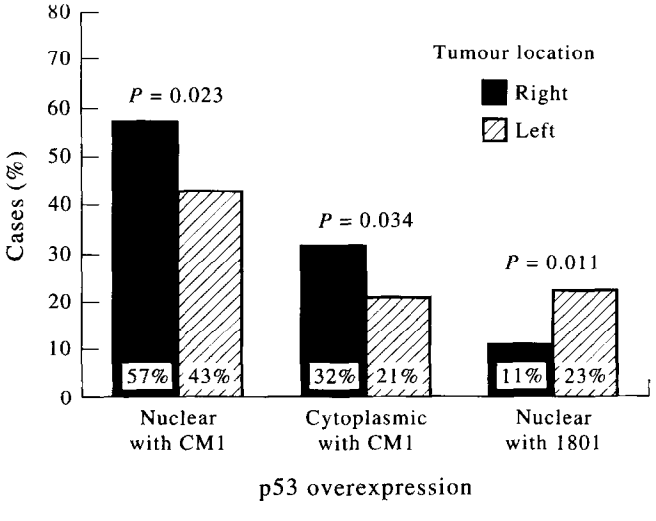

Figure 2. p53 expression detected by different antibodies in relation to tumour location.

cytoplasmic expression in left-sided tumours ($P = 0.04$) retained their prognostic values.

Further multivariate analyses showed that correlations with the prognostic value of nuclear p53 expression differed significantly between patients with right-sided tumours and those with left-sided tumours ($P = 0.018$). Namely, the prognostic value CM1 nuclear expression was greater in right-sided tumours than in left-sided tumours. There was no significant difference in the association of p53 cytoplasmic expression ($P = 0.9$) or PAb 1801 expression ($P = 0.2$) with survival for left- and right-sided tumours.

DISCUSSION

We found that there were variations in the incidence of p53 detection with CM1 and PAb 1801, which is in line with previous results [16, 17]. Since the antibodies recognise different regions of the protein, varied *TP53* mutations in different tumours (or even in different cells of the same tumour) can result in the formation of a changed or truncated protein which would not be detected by all antibodies [16–18]. However, as we also demonstrated that PAb 1801 expression was positively related to CM1 expression, either in the nucleus or in the cytoplasm, it would be tempting to speculate that most tumour cells have similar conformation of p53 proteins. Genetic analyses have shown that the most *TP53* mutations reported so far in tumours are clustered within exons 5–8 and located in four highly conserved domains, although the mutations may occur throughout the whole gene [19, 20].

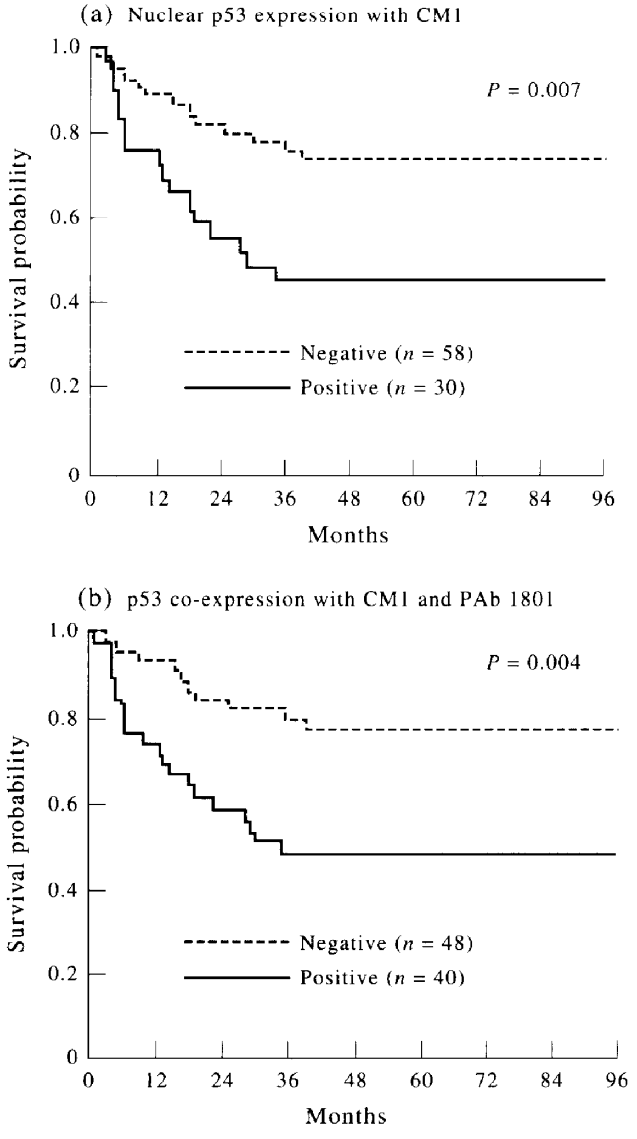

Figure 3. p53 expression in relation to survival in patients with Dukes' stage A–C, right-sided adenocarcinomas.

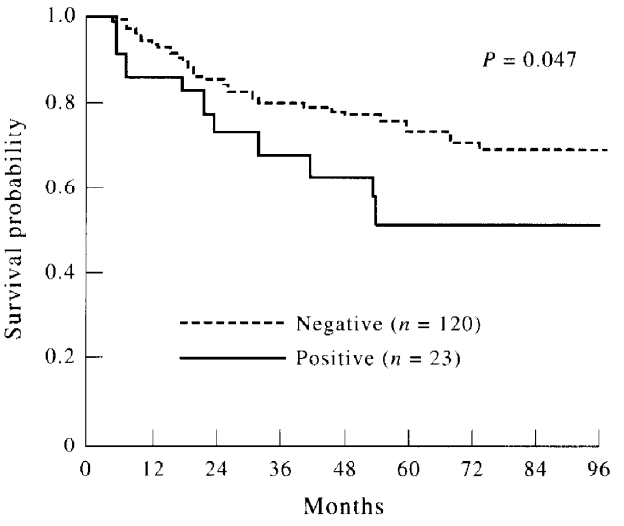

Figure 4. CM1 cytoplasmic p53 expression in relation to survival in patients with Dukes' stage A–C, left-sided adenocarcinomas.

The pattern of p53 expression detected in this study has also been reported by others. Bosari and coworkers [7] found that nuclear p53 detected with PAb 1801 was significantly more prevalent in tumours in left-sided colorectal tumours but there was no difference with CM1 staining according to the tumour sites. Nuclear p53 protein detected with PAb 1801 demonstrated a significantly worse prognosis in patients with right-sided tumours, and cytoplasmic p53 overexpression with CM1 independently predicted a worse prognosis in patients with left-sided tumours. However, they did not find this relationship between CM1 nuclear p53 staining and survival in right-sided tumours. The reasons for this are unclear. However, unlike their study, we did not include cases with Dukes' stage D in the survival analysis, since they had distant metastasis, which affects survival. Also, we had a larger number of cases with a longer follow-up.

Observations compiled from epidemiology, clinical and research, indicate that there are differences in race, sex, age, aetiology, pathology, morbidity, mortality and genetic abnormalities in the right colon and left colorectum [5, 7, 9, 10, 21–23]. This evidence supports the theory that initiation and progression of left- and right-sided colorectal cancers may involve different mechanisms. Our data may add additional means for refining prognostic indicators to evaluate tumour aggressiveness and select patients for adjuvant therapy.

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