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Overexpression of poly(ADP-ribose) polymerase-1 (PARP-1) in the early stage of colorectal carcinogenesis

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ARTICLE INFO

Article history:
Received 5 October 2005
Received in revised form
22 December 2005
Accepted 9 January 2006
Available online 30 June 2006

Keywords:
PARP-1
β-Catenin
Colorectal adenoma
Early invasive colorectal cancer

ABSTRACT

Colorectal carcinogenesis is initiated mainly by aberrant activation of the Wnt signaling pathway, caused by mutation of either *APC* or *β-catenin* (*CTNNB1*) gene. Poly(ADP-ribose) polymerase-1 (PARP-1) is a highly conserved nuclear enzyme, which binds tightly to DNA and plays a role in DNA repair, recombination, proliferation and genomic stability. It has recently been shown that PARP-1 is a novel co-activator of TCF-4/β-catenin-evoked gene transactivation and may play a role in colorectal carcinogenesis. The aim of this study was to examine the PARP-1 expression and determine whether it is correlated with the expression of β-catenin and its target genes such as *c-myc*, *cyclin D1* and *matrix metalloproteinase* (*MMP*)*-7* in the early stage of sporadic colorectal carcinogenesis. Using the semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), 91 colorectal tumours, including 65 adenomas and 26 submucosal (pT1) cancers, were analysed for the expression of *PARP-1*, *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7*. Immunohistochemical analysis of PARP-1 and β-catenin was also performed. *PARP-1* mRNA overexpression was detected in 64 (70.3%) of the 91 tumours. *PARP-1* overexpression was significantly correlated with tumour size and histopathology. Overexpression of *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* mRNA expression was observed in 39.6%, 78.0%, 83.5% and 72.5% of the 91 tumours, respectively. *PARP-1* overexpression was correlated significantly with overexpression of *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7*. Correlation of PARP-1 expression with β-catenin overexpression was also demonstrated by immunohistochemistry. The results suggest that PARP-1, in conjunction with β-catenin, c-myc, cyclin D1 and MMP-7, plays an important role in the early stage of colorectal carcinogenesis.

1. Introduction

Colorectal cancer is one of the most common human malignancies in the world. Although alternative pathways exist, it is generally accepted that most colorectal carcinomas arise in pre-existing adenomas.[1]

Aberrant transactivation of T cell factor (TCF)-4-regulated genes by β-catenin plays a key role in colorectal carcinogenesis.[1] APC dysfunction or abnormalities of the *β-catenin* gene result in cytoplasmic accumulation of unphosphorylated β-catenin. This stabilized β-catenin protein translocates into the nucleus where, it modulates gene transcription by inter-

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doi:10.1016/j.ejca.2006.01.061

acting with TCF-4, resulting in transcriptional activation of target genes such as *c-myc*, *cyclin-D1* and *matrix metalloproteinase* (*MMP*)*-7* (*matrilysin*).[2] Thus, the β-catenin/TCF-4 complex and its associated molecules appear to be candidates for targets of molecular therapy against colorectal cancer. In this regard, it is notable that poly(ADP-ribose) polymerase-1 (PARP-1) was recently identified as a novel co-activator of TCF-4/β-catenin-evoked gene transactivation.[3]

PARP-1 is a highly conserved nuclear enzyme that binds tightly to DNA and plays a role in DNA repair, recombination, proliferation and genomic stability.[4–6] Although PARP-1 has been thought to play a protective role against carcinogenesis, no mutation or loss of heterozygosity (LOH) of the *PARP-1* gene has been reported in clinical cancer tissues. On the other hand, overexpression of PARP-1 has been reported in various human malignancies, such as malignant lymphoma,[7] breast carcinoma,[8] Ewing's sarcoma,[9] hepatocellular carcinoma[10] and endometrial carcinoma.[11] Importantly, aberrant poly (ADP-ribose) metabolism, including enhanced PARP activity and poly(ADP-ribose) synthesis, has been shown by biochemical and immunohistochemical analyses in human colon adenoma and carcinoma.[12]

Recently, Idogawa and colleagues[3] reported that PARP-1 physically interacted with TCF-4 and augmented the transcriptional activity of the β-catenin/TCF-4 complex. Knockdown of PARP-1 by RNA interference (RNAi) significantly suppressed both transcriptional activity and proliferation of colorectal cancer cells. PARP-1 was strongly expressed in nuclei of adenoma cells in the large intestine of all 10 patients with familial adenomatous polyposis (FAP) analysed and in the small and large intestines of *Min* mice. Notably, the expression pattern of PARP-1 in adenoma cells completely parallelled that of accumulated β-catenin protein. Therefore, the expression patterns and functional properties of PARP-1 suggest that PARP-1 plays a role in colorectal carcinogenesis.[3,12] PARP-1 overexpression has been detected in 23 (82.1%) of 28 sporadic colorectal carcinoma tissues.[3] Although enhanced PARP-1 activity and poly(ADP-ribose) synthesis have been reported in a small number of sporadic colon adenoma tissues,[12] it is not known whether PARP-1 overexpression is correlated with β-catenin in sporadic colorectal adenoma.

In an attempt to address these issues, we investigated the expression of *PARP-1*, *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* in 91 early colorectal tumour tissues, including 65 adenoma tissues and 26 cancer tissues with submucosal invasion (pT1), by using the semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). We further analysed immunohistochemically the expression of PARP-1 and β-catenin.

2. Materials and methods

2.1. Patients and tissue samples

Ninety-one paired specimens of colorectal tumour and non-tumour tissues were obtained by polypectomy or surgical treatment. These tumour samples consisted of 65 adenomas and 26 adenocarcinomas with submucosal invasion (pT1 in the TNM classification of the Union International Contre Cancer) (Table 1). Additionally, seven hyperplastic polyp tissues were obtained by endoscopy. Each tissue specimen was divided into two pieces. For total RNA extraction, one sample was immediately frozen in liquid nitrogen at the time of endoscopy or surgery and stored at −80 °C until extraction. The other sample was processed for pathological examination using haematoxylin and eosin staining for the evaluation of the tumour cell content. Only specimens containing more than 70% tumour cells were used for analysis. The histopathological features of the specimens were classified according to the TNM classification system. Locations of the colorectal tumours were divided into proximal colon (cecum, ascending and transverse colon) and distal colon (descending and sigmoid colon and rectum). Macroscopic types were divided into protruded type (height of tumour ≧ 3 mm) and flat type (height of tumour < 3 mm). The clinicopathological characteristics of colorectal tumours are shown in Table 1.

Table 1 – Clinicopathological characteristics in 91 colorectal tumour tissues

Characteristics	Adenoma *n* = 65	Cancer (pTl) *n* = 26
Age (years ± SD)	64.6 ± 11.7	67.9 ± 6.8
Mean size (mm ± SD)	11.0 ± 8.7	20.0 ± 8.1
Gender		
Male	38	13
Female	27	13
Location		
Proximal	24	9
Distal	41	17
Macroscopic type		
Protruded	33	19
Flat	32	7
Lymph node metastasis		
Present		3
Absent		23
Lymphatic invasion		
Present		5
Absent		21
Venous invasion		
Present		5
Absent		21

2.2. Semi-quantitative RT-PCR

Total RNA was extracted from specimens using the acid guanidinum thiocyanate–phenol–chloroform extraction method and treated with DNase I. cDNA was synthesised from 1 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen, San Diego, CA, USA) with random hexamers. PCR was performed using primers specific for each target gene and the *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) genes in duplex PCR.[13,14] *GAPDH* served as an internal control of the resection. The reactions were controlled without reverse transcriptase. Results were analysed using a multi-image analyser (Bio-Rad, Richmond, CA, USA). The levels of gene transcripts were quantified as the ratio of the intensity of the target gene to the intensity of *GAPDH*. Overexpression was judged when the target gene expression in the tumour samples was at least three times higher than that in the corresponding normal sample. To perform semi-quantitative RT-PCR, the ranges of linear amplification for the target gene and for the *GAPDH* genes were studied. The optimal number of PCR cycles and the mixing ratio of primers were determined.

The primers used were 5′-GTGTGGGTACGGTGATCGGTA-3′ and 5′-GCCTGCACACTGTCTGCATT-3′ for *PARP-1*, 5′-GGT CTTCCCCTACCCTCTCAA-3′ and 5′-CGTTGTGTGTTCGCCTCT-TG-3 for *c-myc*, 5′-CCCGCTGGCCATGAACTA-3′ and 5′-CGGAG GCAGTCTGGGTCA-3 for *cyclin D1*, 5′-TCTTTGGCCTACCTA-TAACTGG-3′ and 5′-CTAGACTGCTACCATCCGTC-3′ for *MMP-7*, 5′-GCGTGGACAATGGCTACTCA-3′ and 5′-GAGTTGTAATGGC ATAAAACAAC-3′ for *β-catenin*, 5′-GGCGTCTTCACCACCATG-GAG-3′ and 5′-AAGTTGTCATGGATGACCTTGGC-3′ for *GAPDH*.

2.3. *Immunohistochemistry*

Sixty formalin-fixed, paraffin-embedded colorectal tumour specimens were obtained from patients who had undergone polypectomy or surgical treatment. These tumour samples consisted of 36 adenomas and 24 pT1 adenocarcinomas. Additionally, specimens of seven hyperplastic polyps were obtained. Sections of 5 μm-thickness were dewaxed in xylene and rehydrated in alcohol. The sections were then heated to 105 °C in a target retrieval solution (DakoCytomation, Carpinteria, CA, USA) in an autoclave for 10 min, for antigen retrieval. Endogenous peroxidase activity was suppressed by a solution of 3% (v/v) hydrogen peroxide in methanol for 5 min. After being rinsed twice in phosphate-buffered saline (PBS), the sections were incubated for 18 h at 4 °C with an anti-human PARP-1 mouse monoclonal antibody (10 μg/ml, Lab Vision, Fremont, CA, USA) or anti-human β-catenin mouse monoclonal antibody (10 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The antibodies were diluted in antibody diluent with background reducing components (0.05 mol/L Tris–HCl buffer containing 0.1% (v/v) Tween and 0.015 mol/L sodium azide) (DakoCytomation). Normal mouse immunoglobulins were substituted for each primary antibody as negative controls. After washing three times in PBS, the sections were treated with biotinylated anti-mouse immunoglobulin for 10 min and then with horseradish peroxidase–avidin complex, diluted as recommended by the manufacturer for 10 min. The slides were then washed in PBS and developed in 0.05 M Tris–HCl (pH 7.5) containing 0.6 mg/ml of 3,-3′ diaminobenzidine at room temperature. The sections were counterstained in Mayer's hematoxylin and mounted. The sections were examined microscopically by two well-trained pathologists who were blinded to the clinicopathological characteristics. Nuclear expression of PARP-1 and β-catenin was defined as positive when an immunoreactivity was observed in more than 10% of tumour cells.

2.4. *Statistical analysis*

Expression of each target gene was assessed for associations with clinicopathological characteristics using the following statistical tests: Mann–Whitney *U*-test for age, size and average tumour-normal expression ratios, and the χ^2 two-tailed test or Fisher's exact test for the remaining parameters.

3. Results

3.1. *PARP-1 and β-catenin mRNA expression in colorectal tumour tissues*

To perform semi-quantitative RT-PCR analysis, the ranges of linear amplification for each target gene and for the control

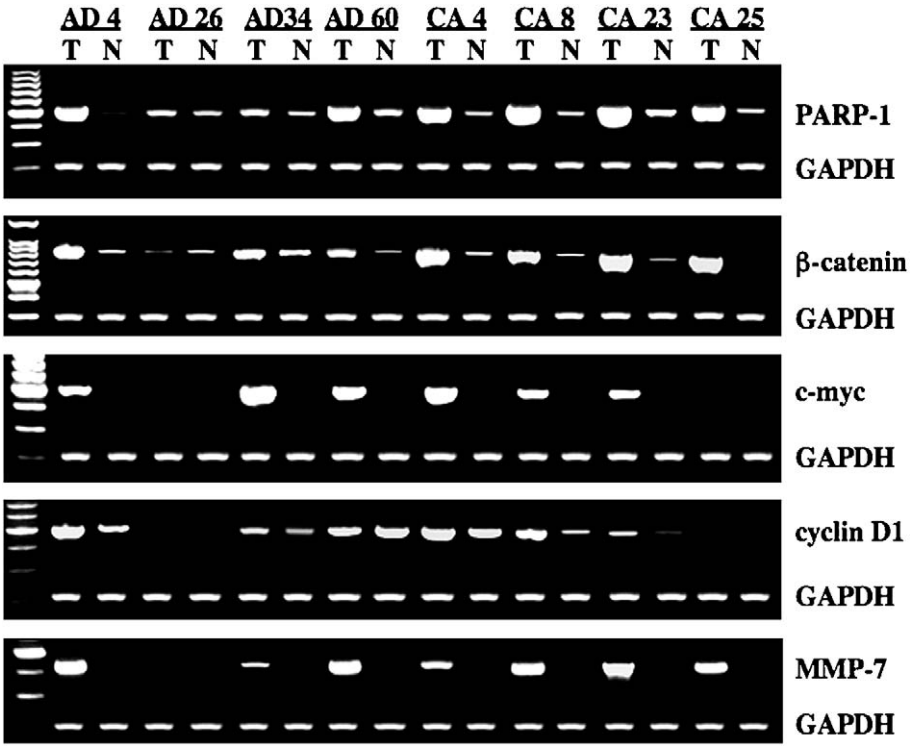

Fig. 1 – RT-PCR analysis of mRNA expression for PARP-1, *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* in colorectal tumour tissues. T and N, matched samples from tumour and non-tumour tissue, respectively. Cases 1–4 (AD4, AD26, AD34 and AD60 in Table 2) are colorectal adenomas and cases 5–8 (CA4, CA8, CA23 and CA25 in Table 2) are colorectal carcinomas (pT1).

Table 2 – Clinicopathological characteristics and mRNA expression profiles in 91 colorectal tumour tissues

Case	Age	Size	Gender	Location	Macroscopic type
No. 1	74	8	F	P	P
2	47	3	M	D	F
3	49	5	M	D	F
4	26	6	M	D	P
5	69	6	M	D	P
6	55	6	F	D	P
7	55	5	F	D	F
8	55	4	F	P	F
9	68	13	F	P	P
10	68	13	F	P	P
11	74	10	M	P	F
12	59	20	M	D	P
13	68	9	F	D	F
14	78	8	F	D	P
15	78	6	F	D	P
16	77	40	M	D	F
17	42	3	F	D	F
18	60	20	M	D	P
19	60	15	M	D	P
20	60	6	M	D	P
21	60	10	M	P	P
22	60	6	M	P	P
23	66	4	M	P	F
24	27	3	F	D	F
25	65	40	M	D	F
26	59	15	M	P	P
27	66	12	M	P	P
28	66	12	M	D	P
29	66	12	M	D	P
30	66	8	M	P	F
31	74	9	M	D	P
32	74	4	M	P	F
33	74	7	M	D	F
34	74	6	M	D	F
35	74	15	M	P	F
36	66	6	M	D	F
37	59	40	M	D	P
38	81	3	F	D	F
39	81	15	F	D	F
40	81	40	F	D	F
41	74	14	F	P	F
42	72	15	F	P	F
43	50	10	F	D	P
44	59	10	M	D	P
45	66	8	M	P	F
46	78	15	M	D	P
47	66	6	F	D	P
48	74	13	M	P	P
49	74	8	M	P	P
50	74	5	M	P	P
51	60	6	F	D	P
52	60	8	F	D	P
53	61	10	F	D	F
54	55	20	F	D	F
55	65	12	F	P	F
56	62	6	F	D	P
57	62	5	F	D	P
58	62	10	F	D	P
59	60	10	F	D	P
60	88	15	M	P	F
61	88	12	M	P	F
62	57	8	M	P	F
63	57	8	M	P	F
64	57	3	M	D	F
65	57	3	M	D	F

Case	Age	Size	Gender	Location	Macroscopic type
No. 1	78	14	F	D	P
2	72	10	M	D	P
3	72	15	M	D	P
4	55	10	F	D	P
5	63	12	M	D	P
6	68	45	F	P	P
7	62	35	F	P	F
8	74	5	M	P	F
9	59	20	M	D	P
10	81	20	F	D	P
11	81	20	F	P	P
12	68	22	M	P	P
13	68	20	F	D	P
14	68	18	F	D	P
15	68	24	M	D	P
16	68	16	M	D	P
17	70	20	F	D	P
18	70	20	M	P	P
19	70	19	M	P	P
20	60	26	F	P	P
21	60	22	M	D	P
22	69	23	M	D	F
23	74	22	F	D	F
24	66	8	M	D	F
25	66	22	F	D	F
26	55	20	F	P	F

Each row is a colorectal adenoma ($n = 65$) or pT1 cancer ($n = 26$). Black rectangles indicate each mRNA expression as positive. Gender (M, male; F, female), location (D, distal; P, proximal), macroscopic type (P, protruded; F, flat).

GAPDH gene were examined. The optimal number of PCR cycles and optimal mixing ratios of primers were determined. The expression of *PARP-1* and *β-catenin* mRNA in 91 colorectal tumour tissues was examined. Fig. 1 shows the representative results. *PARP-1* mRNA expression was detected in 64 (70.3%) of the 91 colorectal tumour tissues but was only faintly detected in adjacent non-tumour tissues. *PARP-1* overexpression was not detected in seven hyperplastic polyps (data not shown). The relationships between *PARP-1* overexpression and clinicopathological characteristics are shown in Table 2. *PARP-1* overexpression was detected in a significantly larger percentage of pT1 cancer tissues (25/26; 96.2%) than in adenoma tissues (39/65; 60.0%) ($P = 0.0006$). The expression was correlated significantly with tumour size (15.7 ± 9.9 mm *versus* 8.1 ± 4.6 mm (standard deviation, SD); $P = 0.0003$). There was no correlation of *PARP-1* overexpression with age, gender, tumour location or macroscopic type. When only adenoma tissues were considered, the expression was correlated significantly with tumour size (13.2 ± 10.2 mm *versus* 7.6 ± 3.8 mm; $P = 0.0088$). *PARP-1* overexpression was detected in 5 of 5 villous adenoma and 36 (56.7%) of 60 tubular adenoma tissues, respectively. Thus, there was a trend towards an association between villous adenoma and *PARP-1* overexpression ($P = 0.0697$). On the other hand, no significant difference was seen between *PARP-1* overexpression and the degree of dysplasia within adenoma tissues.

β-Catenin mRNA expression was detected in 36 (39.6%) of the 91 colorectal tumour tissues but was undetectable or only faintly detected in adjacent non-tumour tissues. The relationships between *β-catenin* overexpression and clinicopathological characteristics are shown in Table 2. *β-Catenin* overexpression was detected in a significantly larger percentage of pT1 cancer tissues (15/26; 57.7%) than in adenoma tissues (21/65; 32.3%) ($P = 0.0253$). There was no correlation of *β-catenin* overexpression with age, tumour size, gender, tumour location or macroscopic type.

3.2. *Expression of c-myc, cyclin D1 and MMP-7 mRNA in colorectal tumour tissues*

Fig. 1 shows representative results of RT-PCR for *c-myc*, *cyclin D1* and *MMP-7*. *c-myc* mRNA expression was detected in 71 (78.0%) of the 91 colorectal tumour tissues but was undetectable in adjacent non-tumour tissues. The relationships between *c-myc* overexpression and clinicopathological characteristics are shown in Table 2. *c-myc* Overexpression was detected in a significantly larger percentage of pT1 cancer tissues (24/26; 92.3%) than in adenoma tissues (47/65; 72.3%) ($P = 0.0374$). There was no correlation of *c-myc* expression with tumour size, age, gender, tumour location or macroscopic type.

Cyclin D1 mRNA expression was detected in 76 (83.5%) of the 91 colorectal tumour tissues. The relationships between *cyclin D1* overexpression and clinicopathological characteristics are shown in Table 2. *Cyclin D1* overexpression was detected in a significantly larger percentage of pT1 cancer tissues (26/26; 100%) than in adenoma tissues (50/65; 76.9%) ($P = 0.0374$). The expression was correlated significantly with gender (female > male; $P = 0.0408$) and tumour location (distal > proximal; $P = 0.0364$). There was no correlation of *cyclin D1* overexpression with age, tumour size or macroscopic type. When only adenoma tissues were considered, *cyclin D1* overexpression was correlated significantly with tumour location (distal > proximal; $P = 0.0347$) but not gender.

MMP-7 mRNA expression was detected in 66 (72.5%) of the 91 colorectal tumour tissues but was undetectable in adjacent non-tumour tissues. The relationships between *MMP-7* overexpression and clinicopathological characteristics are shown in Table 2. *MMP-7* overexpression was detected in a significantly larger percentage of pT1 cancer tissues (25/26; 96.2%) than in adenoma tissues (41/65; 63.1%) ($P = 0.0014$). The expression was correlated significantly with age (67.1 ± 10.4 years old *versus* 61.5 ± 10.5 years old; $P = 0.0256$), tumour size (15.5 ± 9.8 mm *versus* 8.0 ± 5.0 mm; $P = 0.0005$) and tumour location (distal > proximal; $P = 0.0159$). There was no correlation of *MMP-7* overexpression with gender or macroscopic type. When only adenoma tissues were considered, *MMP-7* overexpression was correlated significantly with tumour size (13.1 ± 10.0 mm *versus* 7.3 ± 3.4 mm; $P = 0.0070$) and tumour location (distal > proximal; $P = 0.0275$).

3.3. *Overexpression of β-catenin, c-myc, cyclin D1 and MMP-7 and their relationships with PARP-1 overexpression*

PARP-1 overexpression was correlated significantly with overexpression of *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* ($P < 0.0001$,

Table 3 – Overexpression of β-catenin, c-myc, cyclin D1 and MMP-7 mRNA expression and its relationship with PARP-1 mRNA expression

	PARP-1 mRNA expression		P-value
	Positive $n = 64$	Negative $n = 27$	
β-Catenin mRNA expression			
Present	35	1	<0.0001
Absent	29	26	
c-myc mRNA expression			
Present	61	10	<0.0001
Absent	3	17	
cyclin D1 mRNA expression			
Present	63	13	<0.0001
Absent	1	14	
MMP-7 mRNA expression			
Present	58	8	<0.0001
Absent	6	19	

Table 4 – Overexpression of c-myc, cyclin D1 and MMP-7 mRNA expression and its relationship with β-catenin mRNA expression

	β-Catenin mRNA positive $n = 36$	Expression negative $n = 55$	P-value
c-myc mRNA expression			
Present	34	37	0.0022
Absent	2	18	
cyclin D1 mRNA expression			
Present	35	41	0.0044
Absent	1	14	
MMP-7 mRNA expression			
Present	32	34	0.0047
Absent	4	21	

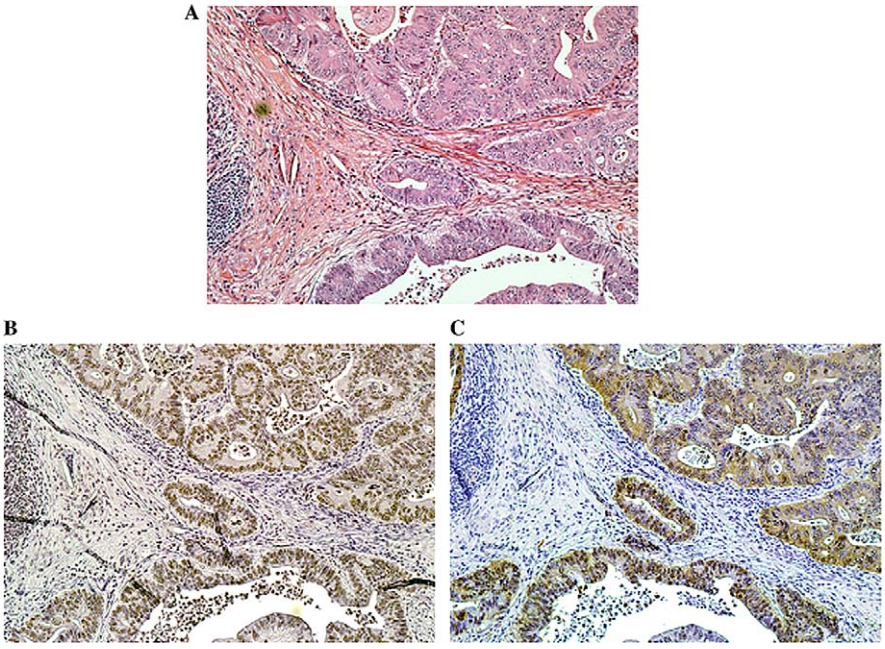

Fig. 2 – Hematoxylin-eosin staining (A) and immunohistochemical analysis for PARP-1 (B) and β-catenin (C) in serial sections of colon cancer tissues (case no. 5 in cancer group). (A) Hematoxylin-eosin stained section. (B) Nuclear expression of PARP-1 in cancer cells. (C) Nuclear expression of β-catenin in cancer cells. (A–C) Original magnification 100×.

$P < 0.0001$, $P < 0.0001$ and $P < 0.0001$, respectively, Table 3). When only adenoma tissues were considered, the correlation between *PARP-1* and *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* expression was still significant ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ and $P < 0.0001$, respectively).

3.4. *Overexpression of c-myc, cyclin D1 and MMP-7, and their relationships with β-catenin overexpression*

β-Catenin overexpression was correlated significantly with overexpression of *c-myc*, *cyclin D1* and *MMP-7* ($P = 0.0022$, $P = 0.0044$ and $P = 0.0047$, respectively, Table 4). When only adenoma tissues were considered, the correlation between *β-catenin* and *c-myc*, *cyclin D1* and *MMP-7* expression was still significant ($P = 0.0237$, $P = 0.0249$ and $P = 0.0090$, respectively).

3.5. *Immunohistochemical expression of PARP-1 and β-catenin in colorectal tumour tissues*

The expression of PARP-1 and β-catenin was analysed immunohistochemically in 60 tumour (36 adenomas and 24 pT1 cancer) specimens. Fig. 2 shows the representative results of immunohistochemical expression of PARP-1 and β-catenin in a patient (case no. 5 in cancer group). PARP-1 was stained in the nuclei of tumour cells. No significant staining was observed in fibroblasts, smooth muscle cells or endothelial cells, although significant staining was found in some lymphocytes. There was no significant difference in stromal lymphocytes infiltration among tumour samples (data not shown). Immunohistochemical expression of PARP-1 and β-catenin was positive in 35 (58.3%) and 17 (28.3%) of the 60 tumour tissues, respectively, but not in seven hyperplastic polyps. PARP-1 expression was correlated significantly with β-catenin expression (14 PARP-1-positive/17 β-catenin-positive *versus* 21 PARP-1-positive/43 β-catenin-negative, $P = 0.0176$). In 8 (22.9%) of 35 PARP-1-positive pT1 cancers, PARP-1 protein expression was stronger at the invasive edge of the tumour as opposed to the more superficial regions. In these cases, nuclear β-catenin expression showed similar expression pattern.

4. Discussion

The issue that we addressed in this study was the expression of PARP-1 and its relationship with the expression of β-catenin and its target genes, such as *c-myc*, *cyclin D1* and *MMP-7*, in the early stage of colorectal carcinogenesis. The reason why we chose pT1 cancer is that it represents the early stage of colorectal cancer.

PARP-1 mRNA overexpression was detected in 64 (70.3%) of the 91 colorectal adenoma and pT1 cancer tissues but was only faintly detected in adjacent non-tumour tissues and hyperplastic polyps tissues. *PARP-1* mRNA overexpression was detected in a significantly larger percentage of pT1 cancer tissues than in adenoma tissues and the expression was correlated significantly with tumour size. Moreover, *PARP-1* expression was detected in a higher percentage of villous adenoma tissues than in tubular adenoma tissues. These results further support the notion that *PARP-1* overexpression plays an important role in the early stage of human sporadic colorectal carcinogenesis.[12]

Overexpression of *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* mRNA was observed in 39.6%, 78.0%, 83.5% and 72.5% of the 91 colorectal tumour tissues, respectively. Expression of *β-catenin*, *c-myc*, *cyclin D1* and *MMP-7* was correlated significantly with histopathology and *PARP-1* expression. Thus, as a co-activator of TCF-4/β-catenin-evoked gene transactivation,

at least in part, PARP-1 may play a role in the early stage of colorectal carcinogenesis. In colorectal carcinogenesis, several genetic and epigenetic alterations such as *K-ras* mutations and *p16* methylation have been reported to be correlated with age, gender and/or tumour location.[15,16] In this context, correlation of overexpression of *cyclin D1* and matrilysin with distal location is interesting. These results as well as correlation of *cyclin D1* with females warrants further analysis.

Hirai and colleagues[12] initially showed nuclear overexpression of poly(ADP-ribose) in colon adenoma and carcinoma tissues. In addition, Idogawa and colleagues[3] reported that the expression of PARP-1 paralleled nuclear expression of β-catenin in adenoma cells of FAP patients and that overexpression of PARP-1 was detected in 23 (82.1%) of 28 sporadic colorectal carcinomas. In the current study, we have shown immunohistochemically PARP-1 overexpression (58.3%) and its correlation with β-catenin overexpression in human sporadic adenomas and pT1 carcinomas. These results further support the notion that PARP-1 and β-catenin play a role in the early stage of human sporadic colorectal carcinogenesis. In a subset of pT1 carcinomas, nuclear expression of PARP-1 and β-catenin was stronger at the invasive edge of the tumour as opposed to the more superficial regions. Further analysis is needed to clarify the mechanisms underlying preferential expression of these genes at the invasive front.

Members of the Ets family of transcription factors have been reported to be key regulators of PARP-1 overexpression in colorectal tumours.[9] Consistent with this report, the mRNA levels of *Ets-1* and *Ets-2* were increased in adenoma tissues of *Min* mice.[3] Moreover, it has been reported that transfection of *Ets-1* or *Ets-2* enhanced the activity of a *PARP-1* gene reporter containing an Ets-binding consensus motif and that a dominant-negative form of *Ets-2* abolished the enhancement.[3] Although *PARP-1* appears not to be a direct target of the TCF/β-catenin complex,[3] *Ets-2* can be a target gene of the TCF-4/β-catenin complex.[17] Moreover, c-myc has been shown to enhance the protein level of PARP-1.[18] These findings suggest that PARP-1 may be indirectly regulated by the TCF-4/β-catenin complex by means of Ets and/or c-myc, establishing a positive feedback loop that enhances PARP-1 expression.[3] Further analyses are required to clarify these issues in colorectal carcinogenesis.

We have recently reported that inducible nitric oxide synthase (iNOS) plays an important role in the early stage of human sporadic colorectal carcinogenesis.[14] Pharmacological inhibition of iNOS with guanidinoethyldisulfide reduced both intestinal tumour development and oxidative stress associated with intestinal polyposis in *Apc min*/+ mice.[19] Interestingly, pharmacological inhibition of PARP by a phenanthridinone derivative, PJ-34, also reduced the intestinal polyposis and oxidative stress in *Apc min*/+ mice, possibly in part through the inhibition of induction of NOS.[19] Therefore, therapeutic agents that inhibit the expression or function of PARP-1 may prove efficacious or might complement agents that compromise β-catenin, c-myc, cyclin D1, MMP-7 and/or iNOS activities in the treatment and/or chemoprevention of colorectal tumours and other tumours characterised by PARP-1 overexpression.

Ethics approval statement

An informed consent was obtained from each patient and the institutional review committee approved this study.

Conflict of interest statement

None declared.

Acknowledgements

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.Y. and K.I.) and Grants-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan (H.Y. and K.I.).

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