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Reciprocity between membranous and nuclear expression of β-catenin in colorectal tumours

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Abstract β-Catenin has a central role not only in linking the cadherin-mediated cell adhesion system but also in the intercellular signalling pathway. To investigate alterations of β-catenin in the development of colorectal carcinoma, the pattern of β-catenin expression was studied using immunohistochemistry in 74 sporadic colorectal adenomas, in histologically normal mucosa adjacent to 65 of these adenomas, and in 52 carcinomas arising in adenomas. All normal epithelia displayed cell boundary staining for β-catenin. Adenomas and carcinomas showed varying degrees of membranous staining. However, some tumours also showed nuclear staining of β-catenin protein. Decreased membranous and increased nuclear β-catenin staining were associated with increasing degrees of dysplasia in adenomas ($P < 0.005$, $P < 0.05$, respectively). Carcinomas manifested significantly reduced membranous, but enhanced nuclear β-catenin expression compared with their associated adenomas ($P < 0.001$, $P < 0.005$, respectively). An inverse correlation was found between decreased membranous and increased nuclear staining of β-catenin in both adenomas and carcinomas ($P < 0.025$, $P < 0.05$, respectively). The data confirm that reduced membranous and increased nuclear expression of β-catenin is associated with the progression of colorectal adenomas to carcinomas. Our results also suggest that decreased membranous expression of β-catenin may result from aberrant localisation of the protein in the cell nucleus.

Key words Adhesion molecules · β-Catenin · Colorectum · Tumours · Adenoma–carcinoma sequence

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Introduction

β-Catenin is a 92,000-KDa cytoplasmic protein, which has high sequence similarity with a *Drosophila* segment polarity gene product Armadillo (71% amino acid identity) [1, 2]. The two proteins have similar biological functions. Experiments have shown that cell–cell adhesion, cell polarity and cytoskeletal integrity are disrupted during *Drosophila* embryogenesis in the absence of Armadillo [3]. Truncated β-catenin also disrupts the interaction between E-cadherin and α-catenin in human cancer cell lines, leading to the loss of intercellular adhesion [4]. Thus, β-catenin/Armadillo has a key role in the cadherin-mediated adhesion system. β-Catenin/Armadillo also participates in intracellular signalling. For instance, Armadillo in *Drosophila* is involved in a signalling cascade initiated by Wingless, a *wnt* protein homologue, and is responsible in part for the formation of the anterior–posterior polarity of the fly segments [5]. Overexpression of β-catenin in the ventral side of the early *Xenopus* embryo also induces the formation of a complete secondary body axis [6]. Furthermore, β-catenin has been shown to interact with the tumour suppressor gene product, adenomatous polyposis coli (*APC*) protein [7, 8], and to be associated with *C-erbB-2* gene product and epidermal growth factor receptor (EGFR), which deal with cascades of intercellular signalling transduction [9–12].

As β-catenin is involved in cell differentiation, adhesion and multiple cellular signalling, and because *APC* mutations are important in the pathogenesis of colonic and other tumours, β-catenin mutations and/or differences in protein expression may be involved in oncogenesis. We have therefore examined the expression of β-catenin in sporadic adenomas and carcinomas. Particular regard has been paid to the subcellular localisation of β-catenin protein in these tumours.

Materials and methods

Specimens

We selected 74 patients with adenomas from the files of the Academic Department of Pathology, St. Mark's Hospital, according to the following criteria: only patients without personal history of malignancies or familial adenomatous polyposis were chosen; all polyps were completely excised by polypectomy or surgical resection; the adenomas selected were the largest and the most severely dysplastic if multiple. Sixty-five histologically normal epithelia adjacent to adenomas and 52 carcinomas arising in adenomas were also included in this study. Serial sections were cut at 4 μm thickness from paraffin-embedded blocks and placed on poly-*l*-lysine-coated (Sigma Chemicals, St. Louis, Mo.) slides. One slide was stained with haematoxylin and eosin and used for histological classification (ICT), and others were used for immunohistochemistry.

Immunohistochemistry

A standard ABC method was employed. Briefly, tissue sections were dewaxed in xylene and were rehydrated through graded alcohol to distilled water. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min. Subsequently, sections were subjected to antigen retrieval by boiling for 10 min in an aluminium pressure cooker at 15 psi in sodium citrate buffer (0.01 M, pH 6.0). Following this, nonspecific staining was blocked by normal horse serum for 10 min. Anti-β-catenin primary antibody (1:2000, Transduction Laboratories, KY 40503) was added to the sections and incubated overnight at room temperature. The slides were sequentially incubated with biotinylated horse antimouse immunoglobin G (Vector Laboratories, Burlingame, CA 94010; 1:200 for 30 min) and stained by using the Vectastain Elite ABC Kit (Vector Laboratories; 1:50 for 30 min). 3-3'Diaminobenzidine (DAB; Sigma Chemicals) was used as the chromogen. Formalin-fixed, paraffin-embedded sections of normal human intestinal mucosa served as positive controls, while negative controls were obtained by using PBS in place of the primary antibody.

Scoring methods

The immunohistochemical results were reviewed by two independent observers (X.P.H., J.P.P.) without knowledge of histopathological features of the tumours. The percentage of cells with membranous positivity was graded as follows: 0 (< 5%), 1 (5–25%), 2 (26–50%), 3 (51–75%), 4 (> 75%), and the staining intensity was graded as negative (0, no staining), weak (+), moderate (++), or intense (+++, as strong as in normal mucosa). Multiplication of the values for the intensity and percentage [13] yielded a score ranging from 12 to 0. Scores of 12–9 were defined as strong staining, 8–6 as reduced and 4–1 as greatly reduced staining, and 0 as negative. Since there was very little difference in immunoreactivity between score 12 and score 9 in comparison with scores in the range 8–0, for statistical reasons, scores of 12–9 were defined as preserved expression while scores of 8–0 were defined as reduced expression. Nuclear staining was considered positive (+) when more than 5% of nuclei were stained and negative (–) when less than 5% were stained.

Statistics

The Chi-square test was used to test correlations between the membranous and nuclear staining and the pathological data.

Results

Normal mucosa

All histologically normal epithelia showed clearly uniform membrane staining along the whole length of the crypts (Fig. 1), and this served as an internal positive control. No background in the stroma or nuclear staining was found at all.

Adenoma

The results of the immunostaining for β-catenin in 74 adenomas are summarised in Tables 1 and 2. Adenomas displayed membranous and nuclear staining (Figs. 2, 3). There were 23 of the 74 (31.1%) adenomas that showed reduced membranous staining, and 34 (45.9%) adenomas manifested nuclear staining. A significant inverse relationship was found between decreased membranous staining and increasing severity of dysplasia ($P < 0.005$; Table 1). Increased nuclear staining was also correlated with degree of dysplasia ($P < 0.05$; Table 2). There was a strong correlation between decreased membranous and increased nuclear staining of β-catenin ($P < 0.025$; Table 3). No correlation existed between adenoma size and membranous or nuclear staining (data not shown).

Table 1 Correlation between membranous staining of β-catenin and dysplasia in sporadic adenomas

	Preserved 12–9 (%)	Reduced		
		8–6	4–1	Total (%)
Dysplasia mild	19 (84.6)	4	0	4 (17.4)
Moderate	17 (41.5)	16	0	16 (48.5)
Severe	5 (27.8)	10	3	13 (72.2)

Chi-square test with 2 *df*, (12–9-v-8–6, 4–1), $P<0.005$

Table 2 Correlation between nuclear staining of β-catenin and dysplasia in sporadic adenomas

	+ (%)	–
Dysplasia mild	7 (30.4)	16
Moderate	21 (63.6)	12
Severe	12 (66.7)	6

Chi-square test with 2 *df*, $P<0.05$

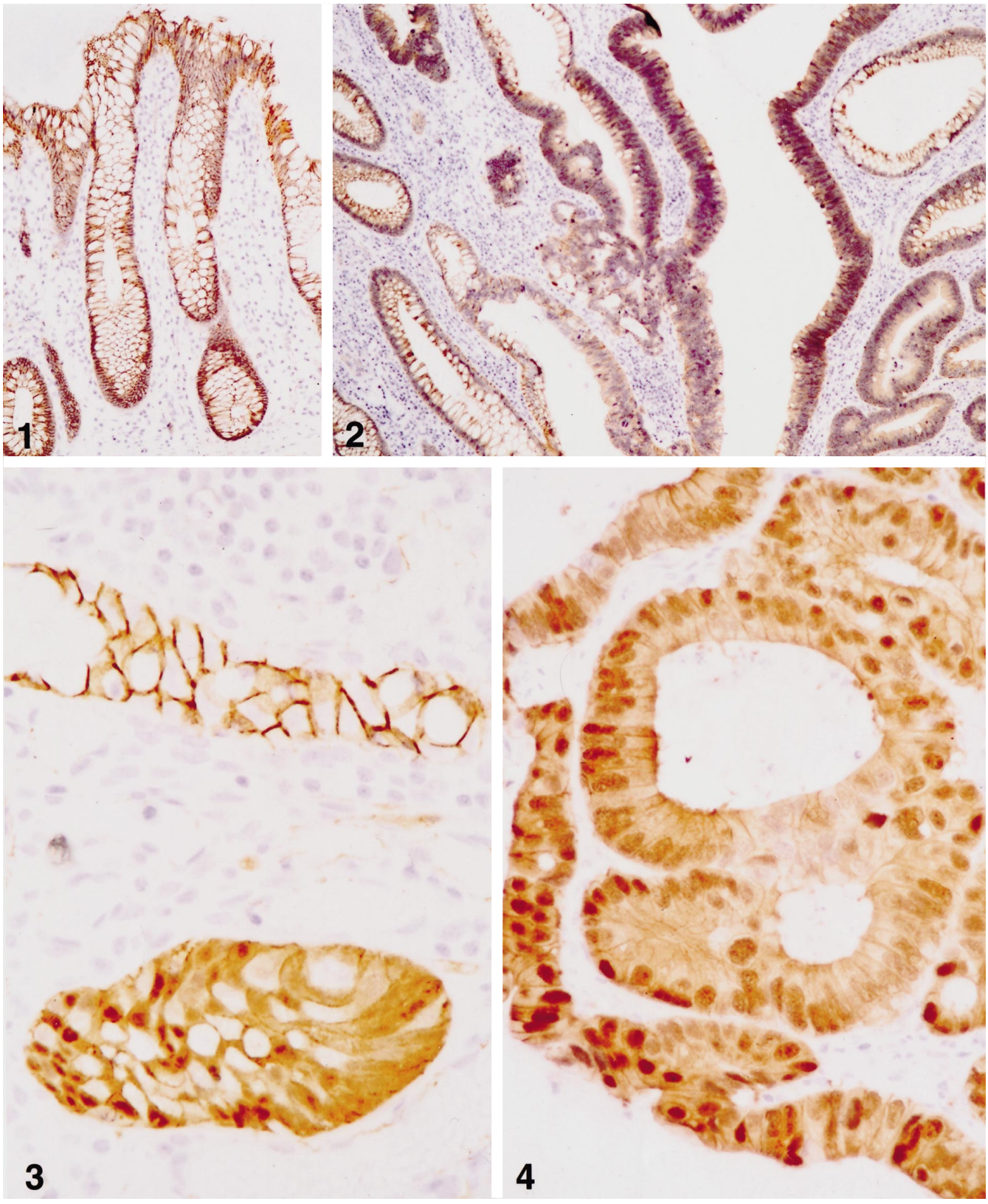

Fig. 1 Normal mucosa showing membranous staining. No nuclear staining is seen. Immunostaining, ×50

Fig. 2 Severe dysplasia showing decreased membrane staining compared with the normal mucosa. Immunostaining, ×25

Fig. 3 Moderate dysplasia manifesting decreased membranous but increased nuclear staining compared with the normal mucosa. Immunostaining, ×125

Fig. 4 Carcinoma cells clearly showing nuclear staining. Immunostaining, ×125

Table 3 Correlation between membranous and nuclear staining of β-catenin in sporadic adenomas

		Nuclear staining	
		+	–
Membranous staining	Preserved	17	24
	Reduced	23	10

Chi-square test with 1 *df*, $P<0.025$

Table 4 Membranous staining of β-catenin in carcinomas and associated adenomas

	Preserved 12–9	Reduced			
		8–6	4–1	0	Total (%)
Adenomas	18	28	6	0	34 (65.4)
Carcinomas	9	17	25	1	43 (82.7)

Chi-square test with 2 *df*, (12–9 vs 8–6 vs 4–0), $P<0.001$

Table 5 Membranous staining of β-catenin in carcinomas and associated adenomas with severe dysplasia

	12–9	8–6	4–1	0
Adenomas	6	26	6	0
Carcinomas	5	14	18	1

Chi-square test with 1 *df* (12–9 vs 8–6 vs 4–0), $P<0.001$

Table 6 Nuclear staining of β-catenin in carcinomas and associated adenomas

	+ (%)	–
Adenomas	26 (50)	26
Carcinomas	41 (78.8)	11

Chi-square test with 1 *df*, $P<0.005$

Table 7 Nuclear staining of β-catenin in carcinomas and associated adenomas with severe dysplasia

	+	–
Adenomas	24	14
Carcinomas	34	4

Chi-square test with 1 *df*, $P<0.01$

Table 8 Correlation between membranous and nuclear staining in carcinomas

		Nuclear staining	
		+	–
Membranous staining	Preserved	6	5
	Reduced	38	7

Chi-square test with 1 *df*, $P<0.05$

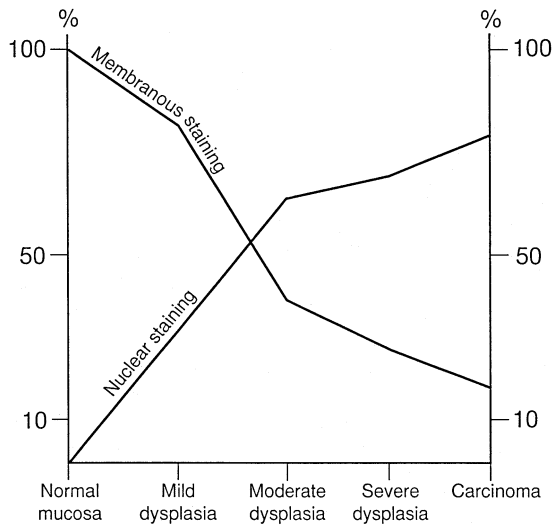

Fig. 5 Correlation of membranous and nuclear staining for β-catenin in sporadic adenomas and carcinomas

Carcinoma and associated adenoma

The results are summarised in Tables 4 and 6. Carcinoma cells manifested the same immunostaining patterns as adenomas (Fig. 4). Most (44/52, or 84.1%) cases showed reduced membranous staining, and 1 case (1.9%) was negative. In contrast, 41 of the 52 (78.8%) displayed nuclear staining. Membranous staining of β-catenin in carcinomas decreased significantly compared with the associated adenomas ($P < 0.001$; Table 4), and even compared with associated adenomas with severe dysplasia ($P < 0.001$; Table 5). Nuclear staining of β-catenin in carcinomas was increased significantly compared with the associated adenomas ($P < 0.005$; Table 6), even when the associated adenomas were severely dysplastic ($P < 0.01$; Table 7). As in the adenomas, there was an inverse correlation between decreased membranous and increased nuclear staining of β-catenin in carcinomas ($P < 0.05$, Table 8, Fig. 5).

Discussion

In this study, all normal epithelia showed uniform membranous staining for β-catenin and no nuclear staining. Membranous staining for β-catenin began to decrease at the mildly dysplastic stage, and became significantly less in parallel with the progression of dysplasia in adenomas. β-Catenin membranous expression in carcinomas decreased further compared with the associated adenomas, even when only the associated adenomas with severe dysplasia were considered. This directly con-

firms that disturbance of β-catenin expression is strongly associated with the development of colorectal carcinomas.

Apart from membranous staining, adenomas and carcinomas also displayed nuclear staining for β-catenin protein, as has already been reported in FAP (familial adenomatous polyposis) patients [14]. We found that nuclear accumulation of β-catenin increased significantly from mild to moderate and severe dysplasia in adenomas, and further enhanced significantly after conversion from adenoma to carcinoma. The role of β-catenin expression in nucleus remains elusive. Recent experiments have shown that at the blastoderm stage of development of the *Drosophila* embryo, preceding the onset of Wingless signalling, Armadillo accumulates in the cytoplasm of all cells and is enriched at cell–cell boundaries, but excluded from nuclei; in contrast, at the embryonic stage, when Wingless signalling is active, Armadillo accumulates in both the cytoplasm and nucleus [15]. This suggests that nuclear accumulation of β-catenin/Armadillo is involved in the transduction of intercellular signalling. Since β-catenin/Armadillo mediates numerous cell fate choices during embryogenesis [16], it is possible that nuclear accumulation of β-catenin may play a part in determining the progression and invasion of cancer.

It is interesting to note that we have demonstrated a reciprocal relationship between membranous and nuclear staining in both adenomas and carcinomas. Moreover, we also find an inverse correlation between membranous staining of E-cadherin and nuclear accumulation of β-catenin in both adenomas and carcinomas (X. Hao et al., unpublished observation). These observations are analogous to the experimental findings. For example, cadherin overexpression in *Xenopus* suppressed β-catenin's role in Wnt signalling [17], whereas in *Drosophila*, reduction in DE-cadherin levels enhanced Armadillo's role in Wingless signalling [3]. These results suggest that the roles of β-catenin/Armadillo in cell adhesion and intercellular signalling are competitive [18], and the reduced membranous staining may result from increased nuclear accumulation. The mechanisms that modulate the levels of β-catenin in the adherens junctions and nuclei may involve many factors, most of which are unknown. Possible influences on β-catenin protein include mutations at the *β-catenin* locus [19], mutations at the *α-catenin*, *γ-catenin* and *E-cadherin* loci [18], *APC* mutations [20] and post-transcriptional modulation of β-catenin protein [11, 21–24].

In summary, we investigated the changes in β-catenin protein expression in colorectal tumorigenesis. An inverse relationship between membranous and nuclear staining of β-catenin was found in both adenomas and carcinomas. Reduced membranous and increased nuclear expression were correlated with the degree of dysplasia in adenomas and with the development of invasiveness in carcinomas arising from adenomas. Further studies are required to reveal the mechanisms that lead to these alterations of β-catenin protein in tumours.

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