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The significance of beta-catenin, E-cadherin, and P-cadherin expressions in neoplastic progression of colorectal mucosa : an immunohistochemical study

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Abstract

Background and study aims : **The purpose of the current study was to investigate the role of β-catenin, E-cadherin and P-cadherin in colorectal carcinogenesis using tissue array method.**

Patients and methods : **Core tissue biopsies were taken from paraffin-embedded tissue blocks of 167 cases including 26 normal mucosae (NM), 99 colorectal polyps (10 hyperplastic polyps (HP), 8 traditional serrated (TSA), 17 tubular (TA), 37 tubulovillous (TVA), and 27 villous adenomas (VA)), 14 adenomas with intramucosal carcinoma (ACA), and 28 colorectal cancers (CCA). Immunohistochemistry was performed using antibodies to β-catenin, E-cadherin, and P-cadherin. Distribution of positivity was assessed using percentage expression while an arbitrary grading scale was used for staining intensity.**

Results : **β-catenin expression was cytoplasmic, membranous, and nuclear. Both E-cadherin and P-cadherin expressions were confined to cytoplasmic-membranous compartments. Membranous expression of β-catenin significantly decreased in CCA ($p < 0.01$). Nuclear β-catenin expression significantly increased in close correlation with neoplastic sequence reaching its highest expression in ACA and CCA ($p < 0.001$). Polyps with intraepithelial neoplasia (IEN) showed significantly higher nuclear β-catenin expression in parallel with increasing grades of IEN ($p < 0.001$). E-cadherin and P-cadherin expression increased in polyps, whereas a significant decrease in their expression was observed in CCA ($p < 0.001$) while E-cadherin expression significantly increased in CCA compared to NM ($p < 0.001$), no such difference was observed in P-cadherin expression.**

Conclusions : **Nuclear β-catenin expression correlating with the grade of IEN in polyps and carcinomas supports its role in colorectal carcinogenesis. E-cadherin and P-cadherin expressions in adenomas suggest that these molecules might have role in adenoma formation though not necessarily be involved in neoplastic progression.** (Acta gastroenterol. belg., **2007**, 70, **339-344**).

Introduction

CRC results from the cumulative effects of multiple sequential genetic alterations. The genetic pathway model for the pathogenesis of CRC proposed by Fearon and Vogelstein is based upon the concept of an adenoma-carcinoma sequence (1). In recent years, a "serrated pathway" involving hyperplastic polyp-serrated adenoma-carcinoma sequence has been proposed as an alternative to the adenoma-carcinoma sequence (2,3,4,5).

Altered APC gene and KRAS mutations are early genetic events resulting in adenoma formation, through increased transcriptional activation of β-catenin and DNA- binding proteins of T-cell factor (TCF)/lymphoid enhancer factor (LEF) (6,7). This mainly occurs through mutations in APC gene and decreased APC-associated degradation of β-catenin. When β-catenin or APC are mutated, or the Wnt pathway is activated, β catenin accumulates in the cytoplasm, translocates to the nucleus, and binds to the TCF/LEF. This results in the up-regulation of genes involved in transcription such as c-myc, c-jun, fra-1 and cyclin D1, thereby causing neoplastic growth (8,9). On the other hand, recent studies have shown that genetic and epigenetic alterations including KRAS mutations, chromosome 1p loss, microsatellite instability (MSI), CpG island methylation of p16 gene and other loci, and CpG island methylator phenotype (CIMP) with concordant methylation of CpG islands are frequently observed in "serrated pathway" of colorectal carcinogenesis (10,11).

Adhesion between epithelial cells is mediated mainly by cadherins, localized largely in adherent junctions (12, 13,14). β-catenin is necessary for cell to cell adhesion and links E cadherin to γ-catenin and α-catenin which mediates the interaction between the cadherin-catenin complex and actin cytoskeleton, helping to regulate cell morphology and polarity. This binding is essential for the adhesive function of E-cadherin and for establishment of tight adhesions between epithelial cells. Cadherins and catenins also have important functions in the biological behaviour of tumours. Changes in the structure of these molecules lead to discordance in cell to cell adhesion and activation of target genes which are involved in regulation of epithelial differentiation and oncogenic transformation (15,16).

Development of colorectal adenoma requires epithelial remodelling and stratification. Recent studies have shown that P-cadherin, normally expressed in squamous epithelia and placenta (17), is aberrantly expressed from the earliest morphologically identifiable stage of colonocyte transformation, prior to changes in E- cadherin, β-catenin and APC mutation (18). Reduced E-cadherin expression accompanied by aberrant P-cadherin expression was shown in colitis (19), breast carcinoma (20) and cervical squamous intraepithelial neoplasia (21).

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These studies suggest interdependence of expression of E- and P-cadherin in some circumstances. Increased nuclear β-catenin expression from early adenomas to adenocarcinomas is predictive of its oncogenic role in tumour progression by transcriptional activation of regulatory genes. However, there is still little information regarding the relationship of E-cadherin and P-cadherin expressions in adenoma formation and progression to carcinoma.

The aim of the present study was, therefore, to determine different expression patterns of β-catenin, E-cadherin and P-cadherin both in classical adenoma-carcinoma sequence and the recently defined serrated neoplasia sequence of the colorectum in a large series of cases using tissue array technique.

Materials and methods

A total of 167 cases including 99 colorectal polyps (10 hyperplastic polyps (HP), 8 traditional serrated (TSA), 17 tubular (TA), 37 tubulovillous (TVA), and 27 villous adenomas (VA)) and 14 adenomas with intramucosal carcinoma (ACA), 28 colorectal cancers (CCA) of which 4 were well-differentiated, 21 were intermediate and 3 were poorly differentiated and 26 normal mucosae (NM) derived from specimens resected for purposes other than cancer were studied. Polyps were further classified according to the degree of glandular intraepithelial neoplasia (IEN/dysplasia) as low, and high grades while carcinomas were graded as well, moderate and poorly differentiated according to WHO classification (22). In polyps showing intramucosal carcinoma and in carcinoma cases a small groups of dedifferentiated tumour cells at the deepest infiltrative part of the tumour was considered as invasive front. After reviewing the slides of each case, representative fields of the lesions were marked and core tissue samples were prepared manually from the paraffin blocks using a 4 mm-diameter dermatologic biopsy needle. A total of 13 array blocks each containing 14 cases that were represented by one core were prepared. Sections of 4 micron thickness were cut and mounted on poly-L-lysine coated slides which were stained with monoclonal antibodies raised against β-catenin (clone : 17C2, 1:100, Novacastra), E cadherin (clone : 36B5, 1:30, Novacastra), and P cadherin (clone : 56C1, 1:75, Novacastra) using Ventana NexEs automated immunostainer for secondary visualization. Antibody detection was performed by using a secondary antibody of Ventana (Ventana Medical Systems Tuscon, AZ, USA) and 3,3'-diaminobenzidine. Positive control tissues were used as recommended by the suppliers whereas exclusion of the primary antibody served as negative control.

The extent of staining was assessed as percentage of positively stained areas within the tissue core while staining intensity was graded as weak (+), moderate (++), and strong (+++) using an arbitrary scale. Expression site within an individual cell was evaluated as nuclear, cytoplasmic, and membranous. An expression score was calculated by multiplying percentage expression by the intensity of staining.

The age and gender of the patients, location, size, number and gross features of the polyps, grade and stage of the carcinoma cases were retrieved from patient files. Location of the polyps was grouped as rectum, left colon including splenic flexura, descending colon and sigmoid, and right colon including caecum, ascending colon and transverse colon. There were 112 males and 55 females with an age range of 21-84 years. Though, mean age was lower in the HP group compared to all other groups no significant age difference was observed between other polyp groups (Table 1). It was significantly lower in polyps without IEN compared to those with IEN and carcinoma ($p < 0.01$). No significant difference was found between male and female patients with regard to the prevalence of different types of polyps. When the location of the polyps was evaluated hyperplastic polyps and carcinomas were found to be more frequently located in the rectum (60% and, 60,7%, respectively) in comparison to TSA, TA, TVA, VA, and ACA (25%, 23.5%, 18.9%, 37%, and 28.6 %, respectively). Adenomas with carcinomatous foci were significantly larger (20.2 mm) when compared with other polyps (HP : 9.38, TSA : 7.6, TA : 8, TVA : 11.06, VA : 11.73 mm) ($p < 0.001$). The site and size of the cases are presented in Table 1. Polyps showing IEN were graded as low (n : 23, 23,2%), and high (n : 62, %62,6) grade. Fourteen of them (14,2%) were free of IEN. No significant association was observed between location, size and IEN in the study groups.

Statistical analysis was performed using Kruskal Wallis variance analysis for the comparison of the groups while Multiple Comparison Tests were used to determine the differences between the groups. Chi square test was used to evaluate the difference between the groups for classified data. A p value of < 0.05 was considered as significant.

Results

Expression of β-catenin was seen both in cell membrane and cytoplasm of normal mucosae, polyps and carcinomas. Nuclear β-catenin was observed only in adenomas and carcinomas. Membranous expression of β-catenin was slightly reduced through the sequence. Both percentage membranous expression and membranous staining intensity of β-catenin were significantly decreased in CCA group compared to all other groups ($p < 0.01$). Cytoplasmic staining of β-catenin increased in parallel with the neoplastic progression while a significant decrease in its expression was observed in CCA ($p < 0.01$). Nuclear β-catenin expression was absent in normal mucosae, HP, and TSA while it significantly increased in close correlation with the neoplastic sequence reaching its highest expression in ACA and CCA ($p < 0.001$). Forty percent of adenomas, 78.6% of

Table 1. — **Clinicopathological features of the study groups**

Groups (n)	Age (years) mean ± SD	Site						Size (mm) mean ± SD
		Rectum		Left		Right		
		n	%	n	%	n	%	
TA (n : 17)	59,83 ± 9,9	4	23,5	10	58,9	3	17,6	8 ± 3,6
TVA (n : 37)	56,46 ± 11,8	7	18,9	22	59,5	8	21,6	11,06 ± 5,5
VA (n : 27)	56,88 ± 16,4	10	37	16	59,2	4	14,8	11,73 ± 5,5
HP (n : 10)	39,62 ± 12,14	6	60	3	30	1	10	9,38 ± 5,4
TSA (n : 8)	55,3 ± 10,1	2	25	6	75	0		7,6 ± 3,5
ACA (n : 14)	59,35 ± 17,5	4	28,6	10	71,4	0		20,2 ± 8,5
CCA (n : 28)	58,28 ± 1486	17	60,7	10	35,7	1	3,6	42,5 ± 14,8

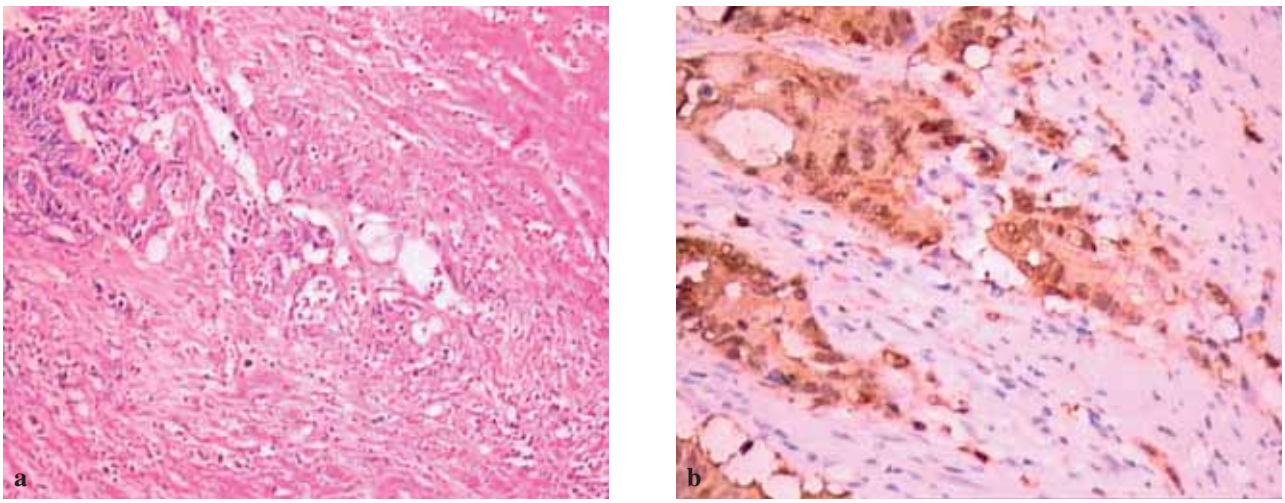

Fig. 1. — Nuclear β-catenin expression in disseminating tumour cells at the invasive fronts of CCA (a : H & E, × 200 ; b : β-catenin, × 400).

ACA and 67.8% of CCA showed nuclear expression of β-catenin. In ACA and CCA, nuclear β-catenin expression was found predominantly in disseminating tumour cells at the invasive fronts (Fig. 1).

E-cadherin expression was observed both in the cell membrane and in the cytoplasm. Normal mucosae showed the weakest E-cadherin expression which increased in HP, TSA and adenomas while a significant decrease in its expression was observed in CCA ($p < 0.001$) in a similar manner as its staining intensity which was also significantly lower in CCA group when compared to all other groups ($p < 0.001$).

P-cadherin expression was also both membranous and cytoplasmic. No significant difference was observed in the expression pattern of P-cadherin between polyp types. Percentage expression of P-cadherin also showed a significant increase ($p < 0.001$), through the sequence except for CCA group which had significantly lower expression staining intensity compared to all other groups except normal mucosae ($p < 0.001$). Expression of β-catenin, E- cadherin and P-cadherin is presented in table 2 and figure 2.

Cytoplasmic and membranous β-catenin expression significantly ($p < 0.01$) decreased in the carcinoma group which showed the highest nuclear expression. Nuclear β-catenin expression significantly correlated ($p < 0,001$) with the grade of IEN in contrast to membranous and cytoplasmic staining. Nuclear β-catenin expression was absent in polyps without IEN whereas polyps with IEN showed significantly higher nuclear β-catenin expression in parallel with the increasing grades of IEN ($p < 0.001$). Percentage expressions of E-cadherin and P-cadherin were significantly higher in polyps with IEN when compared to polyps without IEN ($p < 0.01$). However, no correlation could be observed with the grade of adenocarcinomas and β-catenin, E-cadherin and P-cadherin expressions since the majority of the cases were in intermediate grade. Expression of β-catenin, E and P-cadherin in polyps with IEN is presented in figure 3.

Discussion

Activation of APC/β-catenin pathway plays an important role in colorectal tumourigenesis, and mutations in β-catenin gene occur early in this process, leading to adenoma formation. The demonstration of increased cytoplasmic expression of β-catenin in the earliest identified neoplastic lesion in the colon (aberrant crypt foci-ACF), suggests its early role in colorectal carcinogenesis (23). β-catenin plays a major role in cadherin mediated cell-cell adhesions as well as in transcriptional

Table 2. — **Percentage expression and staining intensity of β-catenin, E-cadherin and P-cadherin in study groups**

Study Groups n	β-catenin (cytoplasmic)		β-catenin (membranous)		β-catenin (nuclear)		E-cadherin		P-cadherin	
	% exp	Intensity	% exp	Intensity	% exp	Intensity	% exp	Intensity	% exp	Intensity
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Normal (n : 26)	75,2 ± 27,2	1,30 ± 0,4	80,1 ± 15,3	1,84 ± 0,46	0	0	35,38 ± 22,8	2 ± 0,77	35,7 ± 25,3	1,92 ± 0,89
HP (n : 10)	86 ± 17,1	1 ± 1	83 ± 14,51	1,2 ± 0,63	0	0	81 ± 23,30	2,6 ± 0,7	69 ± 23,7	1,8 ± 78
TSA (n : 8)	97,5 ± 4,6	1,12 ± 0,35	89,4 ± 5,25	1,87 ± 0,64	0	0	93,75 ± 11,8	2,37 ± 0,7	80 ± 31,6	2,37 ± 0,7
TA (n : 17)	93,5 ± 13,2	2,29 ± 0,58	90,3 ± 8,72	2,29 ± 0,68	8 ± 14,4	0,76 ± 1,1	92,9 ± 24,2	2,7 ± 0,43	96,47 ± 12,2	2,41 ± 0,71
TVA (n : 37)	98,2 ± 6,8	2,35 ± 0,58	91,52 ± 5,38	2,4 ± 0,68	2,97 ± 7,5	0,91 ± 1,3	98,9 ± 5,1	2,67 ± 0,52	94,1 ± 17,1	2,27 ± 0,6
VA (n : 27)	97,7 ± 5	2,18 ± 0,73	89,3 ± 12,4	2,11 ± 0,57	8,96 ± 25,7	1,33 ± 1,3	95,9 ± 7,47	2,6 ± 0,5	92,4 ± 11,7	2,18 ± 0,6
ACA (n : 14)	95,35 ± 9,3	2,14 ± 0,77	83,8 ± 14,2	1,78 ± 0,89	17 ± 26,8	1,92 ± 1,2	96 ± 5,6	2,28 ± 0,61	95 ± 6,5	2,1 ± 0,6
CCA (n : 28)	80,53 ± 17	1,60 ± 0,49	72,2 ± 12,3	0,42 ± 0,5	17,1 ± 25,3	1,53 ± 1,23	74,28 ± 18,8	1,96 ± 0,63	38,2 ± 28,5	1,67 ± 0,66

signalling in colonic epithelium. Alterations in β-catenin expression are likely to affect both of these functions.

Although the mechanisms of cytoplasmic accumulation and nuclear expression of β-catenin are not fully demonstrated, it is known that mutations in APC gene lead to transfer of β-catenin from membrane to cytosol and then to the nucleus. Mutations of APC or β-catenin genes occur in majority of the adenomas and carcinomas of the colorectum (6,24). Previous studies showed that cytoplasmic and nuclear expression of β-catenin increase from early adenomas to carcinomas, and the distribution of its expression within a tumour may vary (25). We found a significant increase in nuclear β-catenin expression throughout the neoplastic progression reaching the highest values in ACA and CCA. While nuclear expression of β-catenin was found predominantly in with adenomas with high grade IEN and/or those with carcinomatous foci as well as in dedifferentiated tumour cells at the invasive fronts of carcinomas, membranous and cytoplasmic β-catenin expression was reduced in these cases. These observations are in accordance with the well known intracellular transfer of β-catenin to the cell nucleus which is predisposed by decreased accumulation of the molecule in the cell membrane and cytoplasm. In a study by Iwamoto et al., immunohistochemical expression of β-catenin was found in the cytoplasm and nucleus of all adenomatous polyps included in the study while APC immunreactivity was completely absent only in 29% of the same polyps (26). Although, mutations of APC occur early in adenoma-carcinoma sequence, loss of normal APC protein does not seem to be the unique determinant for dysregulation of β-catenin. Accordingly, intracellular distribution of β-catenin within adenomas and carcinomas may be explained by additional molecular mechanisms.

In a series of ACF, reduced expression of membranous β-catenin together with increased cytoplasmic and nuclear expressions were found in correlation with the grade of IEN (27). In another study comprised of adenomas and carcinomas both decreased membranous and increased nuclear β-catenin expressions were associated with increasing degrees of IEN (28). In our study, despite the slightly reduced membranous expression of β-catenin in adenomas, higher nuclear expression of β-catenin was observed as the grade of IEN increased. These findings suggest that, nuclear expression of β-catenin can be used as a reliable marker of IEN in colorectal polyps.

Cadherin/catenin complex is essential for the establishment of cell-cell adhesion. E-cadherin has been extensively studied in colorectal carcinomas in the literature (29,30,31), which have showed reduced or absent expression of E-cadherin being inversely proportional to the degree of tumour differentiation (32,33). In our study, expression of E-cadherin and P-cadherin decreased significantly in CCA group while no correlation was found with the grade of adenocarcinomas. However, we found significantly higher expressions of E-cadherin and P-cadherin in polyps with IEN. In contrast to our findings, and to the studies that reported downregulation of E-cadherin and catenins in carcinomas, El-Bahrawy et al., showed over-expression of cytoplasmic E-cadherin and β-catenin in addition to a preserved membranous protein expression in colorectal adenomas and carcinomas of FAP patients, including the poorly differentiated carcinomas (15). However, they did not provide data on E-cadherin expression and IEN in polyps. FAP-associated carcinogenesis can be one of the reasons for this discrepancy. There are only few studies regarding the expression of P-cadherin in colorectal polyps and/or carcinomas. In a study, Hardy *et al.*, reported that P-cadherin is aberrantly expressed in the earliest stage of abnormal colonocyte morphology, prior to changes in E-cadherin and catenins and its expression is unable to determine tissue morphology alone (18). Though, E-cadherin and P-cadherin do not seem to differentiate between subtypes of adenomas, the presence of their expressions in adenomas suggests that these molecules are involved in adenoma formation and neoplastic transformation. The heterogeneous expression

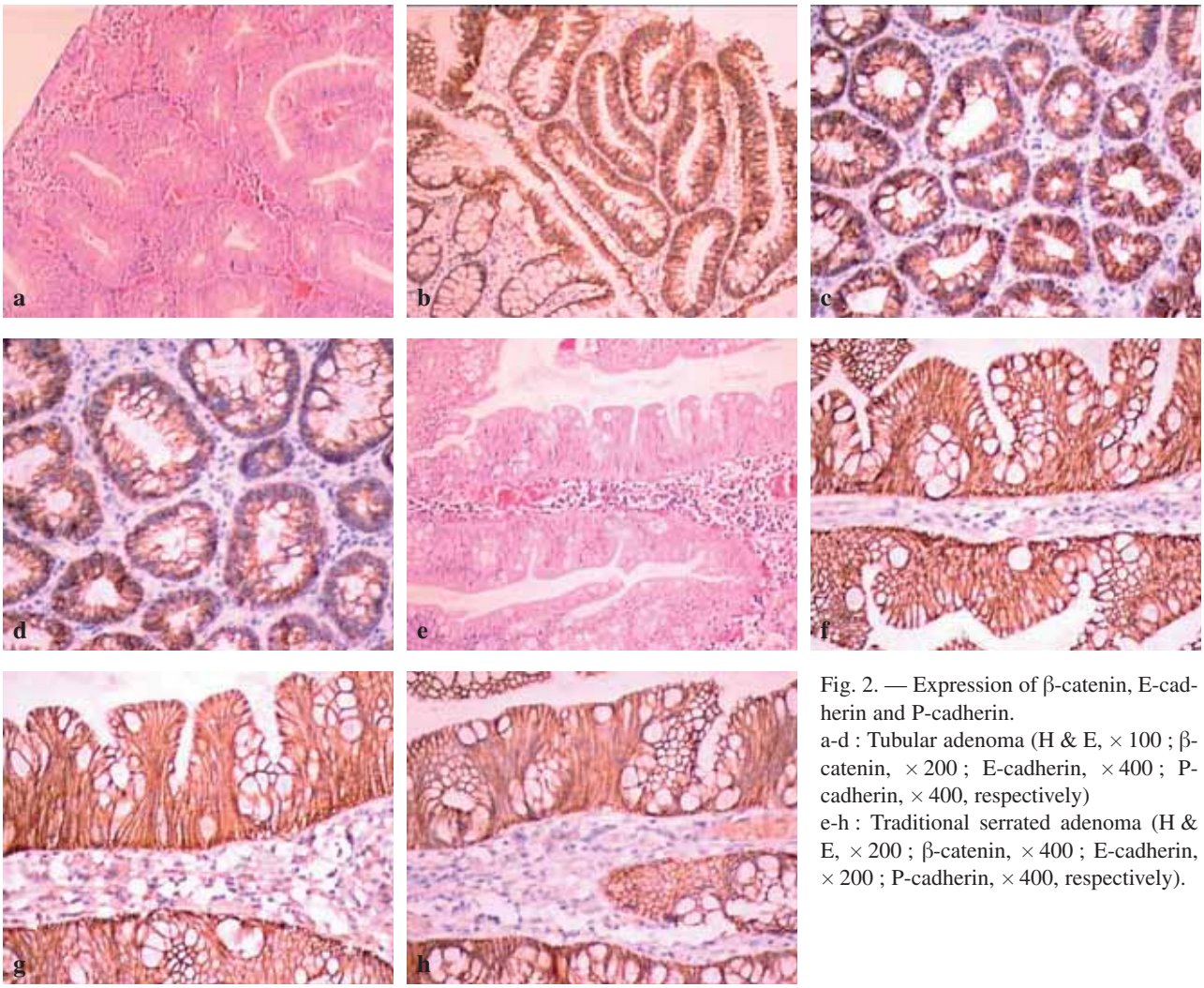

Fig. 2. — Expression of β-catenin, E-cadherin and P-cadherin.
a-d : Tubular adenoma (H & E, × 100 ; β-catenin, × 200 ; E-cadherin, × 400 ; P-cadherin, × 400, respectively)
e-h : Traditional serrated adenoma (H & E, × 200 ; β-catenin, × 400 ; E-cadherin, × 200 ; P-cadherin, × 400, respectively).

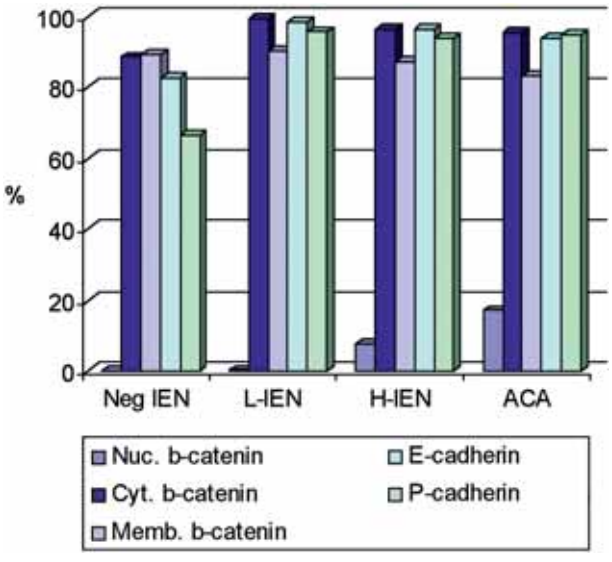

Fig. 3. — Expression of β-catenin, E-cadherin and P-cadherin in polyps with IEN.
(Neg IEN : IEN negative ; L-IEN : low grade of IEN ; H-IEN : high grade of IEN).

patterns of cadherins and catenin in sporadic adenoma and carcinoma may reflect the greater heterogeneity of sporadic colorectal carcinogenesis.

In recent years, a group of colorectal cancers, mucinous type in particular, were believed to follow a different pathway involving hyperplastic polyps, sessile serrated adenomas, and traditional serrated adenomas (3,4, 5). Traditional serrated adenoma is an entity that shares histopathologic features with both hyperplastic polyps and conventional adenomas (4) while sessile serrated adenoma can be simply defined as a "variant" hyperplastic polyp with characteristic differential features such as dilated, boot-shaped basal crypts and an abnormal proliferation zone (3). In the present study, traditional serrated adenomas showed cytoplasmic and membranous expression of β-catenin and E and P-cadherin at levels higher than both hyperplastic polyps and carcinomas. Interestingly, no nuclear β-catenin accumulation was seen in either TSA or HP. High levels of cytoplasmic and membranous expression of β-catenin together with absence of nuclear positivity in TSA suggest that these lesions follow different morphogenetic pathways which have only recently been recognized.

Catenins and cadherins have been extensively studied in many tumour types since they were first discovered. In a majority of these studies, only a small group of specific types of adenomatous polyps or adenocarcinomas were evaluated with different techniques including immunohistochemistry, in situ hybridization and PCR. The present study, however, seems to be unique for the composition of the cases which forms a complete spectrum of colorectal carcinogenesis including, normal colorectal mucosa, hyperplastic polyps, serrated and conventional adenomas, as well as adenomas with carcinomatous foci and advanced adenocarcinomas. The use of tissue array technique also enabled us to study a large number of cases in each group which allowed a reliable statistical analysis. The result of this extensively sampled series of colorectal polyps and carcinomas suggest that differential expression of catenins and cadherins may highlight various stages of neoplastic progression of colorectal mucosa.

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