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The Absence of a Significant Role for β-Catenin in the Tumorigenesis of the Serrated Adenoma of the Colorectum: A Comparative Study with the Traditional Tubular Colorectal Adenoma

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Summary. **The nuclear accumulation of β-catenin due to adenomatous polyposis coli (*APC*) gene alterations plays a central role in the tumorigenesis of the traditional tubular adenoma (TA) through activation of the Wingless/Wnt signal transduction pathway. To determine whether β-catenin plays a role in the tumorigenesis of the serrated adenoma (SA), a newly proposed subtype of colorectal adenoma, we immunohistochemically evaluated the nuclear expression of β-catenin in SA compared with that of the traditional TA. Twenty-three samples of SA without familial adenomatous polyposis (FAP) and 24 samples of TA without FAP and TA with FAP were respectively examined. While nuclear β-catenin expression was demonstrated in 79% (19/24) of TAs without FAP and 91% (22/24) of TAs with FAP, that in SAs was 0% (0/23). The difference in the expression rate in TAs without and with FAP from that in SAs was significant ($p<0.0001$, respectively). These results indicate that the tumorigenesis of the SA is distinct from that of the traditional TA and that the SA does not follow a APC/β-catenin/ Wnt signaling pathway.**

Key words—serrated adenoma, β-catenin, tumorigenesis of colorectal adenoma, immunohistochemistry.

INTRODUCTION

The properties of colonic neoplastic growth are acquired by a series of well-defined mutations during the adenoma-carcinoma sequence. Inactivation of the adenomatous polyposis coli (*APC*) suppressor gene is the first event in this sequence, which results in traditional adenoma formation[1] and has been found in more than 80% of early colorectal adenomas[2]. Recent studies have shown that the intracellular levels of the β-catenin protein that is involved in two major functions -- cell adhesion and mediation of the Wingless/Wnt signal transduction pathway[3,4]-- are regulated by the APC protein dependent degradation[5]. Mutations of the *APC* gene allow an increase in cytoplasmic β-catenin levels, and its translocation and accumulation to the nuclei in colorectal epithelial cells influence those cells to tumorigenesis[6].

The oncogenic potential of β-catenin is associated with the Wingless/Wnt signal transduction pathway. The nuclear pool of β-catenin protein interacts with transcription factors of the T cell factor family[3,7], and the resulting transcription complex can activate target genes, such as c-*myc*[8], *cyclin D1*[9], and matrix metalloproteinase matrilysin[10,11], which lead to tumor proliferation and the malignant progression of

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Abbreviations—APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; MSI, microsatellite instability; SA, serrated adenoma; TA, tubular adenoma; TVA, tubulovillous adenoma.

Table 1. Nuclear expression of β-catenin in SA and TA

	Negative	Sporadic	Clustered	Diffuse	Total
SA	23(100%)*	0	0	0	23
<5 mm	1	0	0	0	1
≧5 mm	22	0	0	0	22
Sporadic TA	5(21%)**	3(13%)	3(13%)	13(53%)	24
<5 mm	1	0	0	6	7
≧5 mm	4	3	3	7	17
FAP TA	2(8%)***	2(8%)	2(8%)	18(76%)	24
<5 mm	2	0	1	14	17
≧5 mm	0	2	1	4	7

SA, serrated adenoma; TA, tubular adenoma; FAP, familial adenomatosis polyposis; *vs**, $p<0.0001$, *vs***, $p<0.0001$, **vs***, not significant.

epithelial cells. β-catenin accumulation has been reported in the nuclei of adenomas from patients with familial adenomatous polyposis (FAP)[12], and a similar nuclear localization has been demonstrated in most sporadic adenomas, as well[13,14].

The serrated adenoma (SA) is a new morphological subtype of colorectal adenoma, as proposed by Longacre and Fenoglio-Preiser in 1990[15]. The SA combines the architectural features of hyperplastic polyp and the cytologic features of the traditional adenoma. It has malignant potential[15], is thought to play a role in the development of colorectal carcinoma[16,17,18], and demonstrates cell kinetics distinct to the traditional tubular adenoma (TA)[19]. Molecular investigations have demonstrated that the genetic background of the SA may differ from that of the traditional colorectal adenoma. In the SA, APC and K-*ras* mutations were reported to be infrequent[16,20,21,22], while microsatellite instability was demonstrated more frequently than in the traditional adenoma[23,24]. However, the status of nuclear accumulation of β-catenin protein has been investigated only in few studies[25,26]. In this study, we examined the immunohistochemical expression of β-catenin in the SA in comparison with the traditional TA, in order to elucidate whether β-catenin protein nuclear accumulation plays a role in the tumorigenesis of the SA.

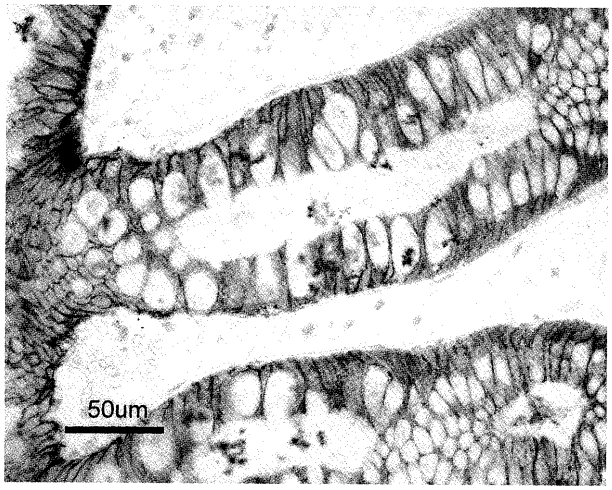

Fig. 1. Basolateral membrane expression of β-catenin within the normal colonic epithelia.

MATERIALS AND METHODS

Specimens

Formalin-fixed, paraffin-embedded tissue of 23 SAs (mean 7.5 mm, range from 3 to 10 mm) from 21 patients without FAP, 24 TAs (mean 6.3 mm, range from 5 to 8 mm) from 22 patients without FAP (sporadic TA), and 24 TAs (mean 4.5 mm, range from 9 to 2 mm) from 3 patients with FAP were examined. SAs containing a traditional adenomatous component (mixed polyp) or an adenocarcinoma were excluded from the study. All adenomas were diagnosed as low-grade atypia[27]. Four μm-thick sections were prepared for hematoxylin and eosin (HE) staining and β-catenin immunohistochemistry.

Immunohistochemistry

The expression of β-catenin protein was examined by a mouse anti-β-catenin monoclonal antibody (1 : 200 clone 17C2; Novocastera, UK) using the streptavidin-biotin immunoperoxidase method (Histofine SAB-PO kit; Nichirei, Japan). The sections

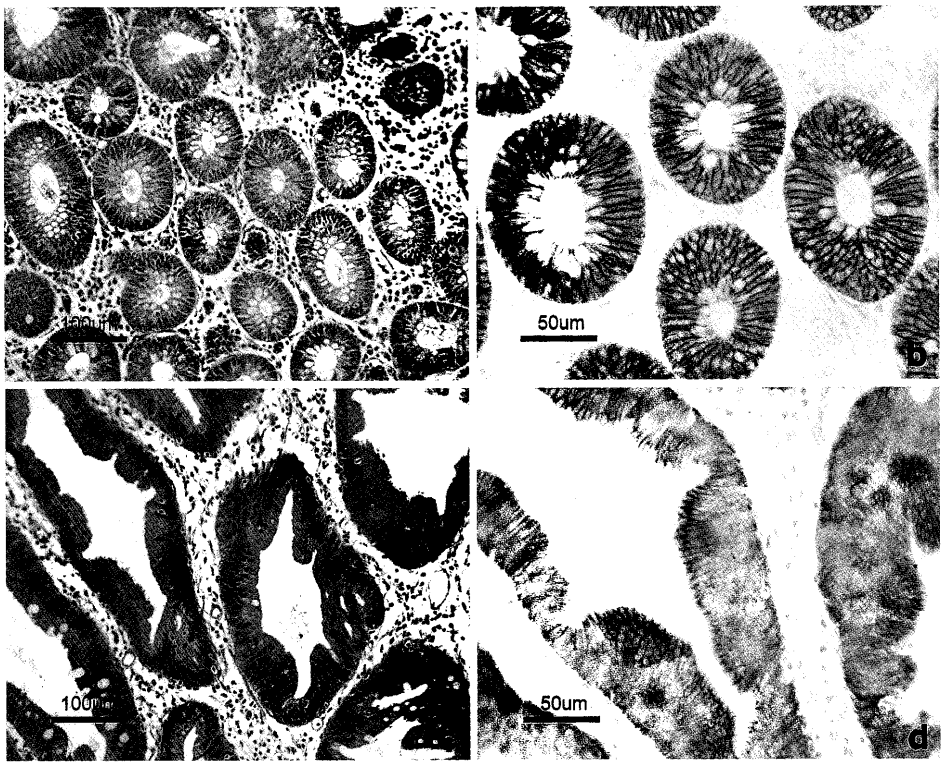

Fig. 2. Negative β-catenin nuclear expression. A tubular adenoma (**a** and **b**) and serrated adenoma (**c** and **d**). β-catenin staining (**b** and **d**) is positive for the basolateral membrane of the tumor cells but the nuclei are negative.

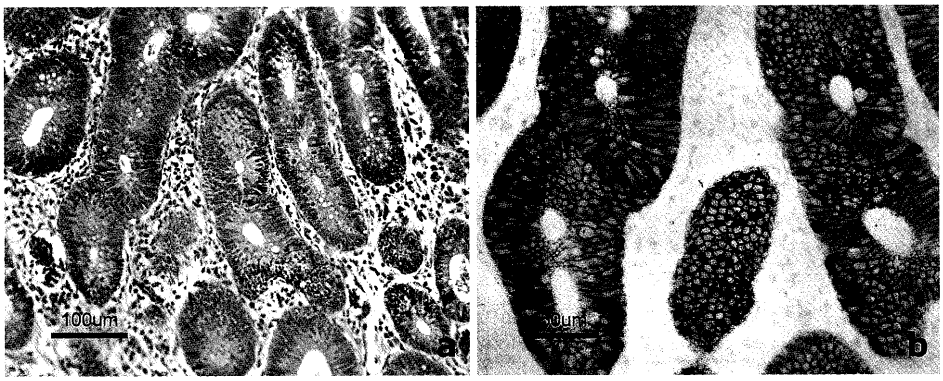

Fig. 3. A tubular adenoma **a** with sporadic β-catenin nuclear expression **b**. Very few positive nuclei are scattered without forming clusters **b**.

were counterstained with 2% methyl green solution (Chroma, Kongen, Germany). Since β-catenin normally functions as a cell adhesion molecule, the basolateral membrane of colonic epithelial cells are positive for β-catenin immunostaining[13]. Therefore, cell membrane staining of normal colonic epithelia adjacent to the adenoma was used as an internal positive staining control (Fig. 1). In each sample, nuclear β-catenin staining was evaluated. Positive staining was defined as that with an intensity equal to or higher than the internal control. Nuclear immunostaining patterns were classified into 4 groups as follows: 1) Negative: no expression (Fig. 2); 2) sporadic: very few scattered positive cells without forming clusters (Fig. 3); clustered: positive cells clustered in focal areas (Fig. 4); diffuse: positive cells diffusely distributed and more than 50% of tumor cells positive (Fig. 5).

Statistical analysis

Statistical analyses were performed by Fisher's

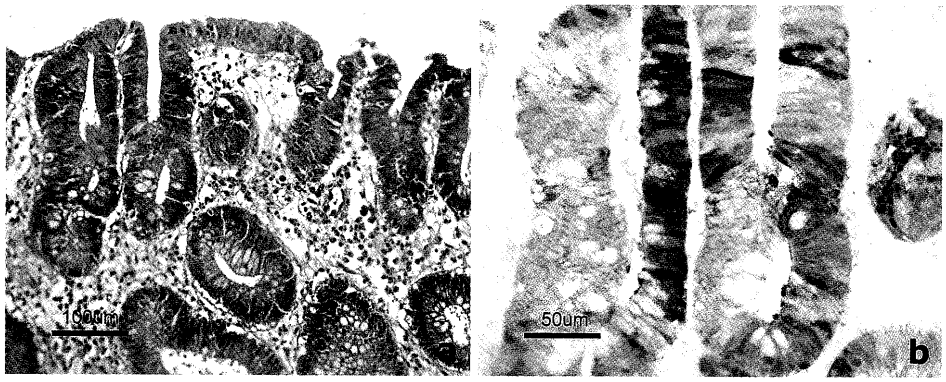

Fig. 4. A tubular adenoma (**a**) with clustered β-catenin nuclear expression (**b**). Positive nuclei are clustered in focal areas (**b**).

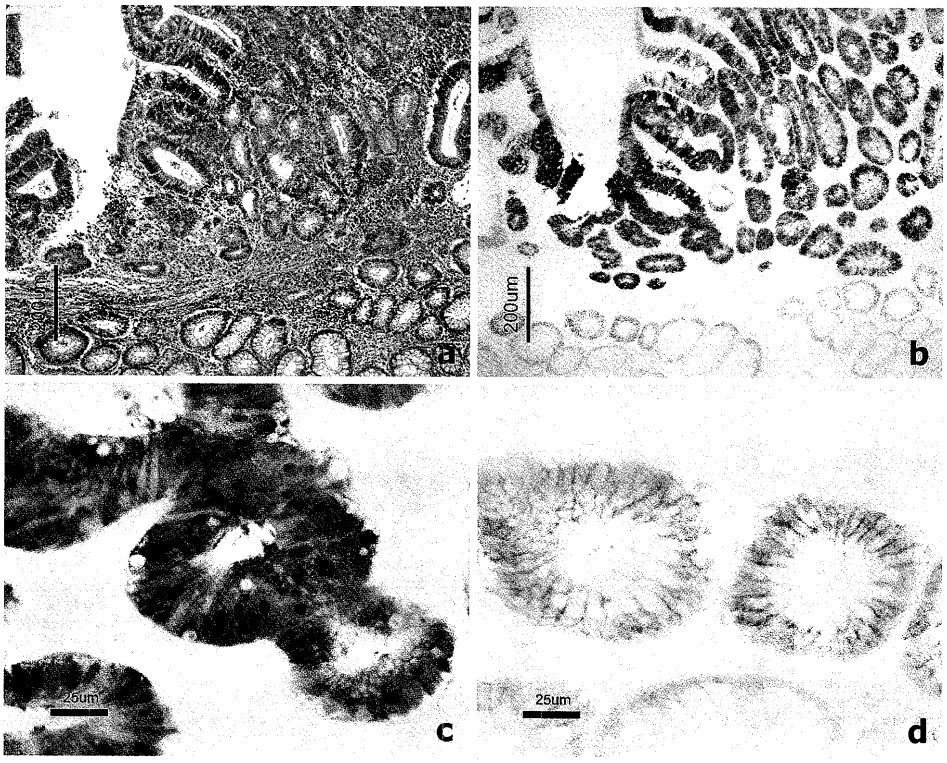

Fig. 5. A tubular adenoma (**a**) with diffuse β-catenin nuclear expression (**b** and **c**). More than 50% of the nuclei are positive and they are diffusely distributed throughout the adenomatous crypts (**c**). β-catenin immunostaining of the adjacent normal colonic crypts as inner staining control (**d**).

exact test. A P value of less than 0.05 was considered significant.

RESULTS

In the normal mucosa, β-catenin expression was seen uniformly along the basolateral membrane of the crypt epithelial cells (Fig. 1), and there was no nuclear staining observed.

The results of immunostaining for SAs and TAs are summarized in Table 1. There was no nuclear β-catenin expression detected in SAs (Fig. 2c and d), while expression was seen in 19/24 (79%) and 22/24 (91%) of sporadic TAs (TA without FAP) and TAs with FAP, respectively (Fig. 3, 4 and 5). There was a significant difference between SAs and sporadic TAs or TAs with FAP ($p<0.0001$, respectively), while sporadic TAs and TAs with FAP did not show any significant difference. In TAs, β-catenin nuclear

expression was seen diffusely throughout neoplastic epithelia in 13/24 (53%) (sporadic) and 18/24 (76%) (with FAP). Such a diffuse expression was demonstrated even in small lesions measuring less than 5 mm, and there was no significant difference in the pattern of β-catenin expression due to size in either sporadic TAs or TAs with FAP.

DISCUSSION

In earlier studies, the nuclear expression of β-catenin protein was demonstrated in 45.9% to 80%[13,14,28] of sporadic TAs by the immunohistochemical method. In the present study, 79% of sporadic TAs showed a nuclear β-catenin expression, which was within the published range. The expression rate in TAs from patients with FAP was slightly higher (91%), but there was no significant difference compared with that of sporadic TAs. Diffuse β-catenin nuclear expression in TAs was detected even in small tumors measuring less than 5 mm, and there was no significant difference compared with the expression in larger tumors. The present data are consistent with the known tumorigenetic mechanism of traditional colorectal adenomas, involving activation of a Wingless/Wnt signal transduction pathway by *APC* mutation and subsequent β-catenin protein nuclear accumulation, which plays a significant role in the initiating neoplastic formation[6]. In contrast to traditional TAs, there was no nuclear β-catenin expression detected in SAs. The present results are consistent with its reported infrequent *APC* mutation, which ranges from 0 to 3.8%[20,21,22], and suggest that the tumorigenesis of SAs does not follow the APC/β-catenin/Wnt signaling pathway. Furthermore, the present findings indicate that β-catenin immunostaining can be a useful tool for the pathological diagnosis of colorectal tumors when the differentiation between SAs and traditional TAs is difficult.

Prior to our study, there were few investigations that focused on nuclear β-catenin expression in SAs. Sawyer et al.[25] reported that 15% of SAs showed a nuclear β-catenin expression, and in Yamamoto's series, the ratio was 11% in tumors measuring 10 mm or more[26]. The probable reasons for the discrepancy between these early studies and the absence of nuclear β-catenin expression in our series could be differences in the sampling of SA, that is, whether or not a pure SA alone was selected for the study. The SA is not necessarily a pure lesion and may contain traditional adenomatous components[23,29], Furthermore, it remains unclear whether the biological nature and genetic alteration of the SA component of mixed polyps and a pure SA is identical. Although we ensured that our SA samples consisted of a pure SA alone, this was not described in the series by Sawyer et al. [25]. In the study by Yamamoto et al. [26], a mixed polyp consisting of SAs and traditional TAs or carcinomas comprised 8 of 45 SA samples. Although Yamamoto et al. suggested that there was no major tumorigenic role for β-catenin in SAs similar to ours, a mixed polyp and pure SA should be investigated separately or a pure SA alone should be selected as material in order to elucidate the biological nature of the SA. For a further investigation, we are planning to extend our materials to mixed polyps (mixed hyperplastic polyp and SAs, and mixed TAs and SAs) and carcinomas developed in SAs, to examine the role of β-catenin in such polyps with a heterogeneous histology and malignant transformation of the SA.

We speculate that the tumorigenesis of the SA is distinct from that of the traditional TA and does not follow the APC/β-catenin/Wnt signaling pathway. This may reflect differences in cell kinetics between the SA and traditional TA, which we have demonstrated previously[19]. In most traditional TAs, the cell proliferative zone is translocated superficially and newly generated tumor cells migrate downward from the surface to the bottom of the crypts. The proliferative zone of the SA is located basally, similar to that of non-neoplastic crypts, and tumor cells migrate from the bottom to the surface of the crypts[19]. The superficial translocation of the proliferative zone seen in the traditional TA was recently described as "top-down morphogenesis"[30], driven by alteration of the *APC* gene. In contrast, the cell kinetics of the SA can be described as "bottom-up morphogenesis", which would be attributed to the lack in APC/β-catenin/Wnt signaling transduction during its tumorigenesis. Further investigation is needed to elucidate the detailed mechanism connecting the molecular histogenetic pathways and cell kinetics of colorectal adenomas.

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