β-Catenin Helices in the Cytoplasm of Sessile Serrated Adenoma/Polyps and Conventional Colorectal Adenomas

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Abstract. *Initiation and progression in conventional adenomas is triggered by deregulation of WNT/β-catenin signaling. In the absence of WNT signal (off-state), β-catenin prevents phosphorylation of GSK3β, leading to aberrant nuclear accumulation in human tumors. It has been postulated that mutations in the β-catenin gene are always associated with a morphologically-neoplastic course. While investigating the nuclear expression of β-catenin in 170 colorectal biopsies, we observed a non-previously reported phenomenon, namely the presence of β-catenin cytoplasmic helices in 29% (n=7) of 24 sessile serrated adenoma/polyps (SSA/P), in 24% (n=13) of 54 adenomas, in 8% (n=3) of 38 specimens with IBD, but in none (0/54) with normal mucosa. The earliest β-catenin helices were found at the bottom of SSA/P glands (the domain of stem cells in the colorectal mucosa). It is submitted that β-catenin helices might highlight a non-previously described cytoplasmic phenomenon evolving during the serrated–carcinoma pathway in SSA/P, and during the adenoma–carcinoma pathway in conventional adenomas.*

Colorectal carcinoma (CRC), the third most commonly diagnosed type of cancer in Europe (1) and the USA (2) and usually evolves *via* the adenoma–carcinoma sequence. Conventional adenomas exhibit tubular or villous structures, either with low-grade or high-grade dysplasia (3), increased cell proliferation and progressive accumulation of *KRAS* mutations and chromosomal instability (4). In this paradigm, hyperplastic polyps are considered innocuous.

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More recently, the serrated–carcinoma pathway of colonic carcinogenesis has received wide acceptance. In this model, hyperplastic polyps, sessile serrated adenoma/polyps with or without dysplasia (SSA/P) and traditional serrated adenomas are regarded as early histological precursors of serrated carcinomas (5, 6). The main molecular aberrations found in SSA/P are *BRAF* mutations and DNA methylation leading to the CpG island methylator phenotype, microsatellite instability and loss of DNA mismatch repair proteins, particularly hMLH-1 and PMS-2 (7). It has been postulated that progression to colonic cancer along the serrated pathway may occur at a faster rate than that with chromosomal instability evolving in the adenoma–carcinoma sequence (8).

In most conventional adenomas, initiation and progression is triggered by de-regulation of WNT/β-catenin signaling causing increased transcriptional activity of the protein β-catenin (9). The WNT pathway is activated *via* the tumor suppressor adenomatous polyposis coli (*APC*) gene, resulting in loss of heterozygosity, inactivation and mutations of the APC protein complex targeting β-catenin (10). The nuclear accumulation of β-catenin elicits the transcriptional activation of T-cell factors that regulate the genes involved in cell proliferation and apoptosis (11). WNT/β-catenin signaling is the custodian of colorectal stem cell regulation (9-11).

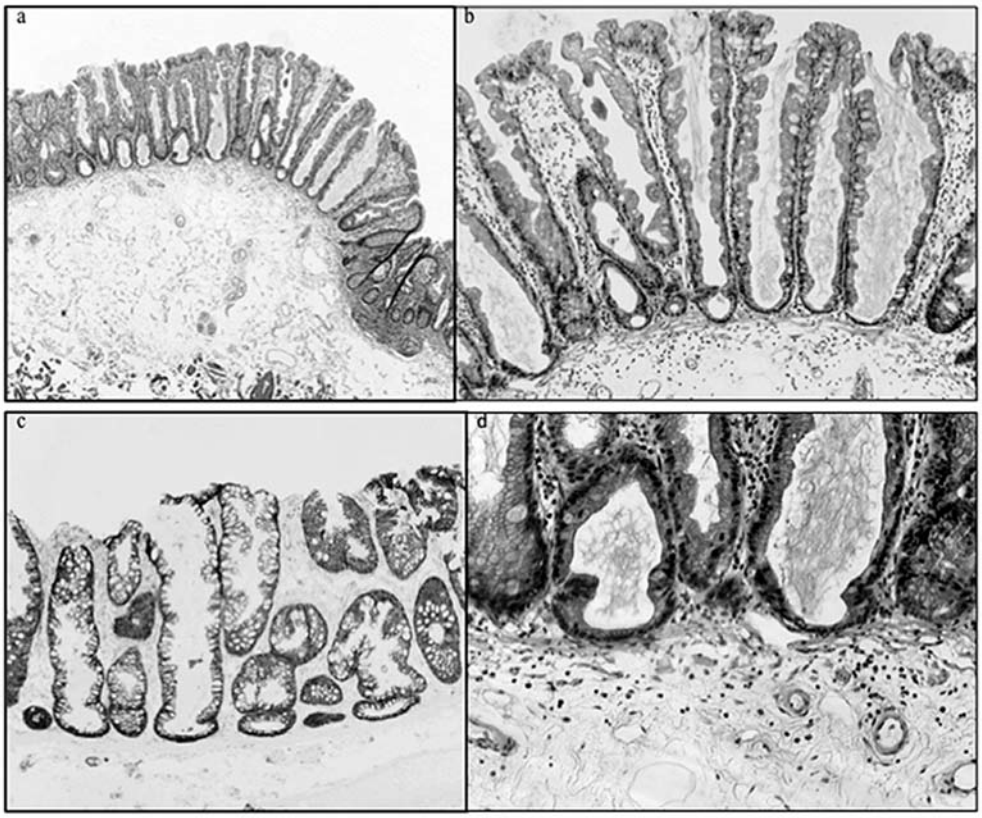
Recently, Murakami *et al*. found that nuclear β-catenin labeling also occurs in SSA/P but at a significantly lower frequency than in their conventional adenoma counterparts (12).

While investigating the nuclear expression of β-catenin in immunostained colorectal biopsies, we observed β-catenin helices in the cytoplasm of colonic SSA/P and of conventional adenomas.

The purpose of the present work is to report and illustrate these novel findings in colorectal SSA/P and conventional adenomas.

Materials and Methods

One hundred and seventy consecutive colorectal biopsies diagnosed by the senior author (CAR) were immunostained with anti-β-



Figures 1. *Four images of sessile serrated adenomas/polyp of the colon. Note crypts filled with mucus, and the basal aspect of the lesion, the serrated crypts being broad and irregular (Hematoxilin and eosin: a: ×4, b: ×10, c: ×10, d: ×20).*

Table I. *Results of 170 biopsies immunostained with anti-β-catenin: 24 with SSA/P, 54 conventional adenomas, 38 of inflammatory bowel disease (IBD)/chronic colitis/proctitis, and 54 with normal colonic mucosa.*

				β-Catenin staining	
Histology	No. of cases	Age (years)	Gender M/F	Nuclear	Helices
SSA/P-TSA	24	63.9 (41-87)	8/16	3 (12.5%)	7 (29.2%)
Conventional adenomas	54	60.5 (25-89)	20/34	30 (55.6%)	13 (24.1%)
IBD, chronic colitis/proctitis	38	48.9 (7-80)	28/10	4 (10.5%)	3 (7.9%)
Normal colonic mucosa	54	42.8 (28-72)	20/34	0 (0%)	0 (0%)

SSA/P, Sessile serrated adenomatous polyps. TSA: Traditional serrated adenomas.

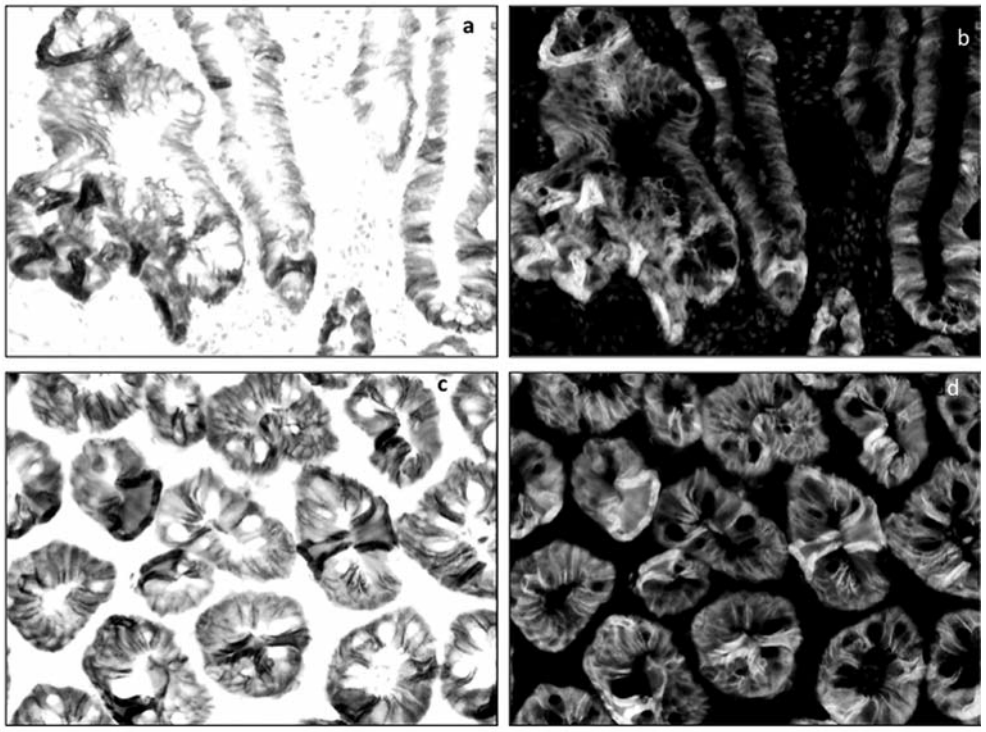
catenin. Out of the 170 cases, 24 were SSA/P, 54 conventional adenomas, 38 IBD or other forms of chronic colitis/proctitis and 54 had normal colonic mucosa.

Diagnostic sections were cut at 4 μm, stained with H&E and immunostained with antibody to β-catenin (ready to use; Bond Reagents, Leica Biosystems AB, Kista, Sweden), together with positive controls. Paraffin blocks of 13 cases exhibiting β-catenin helices in sections cut at 4 μm. were re-cut at 2.5 μm and at 6 μm and immunostained for β-catenin. Sections cut at 2.5 μm inhibited no β-catenin helices and those cut at 6 μm were too dark to detect the helices. Conventional adenomas were defined as reported elsewhere (3, 13) and SSA/Ps according to the WHO (5), Bateman (6), and more recently Rubio and Slezak (13).

Statistical analysis. The non-parametric Mann–Whitney test was used Statistical significance was set at $p<0.05$.

Results

β-Catenin in normal colorectal mucosa. Age and gender of the patients: The mean age of the 54 patients was 42.8 years (range=28-72 years). Out of the 54 patients, 37.0% (n=20)



Figures 2. *Left panel: Images of sessile serrated adenomas/polyp showing activated β-catenin helices (anti-β-catenin immunostain). Right panel: Images are those shown on the left transformed using the invert function of Ps Adobe Photoshop CS3 (extended) to highlight the β-catenin helices or coils Original magnification, ×10.*

were males. None of the biopsies with normal mucosa exhibited β-catenin helices or β-catenin-activated nuclei.

β-Catenin in SSA/Ps. Age and gender of the patients: The mean age of the 24 patients with SSA/P was 63.9 years (range=41-87 years). Of the 24 patients, 33.3% (n=8) were males.

β-Catenin helices: Table I shows that 29.2% (n=7) of the 24 SSA/P. (Figure 1) investigated had β-catenin helices (Figure 2). In one SSP, the helices were found at the bottom of the crypts (Figure 3, upper panel).

β-Catenin-activated nuclei: β-Catenin-stained nuclei were found in 12.5% (n=3) of the 24 cases investigated (Figure 3, lower panel); in two out of these three cases, nuclear staining concurred with the presence of β-catenin helices.

β-Catenin in conventional adenomas. Age and gender of the patients: The mean age of the 54 patients with conventional adenomas was 60.5 years (range=25-89 years). Out of the 54 patients, 37.0% (n=20) were males.

β-Catenin helices: Out of the 54 conventional adenomas, 24.1% (n=13) exhibited β-catenin helices. Out of these 13 cases, 53.8% (n=7) were found in patients with colorectal carcinoma heredity (four of them had FAP and one Lynch syndrome) and one had been operated on years previously for colonic cancer.

β-Catenin-activated nuclei: β-Catenin-stained nuclei were found in 55.6% (n=30) of the 54 cases investigated; in four cases, nuclear staining concurred with the presence of β-catenin helices.

β-Catenin in IBD. Age and gender of the patients: The mean age of the 38 patients was 48.9 years (range=7-80 years). Out of the 38 patients, 70.3% (n=28) were males.

β-Catenin helices: The results are shown in Table I. It is seen that 7.9% (n=3) of the 38 cases investigated showed β-catenin helices. These three cases had IBD, one of them with low-grade dysplasia. The mean age of the three cases with β-catenin helices was 46.7 years (range=42-52 years). Out of the three cases with β-catenin helices, one was male.

β-Catenin-activated nuclei: β-Catenin-stained nuclei were found in 10.5% (n=4) of the 38 cases investigated; only in one of these three cases, did nuclear staining concur with the presence of β-catenin helices. This case had active ulcerative colitis.

The difference in the frequency of activated nuclei and of activated cytoplasmic helices in conventional adenomas vs. SSA/P, and in IBD/chronic colitis/proctitis, was significant ($p<0.05$).

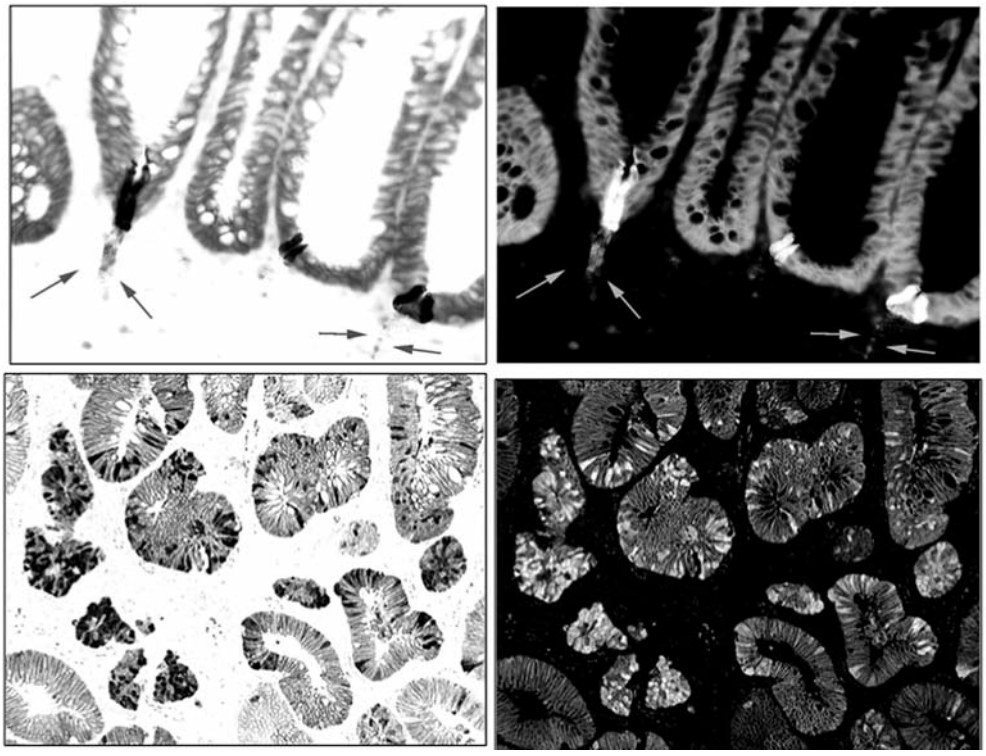


Figure 3. *Upper panel: Bottom of the crypts in a sessile serrated adenoma/polyp showing three β-catenin helices or coils, apparently 'nourished' by small blood vessels (arrows) (anti-β-catenin immunostain, ×40). Lower panel: Conventional tubular adenoma of the colon showing β-catenin-stained nuclei (anti-β-catenin immunostain, ×10). Images shown in the panel on the right are those shown on the left transformed using the invert function of Ps Adobe Photoshop CS3 (extended) to highlight the β-catenin helices and the nuclei, respectively.*

Discussion

The colonic mucosa comprises of a single layer of columnar cells lining millions of regularly-spaced crypts arranged as a 'rack of test tubes' that occupy the thickness of the mucosa, from the lumen to the *muscularis mucosae*. Each crypt contains on average 2,000 cells, and is about 40 cells in circumference and 80 cells in height (14). At the bottom of the crypts, a small number of stem cells divide to generate transient amplifying daughter cells that eventually differentiate into distinctive cell lineages (15, 16).

The WNT/β-catenin pathway (also referred to as canonical WNT signaling) regulates stem cell pluripotency and cell fate decisions. β-Catenin is an integral E-cadherin cell–cell adhesion adaptor protein and transcriptional co-regulator. Cytosolic β-catenin phosphorylation/degradation and its regulation by WNT are crucial in WNT signaling. The transcriptional effect of WNT ligand is mediated *via* Rac1-dependent nuclear translocation of β-catenin. In the absence of WNT signal (off-state), β-catenin prevents phosphorylation of glycogen synthase kinase 3β leading to aberrant nuclear accumulation in human tumors (17). In this survey, β-catenin-positive nuclei were found in 13% of the SSA/P and in 56% of the conventional adenomas. These results substantiate the findings of Murakami *et al.* (12), namely that nuclear β-catenin labeling may be found not only in conventional adenomas, but also in SSA/P. We found, in addition, that 11% of the cases with chronic IBD had β-catenin-positive nuclei. None of these cases had IBD-associated dysplasia. The cause(s) for the presence of β-catenin-positive nuclei in some IBD cases remains poorly understood. Conversely, none of the cases with normal colorectal mucosa exhibited β-catenin-positive nuclei.

Assuming that all nuclei in the dysplastic cells of conventional adenomas are genetically-mutated, the lack of β-catenin-positive nuclei in the remaining 44% of conventional adenomas appears to indicate that the immunostain used in the present work was unable to detect mutated dysplastic nuclei in all lesions.

β-Catenin helices were unexpectedly found in the cytoplasm in 29% of the SSA/Ps, in 24% of the conventional adenomas, and in three (8%) of the cases with chronic mucosal inflammation. One of these latter cases had Crohn´s

colitis with low-grade dysplasia;the other two patients are being followed-up with periodic colonoscopies.In contrast, none of the cases with normal colorectal mucosa (including normal mucosa adjacent to both SSA/P and to conventional adenomas) exhibited cytoplasmic β-catenin helices.

The re-review of diagnostic H&E-stained sections revealed that the nuclei in the crypts and in the glands of SSA/Ps and conventional adenomas were arranged in a picket fence-like fashion. Nuclear disarrangement resembling the helices found on

β-catenin immunostaining was not demonstrated in H&E-stained sections. Sections from two cases with β-catenin helices were de-stained and subsequently re-stained with H&E; nuclei arranged in helices could not be discerned in the H&E re-stained sections. These findings would imply that nuclei of the crypts/glands in these lesions did not participate in the formation of β-catenin helices. If the β-catenin coils are unrelated to an abnormal nuclear distribution at the base of the crypts (as deduced from H&E staining) a rational explanation might be that the helices highlight changes evolving in the cytoplasm of affected cells. The lack of β-catenin helices in >75% of the conventional adenomas here, remains unclear. One plausible option is that the immunostainning used in the present work is not sensitive enough to detect helices in all dysplastic lesions.

The earliest single β-catenin coil occurred at the bottom of some SSA/P glands. This is a remarkable finding, considering that the base of crypts is the domain of stem cells in the colorectal mucosa (16, 18, 19).

The majority of the SSA/Ps and conventional adenomas exhibiting activated β-catenin helices (14/20=70%) exhibited no nuclei with activated β-catenin. The question arises: Is β-catenin activated in the nuclei and in the cytoplasm in these lesions at different time intervals?

In light of the present results, we are inclined to speculate that β-catenin helices might highlight a cytoplasmic phenomenon evolving during the serrated–carcinoma pathway in SSA/P, and during the adenoma–carcinoma pathway in conventional adenomas. This puzzling phenomenon might be of significance considering that β-catenin plays a crucial role in WNT/β-catenin signaling during cancer progression (20, 21). It has been postulated that mutations in β-catenin gene are always associated with a morphologically neoplastic course (22).

Conflicts of Interest

None.

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