β-catenin Helices in the Cytoplasm of Sporadic and FAP Duodenal Adenomas

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Abstract. *Background: 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 glycogen synthase kinase (GSK)-3β leading to aberrant nuclear accumulation in human tumors. While investigating the nuclear expression of β-catenin in biopsies from duodenal adenomas, we observed a non-previously reported phenomenon, namely the presence of β-catenin cytoplasmic helices (coils). Materials and Methods: Sections from 39 biopsies were immunostained with β-catenin: 25 from duodenal adenomas and the remaining 14 had normal duodenal mucosa (n=11) or polypoid gastric duodenal metaplasia (n=3). Results: Eighteen out of the 25 duodenal adenomas (72%) showed β-catenin helices; in contrast, none of the 33 control biopsies (including those with normal duodenal mucosa, gastric duodenal metaplasia and normal mucosa adjacent to 19 adenomas) showed β-catenin helices (p<0.05). The review of diagnostic H&E-stained sections and of β-catenin-stained nuclei revealed that the dysplastic nuclei were arranged in a picket fence-like fashion along the basement membrane of the glands and not as loops within the dysplastic glands; the nuclei of the dysplastic glands were not forming part of the β-catenin helices. Discussion: If these β-catenin coils are unrelated to an abnormal nuclear distribution at the base of the dysplastic glands, the rational explanation might be that the helices highlight changes taking place in the cytoplasm of affected glandular cells. Conclusion: According to some authors, mutations in the β-catenin genes are always associated with a morphologically neoplastic course. It is herein proposed that β-catenin helices in duodenal adenomas might uncover a novel cytoplasmic phenomenon ensuing during the adenoma-carcinoma pathway.*

The histological examination of duodenal polyps may disclose adenomas (tubular, villous, tubulovillous or Brunner's gland adenomas), Peutz–Jeghers hamartomas, polypoid gastric metaplasia of the duodenum (GMD), inflammatory fibroids, carcinoids, lymphomas or mesenchymal tumors (lipoma, leiomyoma, leiomyoblastoma, vascular, fibroma, neurogenic or gangliocytic paraganglioma) (1).

Duodenal adenomas are epithelial polyps with dysplastic glands built of mutated cells having proliferative, biochemical and molecular aberrations (2). Adenomas may evolve sporadically in the general population or through germline mutations as in familial adenomatous polyposis (FAP) (3).

The incidence of duodenal adenomas is 0.1% to 0.3% (4). The prevalence of sporadic duodenal adenomas in patients referred for upper gastrointestinal endoscopy is about 5% (5). On the other hand, 50% to ≥90% of FAP patients exhibit duodenal adenomas (6).

In later years, much research has been done to unveil the molecular aberrations evolving in duodenal adenomas. In these lesions, initiation and progression is triggered by deregulation of Wnt/β-catenin signaling (also referred to as canonical Wnt signaling) leading to increased transcriptional activity of the protein β-catenin (7). The Wnt pathway (the custodian of stem cell regulation (8)) is activated *via* the tumor suppressor *APC* (adenomatous polyposis coli) gene, resulting in loss of heterozygosity (LOH), inactivation and mutations of the APC protein complex targeting β-catenin (7, 9). The cytoplasmic β-catenin protein is constantly degraded and eliminated by the action of the Axin complex that prevents β-catenin from reaching the nucleus (10). In the absence of Wnt signal (off-state), β-catenin precludes phosphorylation of the glycogen synthase kinase 3 beta (GSK-3β) leading to aberrant nuclear accumulation (11). The nuclear accumulation of β-catenin elicits the transcriptional activation of the T-cell factors (TCFs) that regulate the genes involved in cell proliferation and apoptosis (12).

In FAP, the underlying genetic cause is the germline mutation of the APC gene located on 5q21; the transcription

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factor β-catenin is the key effector of the Wnt/β-catenin pathway. β-catenin is stabilized constitutively, providing a permanent mitogenic signal to normally-resting cells. This occurs when the second APC allele is somatically inactivated (7). In most duodenal adenomas, there is a decreased APC-associated degradation of β-catenin. In approximately one half of the remainder of tumors with wild-type APC, this is due to mutations in β-catenin itself (*CTNNB1*) (13).

Several studies have assessed the frequency of β-catenin immunostained nuclei in duodenal adenomas (14-16). While investigating the nuclear expression of β-catenin in immunostained duodenal biopsies, we observed a non-previously described phenomenon, namely the presence of activated β-catenin helices in the cytoplasm of the adenomatous glands.

The purpose of the present work was to report and illustrate this novel finding in duodenal adenomas from patients with FAP, as well as in sporadic cases.

Materials and Methods

Sections from 39 consecutive duodenal biopsies diagnosed by the senior author (CAR) were investigated: 25 adenomas, three polyps exhibiting chronic inflammation and gastric metaplasia (GMD) (17) and 11 with normal duodenal mucosa. In addition, normal duodenal mucosa adjacent to adenomas was present in 19 of the 25 adenomas.

Helices were regarded those β-catenin-positive coils, ringlets or whirls, that could be seen in single crypts or connecting two or more crypts.

Diagnostic sections were cut at 4 μm, stained with hematoxylin and eosin (H&E) and immunostained with anti β-catenin (Ready to use, Bond Reagents; Leica Biosystems AB, Kista, Sweden). β-catenin immunostained sections were scrutinized using a high power objective (×40).

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

Results

β-catenin in the normal duodenal mucosa and in GMD. None of the 33 biopsies with normal duodenal mucosa -including the normal mucosa adjacent to 19 adenomas- as well as three biopsies with GDM displayed β-catenin helices or β-catenin-activated nuclei (Table I).

Duodenal adenomas. All 25 duodenal adenomas were tubular adenomas with low-grade dysplasia: 72% (n=18) were FAP adenomas and the remaining 28% (n=7) sporadic adenomas.

β-catenin in duodenal adenomas.

i) β-catenin cytoplasmic helices: β-catenin cytoplasmic helices were found in 72% (18/25): in 72.2% (13/18) of the FAP adenomas and in 71.4% (5/7) of the sporadic adenomas (Figures 1 to 3 a).

ii) β-catenin activated nuclei: β-catenin-stained nuclei were found in 64% (16/25): in 66.7% (12/18) of the FAP adenomas and in 71.4% (5/7) of the sporadic adenomas (Figure 3 b).

The difference in β-catenin cytoplasmic helices and β-catenin-stained nuclei in FAP and sporadic adenomas *vs*. non-neoplastic duodenal mucosas (normal mucosa, GMD and the normal mucosa adjacent duodenal adenomas, was significant ($p<0.05$). No difference in the frequency of β-catenin cytoplasmic helices and of β-catenin-stained nuclei was found between FAP and sporadic duodenal adenomas.

Discussion

The search for β-catenin-stained nuclei in duodenal adenomas disclosed, unexpectedly, helices of β-catenin in the cytoplasm of a group of dysplastic glands in 72% of the duodenal adenomas investigated but in none of the specimens having polypoid GMD or normal duodenal mucosa (including the normal mucosa adjacent to duodenal adenomas). The review of diagnostic H&E-stained sections and of β-catenin-stained nuclei revealed that the dysplastic nuclei were arranged in a picket fence-like fashion along the basement membrane of the glands (Figure 3c) and not as loops within the dysplastic glands. Hence, nuclear disarrangement resembling helices was not found in H&E sections and in β-catenin immunostains implying that nuclei of the dysplastic glands were not participating in the β-catenin helices. If these β-catenin coils are unrelated to an abnormal nuclear distribution at the base of the dysplastic glands, the rational explanation might be that the helices highlight changes taking place in the cytoplasm of affected glandular cells.

Given the logistical difficulties and resources associated with direct sequencing of β-catenin (CTNNB1), most investigations have used immunohistochemistry with the assumption that the presence of stained nuclei is often associated with β-catenin mutation. Since β-catenin coils were not present in sections with GMD or with histologically-normal duodenal mucosa -not even in the normal mucosa adjacent to duodenal adenomas- the possibility that the β-catenin helices found in the adenomatous tissue could mirror a novel putative mutation in the cytoplasm of adenomatous glands could not be totally rejected. Assuming that all dysplastic cells in duodenal adenomas are mutated, the lack of β-catenin-positive nuclei in the remaining 36% of the adenomas appears to indicate that the immunostain used in the present work was not sensitive enough to detect mutated nuclei in all lesions. Perhaps the same applies for the lack of β-catenin-positive helices in 28% of the adenomas.

In light of the present results, we are prone to speculate that β-catenin helices in duodenal adenomas might highlight a cytoplasmic phenomenon ensuing during the adenoma-

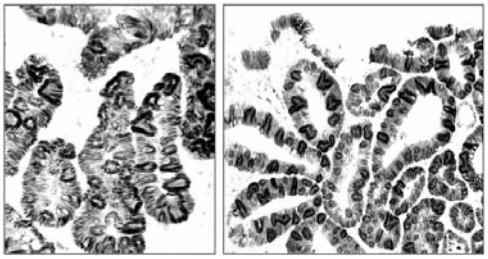

Figure 1. *Two different tubular adenomas of the duodenum showing multiple β-catenin helices in the cytoplasm of dysplastic glandular cells (β-catenin immunostain, ×10).*

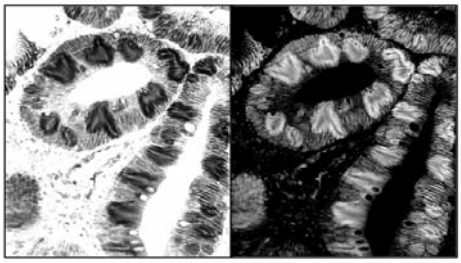

Figure 2. *Left panel: Tubular adenoma of the duodenum showing multiple β-catenin helices in the cytoplasm of dysplastic glandular cells (β-catenin immunostain, ×20). Right panel: Cloned image from the left panel to highlight the β-catenin helices using the INVERT function of a Photoshop Program (Ps Adobe Photoshop CS3 extended).*

Table I. *The frequency of β-catenin-positive nuclei and of β-catenin-positive cytoplasmic helices in 25 duodenal adenomas and in 33 controls*.*

Histology	No. cases	β-catenin (nuclei)	β-catenin (helices)
Duodenal adenomas	25	16 (64.0%)	18 (72.0%)
Duodenal mucosa in controls	33	0 (0%)	0 (0%)

*Includes the normal mucosa in 11 biopsies, the normal mucosa adjacent to 19 duodenal adenomas and three duodenal biopsies with gastric duodenal metaplasia (GDM).

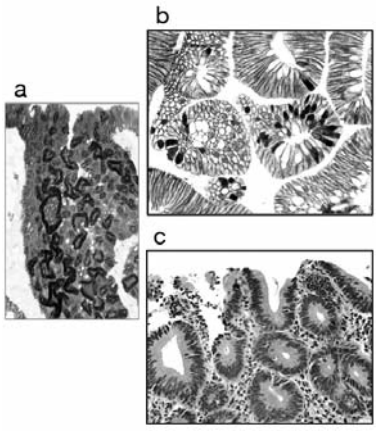

Figure 3. *a: Another tubular adenoma of the duodenum showing multiple β-catenin helices in the cytoplasm of dysplastic glandular cells (β-catenin immunostain, ×10), b: Tubular adenoma of the duodenum showing multiple β-catenin-stained nuclei in dysplastic glandular cells (β-catenin immunostain, ×20), c: Tubular adenoma of the duodenum. Note the regular "picket-fence" nuclear disposition in the glands (H&E ×20).*

carcinoma pathway. It is at present unclear whether the cytoplasmic helices in these neoplasias occur already at the stem cell level (18) or following one or more mitoses in dysplastic cells (19). This puzzling phenomenon might be of significance considering that β-catenin plays a crucial role in the Wnt/β-catenin signaling pathway during cancer progression (20, 21). It has been postulated that mutations in the β-catenin genes are always associated with a morphologically neoplastic course (22).

It should be mentioned that similar cytoplasmic β-catenin helices were recently found in small sessile serrated adenoma/polyp (SSA/P) and conventional colorectal adenomas (23).

Conflicts of Interest

None.

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