

## SUBCELLULAR LOCALIZATION OF BETA CATENIN IN COLORECTAL NON NEOPLASTIC AND NEOPLASTIC LESIONS

Diah Rini Handjari<sup>1</sup>, Pamela Abineno<sup>2</sup>, Budiningsih Siregar<sup>1</sup>

1. Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia, Jakarta 10430, Indonesia

2. Laboratory of Anatomical Pathology, Prof. Dr. W. Z. Johannes District General Hospital (RSUD),  
Kupang 85112, Indonesia

*\*E-mail: rinidiah@yahoo.com*

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### Abstract

Loss of adenomatous polyposis coli (APC) function is typically an early event in sporadic colorectal cancer (CRC) pathogenesis. The key tumor suppressor function of the APC protein lies in its ability to destabilize free cytoplasmic beta catenin. This leads to the accumulation of nuclear beta catenin, and together with the DNA binding protein Tcf-4, functions as a transcriptional activator. Accumulation of stabilized free  $\beta$ -catenin is considered as an early event and perhaps initiating the process in intestinal tumorigenesis. Neoplastic transformation in the CRC associated chronic colitis is considered similar to the adenoma-carcinoma sequence in sporadic CRC. The distinguishing feature from the CRC-related colitis is the difference in time and frequency changes. Loss of APC function, regarded as the beginning of a very common event in sporadic CRC, but the CRC associated chronic colitis generally occurs at the end of the dysplasia-carcinoma sequence. This research was conducted to determine the subcellular location of beta catenin expression in chronic colitis, colorectal adenomas and carcinomas that were evaluated by immunohistochemical staining. It can be concluded that beta-catenin is a component that plays a role in the development of the CRC and the subcellular location of beta-catenin can describe its oncogenic activity.

*Keywords: beta catenin, chronic colitis, colorectal adenoma, colorectal cancer*

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### Introduction

Colorectal cancer (CRC) is a significant cause of morbidity and mortality in the developed world with over 142,570 new cases diagnosed annually around the world.<sup>1</sup> Although patients diagnosed with early stage disease have a high cure rate, many present later when five year survival is poor.<sup>2</sup> Increased understanding of the changes in specific molecular pathways that are responsible for disease progression and poor prognosis may prove essential in the development of more effective targeted treatment.

It has been suggested that colorectal cancer (CRC) progression arises from an accumulation of several genetic changes from normal mucosa to adenoma and then carcinoma. This pathway requires the accumulation of genetic alteration which preceded by the inactivation of tumor suppressor gene adenomatous polyposis coli (APC). Mutation of APC was found in up to 80% of sporadic CRC and adenoma and was thought to be critical in the development of colorectal cancer.<sup>3</sup>

Beta catenin is a multifunctional protein that is involved in cell-cell interaction and transcriptional signaling. In

normal colonic epithelial cells, beta catenin is predominantly bound to E-cadherin as part of a cell to cell adhesion complex on the lateral cell membrane. Cytoplasmic beta catenin levels are regulated by binding to a protein complex consisting of APC, glycogen synthase kinase-3 beta, and axin, followed by ubiquitin/proteasome degradation.<sup>4</sup>

In colorectal cancers, mutations of APC or beta catenin result in stabilization of beta catenin and a significant accumulation of this protein within the cytoplasm. Furthermore, increased beta catenin may translocate to the nucleus and could serve as a transcriptional factor by binding to the T-cell factor/lymphoid enhancing factor (Tcf-Lef) family, leading to transcription of specific genes stimulating tumor formation, such as *cyclin-D1*, *c-myc*, *c-jun*, and *fra-1*.<sup>5</sup>

Chronic inflammation has been known to have an important role in the development of various types of cancer, including colon cancer carcinogenesis through chronic colitis associated CRC.<sup>6</sup> Chronic colitis-related neoplastic progression may be associated with different beta catenin pathway alterations than sporadic carcinogenesis because of its origin in a field of chronic

inflammation.<sup>7</sup> Although the role of the APC gene mutations in carcinogenesis is still unclear, but it has been reported frequency of APC gene mutations in ulcerative colitis-related neoplasia varies from 0-50%.<sup>8</sup> Other studies show the APC protein expression in 80% of cases of ulcerative colitis-related carcinoma. This indicates that the path APC beta catenin may play a more important role in relation to carcinogenesis chronic colitis than those found before.<sup>7-8</sup> In this study, we investigated the expression pattern of beta catenin in colorectal non neoplastic and neoplastic lesion by immunohistochemical staining.

## Methods

**Samples.** The study population consisted of four groups of each 30 patients retrieved by a retrospective search through the pathology files of the Department of Anatomical Pathology, University of Indonesia in 2009. The first group consisted of patient with clinically, endoscopically and pathologically confirmed CRC. All specimen from this group of patients consisted of colonic resection samples. Invasive adenocarcinoma was diagnosed when atypical cells or glands infiltrated into the submucosa.<sup>9</sup> The adenocarcinomas were graded as well-differentiated (grade 1), moderately differentiated (grade 2), or poorly differentiated (grade 3), and the depth of invasion was determined.<sup>9,10</sup> The second group consisted of adenoma. An adenoma was defined as a discrete, well defined, sessile or pedunculated polyp and histological architecture (tubular, tubulovillous, villous), and the dysplasia was graded as low- or high-grade.<sup>9</sup> The patient had no history of pancolitis. All specimens consisted of polypectomy samples. The third group consisted of patient with chronic colitis. Endoscopically biopsy samples of chronic colitis were confirmed when there was expansion of the lamina propria by plasma cells or eosinophils or accompanying glandular distortion.<sup>11</sup> The fourth group consisted of normal colorectal mucosa of the resection margin.

**Beta catenin immunostaining.** Briefly, sections were deparaffinized and rehydrated through graded alcohol. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in methanol for 20 minutes. Antigen retrieval was used by microwave treatment; dilution was 1:100 for beta catenin (Novocastra, United Kingdom). Biotinylated universal antiserum (Starr Trek HRP Universal, Biocare Medical, CA) was used as the secondary antibody. After washing, the slides were incubated for 20 minutes at room temperature with Trekavidin HRP label (Starr Trek HRP Universal Biomedical CA) and developed for 10 minutes using 3-3'-diaminobenzidine as chromogen. After rinsing in water, the sections were counterstained with Meyer haematoxylin, dehydrated, and mounted. Appropriate positive and negative controls were included in each run of immunohistochemistry.

**Beta catenin evaluation.**<sup>12,13</sup> Normal colonic epithelial cells served as internal positive controls with membrane staining (Figure 1). Cytoplasmic, nuclear, and membrane expressions were recorded separately. The percentage of cells with membranous, cytoplasmic and nuclear positivity was graded as follows: 0) <5%, 1) 5–25%, 2) 26–50%, 3) 51–75%, 4) >75%. The staining intensity was graded as (0) negative, no expression, or (1) positive, positive expression. Cytoplasmic staining was graded to 3 categories: (0) negative, no detectable staining, (1) weak, but still detectable staining, (2) heavy staining, intense. The nuclear staining was also graded to into 3 categories: (0) negative, only blue staining seen, (1) weak, blue staining clearly seen through brown staining, and (2) heavy staining, blue scarcely seen through brown staining, nuclei appear darker than the cytoplasm, or no blue seen through brown staining. Immunohistochemistry scores obtained by multiplying the “percentage of positive cells” by the “staining intensity” and expressed as negative (score 0), weak (score 1–4), strong (score 5–8).

All immunohistochemically stained slides were evaluated using regular light microscopy by one of the investigators blinded from any other clinical and laboratory data. A second observer examined 30 cases randomly, and kappa coefficients for agreement in these 30 cases were as follows: 0.525 for cytoplasmic positivity, 0.753 for nuclear positivity, 0.737 for membrane positivity, indicating overall moderate to substantial agreement.

Kruskal-Wallis test was used to evaluate the relationship between the expression of beta catenin and progression and expression of beta catenin and differentiation. Mann-Whitney test was used to evaluate the relationship between the expression of beta catenin and degree of dysplasia. SPSS for Windows 13.0 (SPSS, Chicago, IL) software was used to analyze the data. For statistical significance, the cut off point for p-value was set at 0.05.

## Results and Discussion

A summary of the clinicopathologic features of the specimens are illustrated in Table 1. Beta catenin immunostaining in normal colonic mucosa is performed and give similar results with previous studies.<sup>12-14</sup> All normal colorectal mucosal showed membranous expression of beta-catenin and was used as an internal positive control (Figure 1).

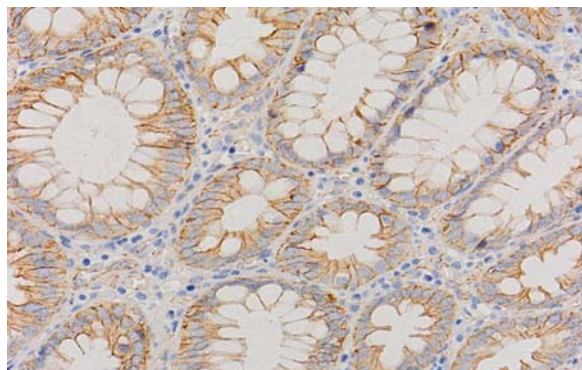
This study showed a significant increase for beta-catenin expression during the progression from normal epithelium to carcinoma ( $p = 0.000$ ). Membranous and cytoplasmic expression of beta-catenin was increased with progression of carcinogenesis (Table 2, Figure 2). Hao *et al.* found an association between

cytoplasmic expression of beta-catenin in 54% cases of dysplastic aberrant crypt foci and concluded that this process is the earliest changes in colorectal carcinogenesis.<sup>12</sup> Similar results also come from

Elzagheid *et al.* who discovered nearly 100% membranous and cytoplasmic expression of beta-catenin in CRC and adenoma.<sup>14</sup>

**Table 1. Clinicopathologic Characteristic**

Parameter	Normal n (%)	Colitis n (%)	Adenoma n (%)	Carcinoma n (%)
Sex				
Male	15 (53)	13 (21)	12 (40)	16 (53)
Female	14 (47)	17 (29)	18 (60)	14 (47)
Age (year)				
<40	5 (17)	12 (40)	4 (13)	5 (17)
41 – 50	5 (17)	3 (10)	5 (17)	5 (17)
51 – 60	12 (40)	9 (30)	12 (40)	12 (40)
>60	8 (26)	6 (20)	9 (30)	8 (26)
Tumor location				
Right sided		4 (13)	2 (6)	7 (23)
Left sided		11 (37)	8 (26)	14 (46)
Rectum		15 (50)	20 (66)	9 (30)
Histopathologic type				
Adenocarcinoma				29 (96)
Mucinous carcinoma				1 (3)
Tubular adenoma			4 (13)	
Tubulovilous adenoma			6 (20)	
Vilous adenoma			3 (10)	
Tumor differentiation				
Well				13 (43)
Moderate				9 (30)
Poor				7 (23)
Dysplasia degree				
Low grade			6 (20)	
High grade			24 (80)	
Tumor staging				
T1				0 (0)
T2				8 (26)
T3				22 (73)
T4				0 (0)
Lymph node status				
Nx				11 (37)
N0				4 (13)
N1				12 (40)
N2				3 (10)



**Figure 1. Membranous Expression of Beta Catenin in Colorectal Normal Mucosa**

Beta catenin is a multifunctional protein complex which is a component of cell-to-cell adhesion and part of the WNT signaling pathway to be degraded by the degradation complex APC-Axin-GSK-3B. In cells with two mutant APC alleles, beta-catenin degradation does not occur and cause increased accumulation of beta-catenin in the cytoplasmic.<sup>15</sup>

Accumulation of beta-catenin in the nucleus was detected in almost all (96%) CRCs (Figure 3). This proves that beta-catenin is a key factor in colorectal carcinogenesis. Cheah *et al.* and Baldus *et al.* reported the nuclear expression of beta catenin in sporadic CRC between 21-100% and support our results (Table 2). Some explanation of the variation results including the heterogeneity of the tumor, different immunostaining procedure, lack of standardization evaluation of positive and negative results as well as different methods of determining the degree of differentiation.<sup>16,17</sup>

This study did not find the relationship between beta-catenin expression (membrane, cytoplasm and nucleus) with the degree of dysplasia ( $p > 0.05$ ) (Table 3). This is similar with the result from Elzagheid *et al.* and Hao *et*

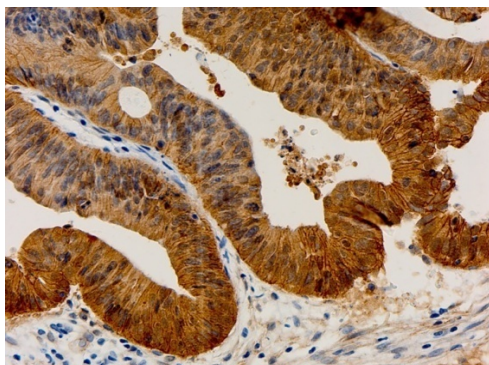
*al.*<sup>12,14</sup> Beta-catenin expression is increased in the membrane and cytoplasm can be detected at an early stage, while the nucleus translocation occurs at an advanced stage to carcinoma and this illustrates the stages of genetic mutations in normal epithelium developed into CRC.<sup>12</sup>

Wanitsuwan *et al.* discovered a correlation between beta-catenin expression with the degree of differentiation. This suggests that beta catenin may function to maintain the status of cell differentiation in carcinoma.<sup>18</sup> Although this study did not find the relationship between expression of beta-catenin with tumor differentiation (Table 3), but in carcinomas with mucinous component and poorly differentiated tumors generally showed lower frequency of beta-catenin expression and a higher membrane expression compared with carcinoma without mucinous components and moderate or good differentiation.

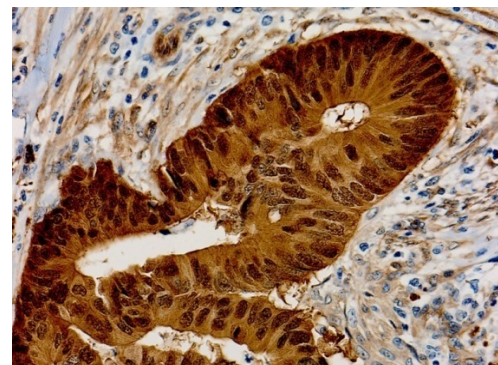
To the best of our knowledge, there were no studies that investigated the expression of beta-catenin in non-neoplastic lesions of the colon, such as chronic colitis. We used a chronic colitis with glandular distortion as

**Table 2. Beta Catenin Expression in Normal Mucosa, Chronic Colitis, Colorectal Adenoma and Carcinoma**

	Normal	Colitis	Adenoma	Carcinoma	<i>p value</i>
Membrane percentage					
<5	0 (0)	0 (0)	3 (10)	0 (0)	
6-25	0 (0)	0 (0)	6 (20)	3 (10)	
26-50	1 (3)	0 (0)	3 (10)	3 (10)	
51-75	0 (0)	1 (3)	7 (23)	11 (36)	
>75	29 (97)	29 (97)	11 (36)	13 (43)	
Cytoplasmic score					<i>p = 0.000</i>
Negative	12 (40)	10 (33)	0 (0)	0 (0)	
Weak	9 (30)	19 (63)	13 (43)	5 (16)	
Strong	9 (30)	1 (3)	17 (56)	25 (83)	
Nuclear score					
Negative	30 (100)	29 (97)	8 (26)	1 (3)	
Weak	0 (0)	1 (3)	10 (33)	12 (40)	
Strong	0 (0)	0 (0)	12 (40)	17 (56)	



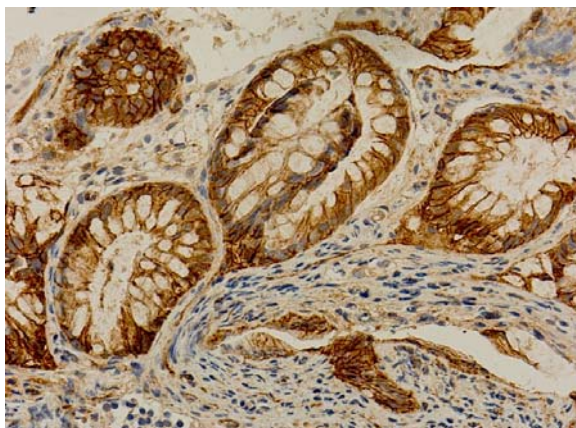
**Figure 2. Membranous and Cytoplasmic Expression of Beta Catenin in Colorectal Adenoma**



**Figure 3. Strong Cytoplasmic and Nuclear Expression of Beta Catenin in Colorectal Carcinoma**

**Table 3. Beta Catenin Expression in Colorectal Adenoma and Carcinoma**

	Dysplasia degree		Tumor differentiation			<i>p value</i>
	Low	High	Well	Moderate	Poor	
Membrane percentage						
<5	0 (0)	3 (10)	0 (0)	0 (0)	0 (0)	
6-25	0 (0)	6 (20)	2 (6)	1 (3)	0 (0)	
26-50	1 (3)	2 (6)	1 (3)	0 (0)	2 (6)	
51-75	2 (6)	5 (16)	4 (13)	4 (13)	3 (10)	
>75	3 (10)	8 (27)	6 (20)	4 (13)	3 (10)	
Cytoplasmic score						<i>p</i> > 0.05
Negative	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Weak	4 (13)	9 (30)	3 (10)	1 (3)	2 (6)	
Strong	2 (6)	15 (50)	10 (33)	8 (27)	6 (20)	
Nuclear score						
Negative	2 (6)	6 (20)	1 (3)	0 (0)	0 (0)	
Weak	3 (10)	7 (23)	4 (13)	3 (10)	5 (16)	
Strong	1 (3)	11 (37)	8 (27)	6 (20)	3 (10)	



**Figure 4. Membranous and Cytoplasmic Expression of Beta Catenin in Chronic Colitis. No Nuclear Expression of Beta Catenin**

one of research materials to investigate the beta-catenin expression changes due to the chronic inflammatory process that can cause carcinogenesis. Inflammation through the free radicals can cause damage to DNA, fats, and proteins. DNA damage with the increase proliferation of can cause persistent gene mutations.<sup>6,7</sup>

Our research shows extensive expression of the membrane in all cases (100%) of chronic colitis (Table 2, Figure 4). We found cytoplasmic expression of beta catenin in 20 cases (66.7%) and weak nuclear expression in one case (3.3%). Cooper *et al.* who investigated the dysplastic lesions in chronic colitis using mouse model discovered alteration in cytoplasmic and/or nuclear expression of beta-catenin in 10 cases (100%) of DALM (Dysplasia Associated Lesion/Mass).<sup>19</sup> Sixteen of 17 cases (94.2%) flat dysplasia showed membrane expression of beta-catenin and in one of 17 cases (5.8%) gave cytoplasm and nuclear expression.

These results indicate translocation of beta-catenin is involved in polypoid dysplastic lesions rather than flat dysplastic lesions and suggest an important role of beta-catenin in the early molecular events in polypoid lesions. This study found no association between the location of expression (membrane, cytoplasm, and nucleus) beta-catenin with the clinical variables (age, gender, and location of the tumor). Some studies find similar results except for the tumor location. Kawasaki *et al.* and Elzagheid *et al.* reported the increased cytoplasm and nuclear expression of beta-catenin in right sided tumor than the left side and rectum.<sup>13,14</sup> The involvement of different molecular mechanism in colorectal carcinogenesis, in which the adenoma carcinoma sequence of carcinogenesis generally occurs on the left side of the colon and rectum while the mutator phenotype occurs in the right side.

The relationship between the location of beta-catenin expression and clinical outcome is still the subject of controversy. In a study involving 60 CRC, nuclear beta-catenin expression predicts a poorer survival.<sup>20</sup> Research in 650 CRC showed beta-catenin expression was not associated with survival rates at.<sup>21</sup> Recent research involving 60 CRC found no predictive value for the nuclear beta catenin expression, but the expression on the membrane, cytoplasm and nucleus associated with better differentiation and lower stage of disease.<sup>22</sup> In a study for 95 stage IV CRC membrane beta catenin expression associated with higher cure rates.<sup>23</sup> Recent data show suppression of beta-catenin can inhibit the development of mutant APC CRC.<sup>24</sup>

## Conclusion

Subcellular localization of beta-catenin can reflects its oncogenic activity thus beta-catenin is a component that plays a role in the development of the CRC. Although



the role in chronic inflammatory setting can not be determined yet, it is acceptable that beta catenin involved in the inflammatory process and may play an important role. Further studies are needed to see the relationship of other factors that played a role in Wnt signaling pathway/beta-catenin, like APC protein, beta-catenin, axin and GSK-3b to get a comprehensive understanding of beta-catenin activity that can be used for the development of preventive agent in the future.

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