Alteration of Subcellular Beta Catenin Expression in Normal Mucosa, Adenoma and Carcinoma in Relation to Colorectal Carcinogenesis

Pamela Damaledo Abineno, Diah Rini Handjari, Budiningsih Siregar

Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia
Dr. Cipto Mangunkusumo General National Hospital, Jakarta

ABSTRACT

Background: *Adenomatous polyposis coli (APC) gene mutation was found in up to 80% of cases of sporadic colorectal cancers and adenomas. Loss of APC protein function has been known as one of the early process in colorectal carcinogenesis. This event leads to the accumulation of beta catenin in the cytoplasm and nucleus and subsequently activates target genes that regulate cell proliferation and apoptosis. The aim of this study was to investigate the alteration of subcellular beta catenin expression in the progression of colorectal cancer.*

Method: *This cross-sectional study was conducted with 30 paraffin-embedded tissue sections each of normal colorectal mucosa, adenomas and carcinomas. Alteration of beta catenin expression in membranous, cytoplasmic, and nuclear compartments were evaluated by immunohistochemical staining.*

Results: *Beta catenin immunoreactivity was detected in all cases, of which 87 (96.7%) cases showed membranous expression, 78 (86.7%) cases had cytoplasmic and 51 cases (56.7%) had nuclear expression. Such results were statistically significant ($p < 0.000$). All normal colorectal epithelium showed membranous beta catenin expression with 18 (60.0%) cases showed cytoplasmic and no nuclear beta catenin expression was found. Strong cytoplasmic expression was found in 17 (56.7%) adenomas and 25 (83.3%) carcinomas; while strong nuclear expression was found in 12 (40.0%) adenomas and 17 (56.7%) carcinomas. There was no statistical significant association between beta catenin expression in the membranous, cytoplasmic and nuclear compartment with the degree of dysplasia or differentiation of tumor ($p > 0.05$).*

Conclusion: *Altered subcellular expression of beta catenin occurs as the oncogenic process develops from adenoma into carcinoma. Such finding reflects the importance of beta-catenin in colorectal carcinogenesis.*

Keywords: *beta catenin, colorectal cancer, adenoma, colorectal cancer progression*

INTRODUCTION

Colorectal carcinoma (CRC) is the third major malignancy worldwide after lung cancer and breast cancer and it is the third leading cause of cancer death from all cancers.[1] Until now, the curative treatment is surgical resection although adjuvant treatment has been growing rapidly. However, there is little that can improve the survival rate of patients in advanced stages of the CRC.[2] The detection of new cases in the early stages should be developed as a preventive action that can reduce morbidity and mortality in CRC patients.[3]

Colorectal carcinogenesis is a multistep processes that occurs due to genetic changes from normal mucosa to adenoma and then carcinoma. This pathway requires the accumulation of genetic changes which proceeded by the inactivation of tumor suppressor gene adenomatous polyposis coli (APC).[4] Beta catenin has recently been the object of increasing interest because of the discovery of additional functions of this protein apart from its well-known role in cell adhesion.[5] The implication of beta catenin in the transduction of Wingless/Wnt-dependent cell–cell signaling has been demonstrated.[6] Furthermore, beta catenin may also

Correspondence:
Diah Rini Handjari
Department of Anatomical Pathology
Dr. Cipto Mangunkusumo General National Hospital
Jl. Diponegoro No. 71 Jakarta 10430 Indonesia
Phone: +62-21-31905888 Fax: +62-21-31934465
E-mail: rinidiah@gmail.com

play a direct role in colorectal carcinogenesis because it binds the product of the tumor supressor gene APC. Glycogen synthase kinase-3 beta (GSK-3-beta) and wild-type APC regulate the level of free cytoplasmic beta catenin by promoting its degradation through the ubiquitin proteasome pathway.[5]

When APC is mutated, as occurs in 85% of CRCs and adenomas, beta catenin accumulates in the cytoplasm and can translocate into the nucleus, where it binds transcription factors of the T-cell factor (TCF)/ lymphoid enhancer factor (LEF) gene family and activates the expression of target genes. Therefore, it is proposed that beta catenin plays a dual role, not only in the formation and maintenance of cells interactions but also in the regulation of gene activity with a dominant oncogenic effect on carcinogenesis.[5,6]

Since it is important to understand beta catenin nuclear localization in the development of colorectal cancers, we have conducted a detailed comparison of beta catenin distribution in normal colorectal mucosa, adenomas and carcinomas

METHOD

Human tissue samples were obtained from surgical resection of 30 patients with colorectal cancer and colonic biopsies of 30 patients with adenomas from the Department of Anatomical Pathology, University of Indonesia. Thirty histologically normal colorectal mucosa of the resection margin were also included in this study. Serial sections were cut at 4 ìm thickness from paraffin-embedded blocks and placed on poly-L-lysin-coated slides. One haematoxylin/eosin stained slide was reviewed for histological classification and other slides were used for immunohistochemical analysis.

Beta catenin immunostaining: in brief, sections were deparaffinized and rehydrated in 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 diaminobenzidine as chromogen. After rinsing in water, the sections were counterstained with Meyer haematoxylin, dehydrated, and coverslipped. Appropriate positive and negative controls were included in each run of immunohistochemistry.

Beta catenin evaluation: 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%, and the staining intensity was graded as (0) negative for no expression, or (1) positive for positive expression. Cytoplasmic staining was graded into 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, (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 who had been 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, which indicated overall moderate to substantial agreement.

Kruskal-Wallis test was used to evaluate the relationship between the expression of beta catenin as well as progression and expression of beta catenin and its differentiation. Mann-Whitney test was used to evaluate the relationship between the expression of beta catenin and degree of dysplasia. All statistical tests were performed by using the SPSS package version 13.0 (SPSS, Chicago, IL), $p < 0.05$ was considered significant.

RESULTS

The clinicopathologic data are summarized in table 1. The mean age of the patients was 53.9 years, ranged from 24 to 99 years (standard deviation 14.078). All histologically normal epithelia showed clearly uniform membrane staining along the whole length of the crypt (figure 1), which served as an internal positive control. No background in the stroma or nuclear staining was found at all.

Table 1. Clinicopathologic characteristic

Parameter	Carcinoma n (%)	Adenoma n (%)
Sex		
- Male	16 (53.3)	12 (40.0)
- Female	14 (46.7)	18 (60.0)
Age (year)		
- <40	5 (16.7)	4 (13.3)
- 41 – 50	5 (16.7)	5 (16.7)
- 51 – 60	12 (40.0)	12 (40.0)
- >60	8 (26.7)	9 (30.0)
Tumor location		
- Right sided	7 (23.3)	2 (6.7)
- Left sided	14 (46.7)	8 (26.7)
- Rectum	9 (30.0)	20 (66.7)
Histopathologic type		
- Adenocarcinoma	29 (96.7)	
- Mucinous carcinoma	1 (3.3)	
- Tubular adenoma		4 (13.3)
- Tubulovilous adenoma		6 (20.0)
- Vilous adenoma		3 (10.0)
Tumor diferentiation		
- Well	13 (43.3)	
- Moderate	9 (30.0)	
- Poor	7 (23.3)	
Dysplasia degree		
- Low grade		6 (20.0)
- High grade		24 (80.0)
Tumor staging		
- T1	0 (0)	
- T2	8 (26.7)	
- T3	22 (73.3)	
- T4	0 (0)	
Lymph node status		
- Nx	11 (36.7)	
- N0	4 (13.3)	
- N1	12 (40.0)	
- N2	3 (10.0)	

The results of the immunostaining for beta catenin in each 30 normal mucosa, adenomas and carcinomas are summarized in table 2. In adenomas and carcinomas, three phenomena were observed: (a) membranous expression of beta catenin; (b) the appearance of cytoplasmic expression of beta catenin; (c) the appearance of nuclear expression of beta catenin.

In all 30 of adenomas, beta catenin expression was present in both the cytoplasm (100%) and nuclear (73.3%). (table 2 and figure 2). Of the 24 cases with high grade dysplasia, intense cytoplasmic expression of beta catenin was found in 50% cases and intense nuclear expression present in 36.7% cases. There was no association between degree of dysplasia and expression of beta catenin ($p > 0.05$) (table 3).

Beta catenin expression was also cytoplasmic (100%) and nuclear (96.7%) in all 30 carcinoma examined (table 2 and figure 3). The intensity of beta catenin staining in the cytoplasm and nuclear of the colorectal cancers was generally greater than those seen in adenomas ($p < 0.000$). There was no association between tumor differentiation and expression of beta catenin ($p > 0.05$) (table 3).

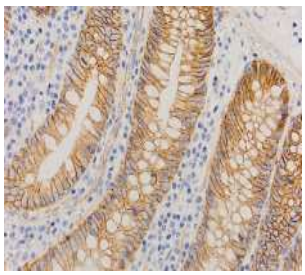

Figure 1. Membranous expression of beta catenin in colorectal normal mucosa

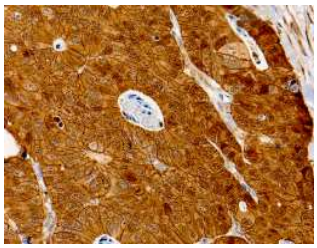

Figure 2. Membranous and cytoplasmic expression of beta catenin in colorectal adenoma

Table 2. Beta catenin expression in normal mucosa, colorectal adenoma and carcinoma

		Membranous					Cytoplasmic			Nuclear		
		0	1	2	3	4	0	1-4	5-8	0	1-4	5-8
Normal		0 (0)	0 (0)	1 (3.3)	0 (0)	29 (96.7)	12 (40)	9 (30)	9 (30)	30 (100)	0 (0)	0 (0)
Adenoma	n (%)	3 (10)	6 (20)	3 (10)	7 (23.3)	11 (36.7)	0 (0)	13 (43.3)	17 (56.7)	8 (26.7)	10 (33.3)	12 (40)
Carcinoma		0 (0)	3 (10)	3 (10)	11 (36.7)	13 (43.3)	0 (0)	5 (16.7)	25 (83.3)	1 (3.3)	12 (40)	17 (56.7)
		p = 0.000					p = 0.000			p = 0.000		

Table 3. Beta catenin expression in colorectal adenoma and carcinoma

		Membranous					Cytoplasmic			Nuclear		
		0	1	2	3	4	0	1-4	5-8	0	1-4	5-8
Dysplasia												
Low grade	n (%)	0 (0)	0 (0)	1 (3.3)	2 (6.7)	3 (10)	0 (0)	4 (13.3)	2 (6.7)	2 (6.7)	3 (10)	1 (3.3)
High grade		3 (10)	6 (20)	2 (6.7)	5 (16.7)	8 (26.7)	0 (0)	9 (30)	15 (50)	6 (20)	7 (23.3)	11 (36.7)
Differentiated												
Well		0 (0)	2 (6.7)	1 (3.3)	4 (13.3)	6 (20)	0 (0)	3 (10)	10 (33.3)	1 (3.3)	4 (13.3)	8 (26.7)
Moderate	n (%)	0 (0)	1 (3.3)	0 (0)	4 (13.3)	4 (13.3)	0 (0)	1 (3.3)	8 (26.7)	0 (0)	3 (10)	6 (20)
Poor		0 (0)	0 (0)	2 (6.7)	3 (10)	3 (10)	0 (0)	2 (6.7)	6 (20)	0 (0)	5 (16.7)	3 10)
		p > 0.05					p > 0.05			p > 0.05		

DISCUSSION

Beta catenin is currently regarded as the component that plays a role in the development of the CRC which is the third leading cause of death from cancer worldwide.[6] Early detection and prompt treatment will greatly affect the cure rate. This led to the rapid development of diagnostic and prognostic factors that can help us to detect asymptomatic CRC and the precursor lesions, such as adenomas, and to predict disease progression of CRC accurately.

This study was expected to provide evidence on the relationship of beta catenin expression caused by APC gene mutation in colorectal carcinogenesis. The hypothesis underlying this study was that loss of function of APC protein early in the process of colonic carcinogenesis should result in the accumulation of cytoplasmic and nuclear beta catenin that would be detected by immunohistochemical analysis. We did not expect the relationship would be perfect because other mechanism that affects beta catenin degradation pathway may occur, such as the mutation in the beta catenin gene or activation of WNT signaling.

Our study showed significant increase regarding beta catenin expression during the progression from normal epithelium into carcinoma as reported by other studies emphasizing its role as a key oncogenic factor in colorectal carcinogenesis.[7]

Although the study did not find a relationship between beta catenin expression and tumor differentiation as in other study, but carcinomas with mucinous components and poorly differentiated tumors, generally indicate reduced beta catenin expression and increased membrane expression as compared with well-to-moderate carcinoma and non-mucinous carcinoma.[8] Such finding suggests that beta catenin serves to maintain the state of cell differentiation.[8]

Hao et al demonstrated that nuclear beta catenin expression was found in 6/46 (13%) cases of aberrant crypt foci (ACF), which was associated with the progression from ACF into adenoma and carcinoma.[9] Another study from Wong et al found nuclear beta catenin expression in 5/60 cases (8%) colorectal polyps (Peutz-Jegher and hyperplastic polyps).[10] It indicated the presence of beta catenin deregulation that occur early in colorectal carcinogenesis.[9,10]

In addition to the nuclear expression, our study also showed both cytoplasmic and membrane expressions of beta catenin, which increases with the progression of carcinogenesis. Similar results were also obtained from Elzagheid et al that found a nearly 100% expression of beta catenin in the membrane and cytoplasm of the CRC and adenoma.[11] Beta catenin is a multifunctional protein complex, which is a component of adhesion between cells and part of the WNT signaling pathway to be degraded by APC degradation complex - Axin-GSK-3-beta. In cells with two alleles, APC mutant beta catenin degradation does not occur and cause increased accumulation of beta catenin in cytoplasm.[12]

Our study demonstrated that there is no association between the location of beta catenin expression (membrane, cytoplasm, and nucleus) and the existing clinical variables (age, gender, and location of the tumor). Some studies have also found similar results except for the tumor location. Kawasaki et al and Elzagheid et al reported increased nuclear and cytoplasm expression of beta catenin in the right-sided tumor location compared to the left-sided and rectum.[11,13] Different molecular mechanisms are involved in colorectal carcinogenesis, in which the adenoma carcinoma sequence of carcinogenesis pathway generally occurs on the left side of the colon and rectum; while the mutator phenotype occurs in the right side.[13]

The relationship between the location of beta-catenin expression and clinical outcome is still a subject of controversy. In a study involving 60 cases of CRC, nuclear beta catenin expression predicted a poorer survival.[14] Another study in 650 cases of CRC showed that beta catenin expression was not associated with survival rates.[15] A recent study involving 60 cases of CRC found no predictive value of nuclear beta catenin expression, but its expression on the membrane, cytoplasm and nucleus was associated with a better differentiation and lower stage of disease.[8] In a study with 95 cases of stage IV CRC, the membrane beta catenin expression was associated with a higher cure rates.[11] Recent data show that suppression of beta catenin may inhibit the development of mutant APC CRC.[16-18]

CONCLUSION

It can be concluded that beta catenin is a component that plays a role in the development of the CRC and that the subcellular location of beta-catenin may describe its oncogenic activity. Further studies are needed to recognize the relationship of other factors that also play roles in Wnt signaling pathway/beta catenin, including APC protein, beta catenin, axin and GSK-3-beta in order to get a comprehensive understanding of beta-catenin activity that can be used for the development of preventive agent in the future.

REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics 2010. CA Cancer J Clin 2010;60:277-300.
2. Wolpin BM, Meyerhadt JA, Mamon HJ, Mayer RJ. Adjuvant treatment of colorectal cancer. CA Cancer J Clin 2007;57;168-85.
3. American Cancer Society. Colorectal cancer facts & figures 2008 – 2010. Atlanta: Am Can Soc 2008.

4. Jass JR. Pathogenesis of colorectal cancer. Surg Clin North Am 2002;82:891-904.
5. Behrens J. The role of the Wnt signalling pathway in colorectal tumorigenesis. Biochem Soc Trans 2005; 33:672-5.
6. Lo Muzio L. A possible role for the WNT-1 pathway in oral carcinogenesis. Crit Rev Oral Biol Med 2001;12:152-65.
7. Wong SC, Lo ES, Lee KC, Chan JK, Hsiao WL. Prognostic and diagnostic significance of beta-catenin nuclear immunostaining in colorectal cancer. Clin Cancer Res 2004;10:1401-8.
8. Wanitsuwan W, Kanngurn S, Boonpipattanapong T, Sangthong R, Sangkhatnat S. Overall expression of beta catenin outperforms its nuclear accumulation in predicting outcomes of colorectal cancers. World J Gastroenterol 2008;14:6052-9.
9. Hao XP, Pretlow TG, Rao JS, Pretlow TP. Beta-catenin expression is altered in human colonic abberant crypt foci. Cancer Res 2001;61:8085-8.
10. Wong SC, Lo ES, Chan AK, Lee KC, Hsiao WL. Nuclear beta catenin as a potential prognostic and diagnostic marker in patients with colorectal cancer from Hong Kong. Mol Pathol 2003;56:347-52.
11. Elzagheid A, Buhmeida A, Korkeila E, Collan Y, Syrjanen K, Pyrhonen S. Nuclear beta catenin expression as a prognostic factor in advanced colorectal carcinoma, World J Gastroenterol 2008;14:3866-71.
12. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J Cell Sci 2007;120:3327-35.
13. Kawasaki T, Katsuhiko N, Ohnishi M, Suemoto Y, Kirkner GJ, Dehari R, et al. Correlation of beta catenin localization with cyclooxigenase-2 expression and CpG island methylator phenotype (CIMP) in colorectal cancer. Neoplasia 2007;9:569-77.
14. Hugh TJ, Dillon SA, Taylor BA, Pignatelli M, Poston GJ, Kinsella AR. Cadherin – catenin expression in primary colorectal cancer: a survival analysis. Brit J Cancer 1999;80:1046-51.
15. Lugli A, Zlobec I, Minoo P, Baker K, Tornillo, Terracciano L, et al. Prognostic significance of the wnt signalling pathway moleculer APC, beta catenin and E-cadherin in colorectal cancer – a tissue microarray-based analysis. Histopathology 2007;50:453-64.
16. Castellone MD, Teramoto H, Gutkind JS. Cyclooxygenase-2 and colorectal cancer chemoprevention: the beta catenin connection. Cancer Res 2006;66:11085-8.
17. Roh H, Green DW, Boswell CB, Pippin JA, Drebin JA. Suppression of beta catenin inhibits the neoplastic growth of APC mutant colon cancer cells. Cancer Res 2001;61:6563-8.
18. Dvory-Sobol H, Sagiv E, Kazanov D, Ben-Ze'ev, Arber N. Targeting the active beta catenin pathway to treat cancer cells. Mol Cancer Ther 2006;5:2861–71.