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Immunohistochemical detection of Beta Catenin in colon tumors among Sudanese patients

Shahinaz S. Salih*, Abu Elgasim A. Awad El- kareem.

Department of histopathology and cytology, College of medical Laboratory sciences, Sudan University of Sciences and Technology
* Corresponding author: shahinaznour86@gmail.com
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ABSRTACT

Colon cancer, or bowel cancer, is a cancer from uncontrolled cell growth in the colon or rectum. Many genes and proteins intervene in the regulation of cell growth and one of the most interesting multifunctional protein is Beta-catenin (β-catenin) which is a dual functional protein, regulating the coordination of cell–cell adhesion, gene transcription and it supposedto have great role in colon tumorigenesis. This study aimed to detect the expression of Beta catenin in colon tumors. Forty colon samples were used and divided as 10 benign colon, 10 non- adenomatous polyps, 20 colon cancer in, all were initially diagnosed by conventional histological techniques then examined immunohistochemically for beta catenin that expressed different patternsand subcellular localization which varies throughout the stages of tumorigenesis sequence as 3/10 (30 %) of benign inflammatory tissues showed membranous reaction and only 2/10 (20%) expressed membranous/cytoplasmic reactivity. The majority of non adenomatous polyps (80%) revealed cytoplasmic reactivity; also the majority of malignant tissues (75%) showed over expression of cytoplasmic reactivity while the rest (25%) showed prominent nuclear staining (P< 0.000). These results suggested the potential role of β-catenin localization in colon tumorigenesis and cancer transformation and showed the magnitude of it as diagnostic and prognostic marker.

المستخلص

سرطان القولون أو سرطان الأمعاء هو السرطان الناتج من نمو الخلايا غير المنضبطفي القولون أو المستقيم، العديد من الجينات والبروتينات تتدخل في تنظيم نمو الخلايا و أحد البروتينات المتعددة الوظائف الاكثر أثارة للاهتمام هو بيتا كاتينين (β – كاتينين)،البروتين الوظيفي المزدوجالذي ينظم وينسق التصاق الخلايا و النسخ الجينات ويتوقع أن يكون له دور كبير في تكون أورامالقولون . هدفت هذه الدراسة الي الكشف عن ظهور β-catenin القولون. استخدمت أربعين عينة قولون قسمت إلى 10 عينات قولون حميدة، 10 بوليباتغيرالغدية ، 20 سرطان قولون، جميعها تم تشخيصه مبدئيا من التقنيات النسيجية التقليدية ثم درست بالصبغ المناعي لبيتا كاتينين واعرب عن أنماط ظهور و توطين تحت الخلوي يختلف باختلاف مراحل تكون الأورام. (30 ٪) من أنسجة الالتهابات الحميدة أظهرت التفاعلا غشائي و فقط (20٪) غشائي/ حشوي التفاعل. وكشفت غالبية الاورام الحميدة (80 ٪) عن التفاعل الحشوي . وأظهرت غالبية الأنسجة الخبيثة (75 ٪) التفاعل الحشوي

والباقي (25٪) أظهرت التلطيخ النووي ($P < 0.000$). وتشير هذه النتائج إلى الدور المحتمل لتموضعبيتا-كاتينين في تكون أورام القولون والتحول السرطاني و أظهرت أهمية أعتباره علامة تشخيصية وتنبؤية.

KEYWORDS: *Colorectal, adenocarcinoma, polyps, Beta catenin*

INTRODUCTION

Colon cancer or bowel cancer is a cancer from uncontrolled cell growth in the colon or rectum or in the appendix[1]. β-catenin is a subunit of the cadherin protein complex and acts as an intracellular signal transducer in the Wnt signaling pathway[2-4]; the pathway that causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional co-activator of transcription factors that belong to the TCF/LEF family[5]. β-catenin participates in the Wnt signaling pathway as a downstream target [6], when Wnt is present; β-catenin will not to be phosphorylated, and thus not ubiquitinated; as a result, its levels in the cell are stabilized as it builds up in the cytoplasm. Eventually, some of this accumulated β-catenin will move into the nucleus with the help of Rac1 subsequently becomes co-activator for TCF and LEF to activate Wnt genes by displacing Groucho and HDAC -the transcription repressors-[7]. Stimulation of the Wnt/β-catenin pathway, and its role in promoting malignant tumor formations and metastases, has been implicated in cancers [8]. β-catenin is thought to play a critical role in sporadic colorectal tumorigenesis if not degraded [9], as nuclear β-catenin localization has also been demonstrated in colorectal adenomas but not in hyperplastic or inflammatory colorectal polyps; suggesting that it is a relatively early and specific event in the pathogenesis of colorectal cancer [10], nuclear staining for β-catenin may serve as an additional parameter to help distinguish colorectal adenocarcinoma from adenocarcinoma of other tissue sites [11]. On the other hand sentinel lymph node biopsy in colon cancer is considered to be highly valuable in clarifying the prognostic role of micro- metastases/isolated tumor cells [12]. In this study we target the demonstrating expression pattern of beta catenin immunohistochemically through out different stages of colon tumorigenesis sequence.

MATERIALS and METHODS

Clinical Samples

This descriptive retrospective case study carried out in National Health Laboratory during the period from March to July 2013. Forty colorectal paraffin embedded tissue blocks were used; ten of which were benign tissues; ten polyps and twenty malignant colon tissues all were primarily diagnosed by haematoxylin and eosin; data upon staging were obtained according to Duke's classification. All samples were stained using immunohistochemistry.

Immunohistochemical Testing

The two micrometer sections for IHC incubated for overnight in an oven at 65ºc then brought to water graduallystarting with two changes of xylene; three minutes for each, two changes of absoluteethanol three minutes each; two minutes for each grade of ethanol (90%, 70%, 50%) and finally sections re-hydrated in distilled water. Then antigens were retrieved in Tris\EDTA pH 9.0 according to DAKO protocol for Envision FLEX; antigen retrieval spent 30 minutes in water path at 95ºc then equilibrated in phosphate buffer saline(PBS pH6.0) at room temperature for 5-10 min. Internal peroxidase activity was blocked for 10mins using Dako peroxidase blocker followed by washing in PBS for 3mins; the Ag\ Ab reactivity was hold usingthe primary antibody Beta catenin(monoclonal mouse anti-human IS 702

from DAKO FLEX) applied for 20 min, washed 3 min in PBS.Secondary labeled antibodywas applied onto sections for another 20 min, washed in PBS another 3min and finally the color developed by incubation in DAB reagent for 10 min then slides washed in tap water for 5min. The nuclei were counter stained briefly in Mayer's Haematoxylin for 2-3 min and then blued for 5min in running water then dehydrated, cleared and mounted in resinous mountant.

Data Analysis

The data were analyzed using version 16 SPSS computer program; frequencies and chi-square correlations were calculated.

RESULTS

Correlation of β-catenin expression pattern to clinicopathological features:

Beta catenin expressed along normal epithelia at cellular membranes as it does serve in intercellular junction and cell to cell contact. Here in this study we correlate the expression of β-catenin to benign, polyps and malignant features of colon and to stage and grade of malignant colon. There was 10 benign colon samples (i.e. inflammatory bowel diseases) 5 of which expressed weak/negative beta catenin staining representing (12.5%), 3 (7.5%) showed pure membranous staining and only 2 (5%) expressed membranous/cytoplasmic staining. From the other aspect 8(20%) of Polypsexpressed membranous/cytoplasmic immune-staining and the rest of them 2 (5%) showed weak/negative staining; whereas 15 out of20 malignant colon(37.5%)showed obvious cytoplasmic staining, on the other hand the nuclear staining of β-catenin was noted in the rest 5/20 (12.5%) with significant correlation(P=0.000) table (1) and figure(1) demonstrating the expression pattern. The clinicopathological features of malignant colon that based on staging and grading showed significant correlation of β-catenin staining pattern to stage only but not to the grade of the tumor. The Duke's stage A 3/5 (15%) and Duke's stage B 5/6 (25%) both expressed membranous/cytoplasmic β-catenin, whereas Duke's stage C expressed the highest and exclusive reactivity towards the nuclear translocated beta catenin and subsequent nuclear staining as seen in 5out of 8 (25%), the latest 3 (15%) showed membranous/cytoplasmic staining with significant correlation (P value <0.05). Notably there was only one colorectal cancer specimen has been diagnosed as showing far metastasis(Duke D); it showed only very weak cytoplasmic staining and constituted only 1 (5%) as shown in table (2). On behalf of association between grade and β-catenin expression there was no significant correlation table(3).

Table1: Correlation of beta catenin expression pattern to clinicopathological characters.

Clinical features	**β- catenin pattern P= 0.000**			
	- / + weak	membranous	cytoplasmic	Nuclear
Benign Tot=10	5 12.5%	3 7.5%	2 5%	0
Polyp Tot=10	2 5%	0	8 20%	0
Malignant Tot=20	0	0	15 37.5%	5 12.5%

Table2: Association between β-catenin and stages of Colon cancer.

β-catenin pattern	**Duke's Stage P= 0.019**			
	Duke's A Tot=5	Duke's B Tot=6	Duke's C Tot=8	Metastatic(Duke D)
- / +	2 10%	1 5%	0 0.0%	1 5%
cytoplasmic	3 15%	5 25%	3 15%	0 0.0%
nuclear	0 0.0%	0 0.0%	5 25%	0 0.0%

Table 3: Association between β-catenin and grades of colon cancer.

β-catenin pattern	**Grade P= 0.103**			
	well	moderate	poor	Undifferentiated cancer cell.
- / +	3 15%	0 0.0%	0 0.0%	1 5%
cytoplasmic	5 25%	5 25%	1 5%	0 0.0%
nuclear	5 25%	0 0.0%	0 0.0%	0 0.0%

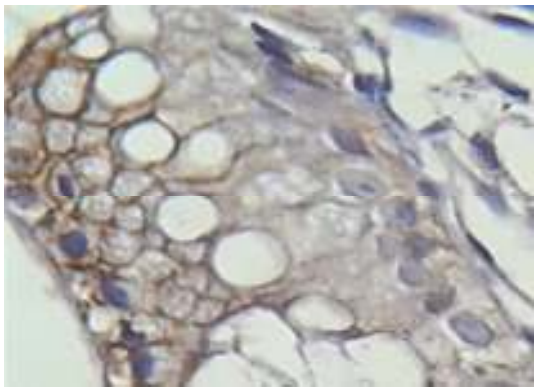

Figure1: Benign colorectal tumor showingmembranous expression of Beta catenin (40x)

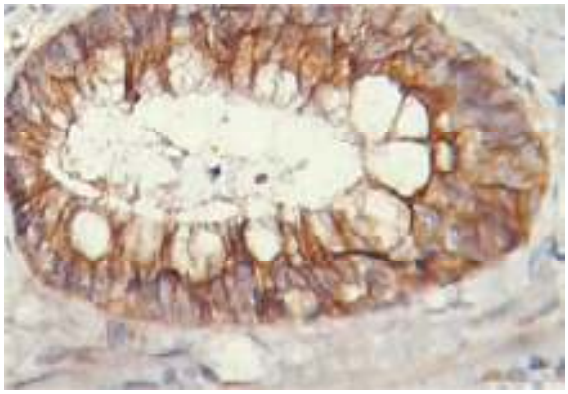

Figure2: Colorectal polyp showing cytoplasmic expression of Beta catenin (40x)

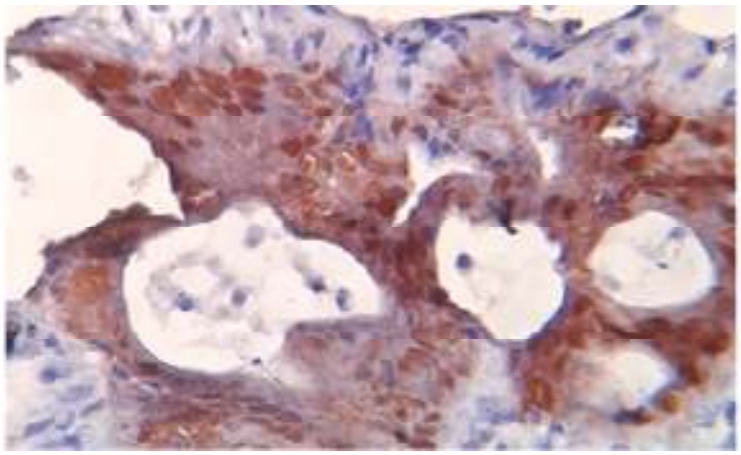

Figure3: Malignant colorectal tumor showing nuclear expression of Beta catenin (40x)

DISCUSSION

Globally greater than 1 million people get colorectal cancer yearly. Estimations of 2008 concluded it is the second most common cause of cancer in women and the third most common in men [(13)]. The expression pattern and subcellular localization of beta catenin was significantly related to the stages of progression from benign to malignant tissue in colon and rectum; namely colorectal tumorigenesis sequence (clinicopathological features); with high significant association (p = 0.000) this argued the significant correlation between gradual translocation of β-catenin in their subcellular locations and the gradual cellular malignant transformation process. These findings agree with the findings reported by Sze, et al. [(11)] who also concluded that the levels of β-catenin in all threeof the subcellular localizations were elevated from normal topolyp, adenoma and carcinoma; thus all of the subcellular expressions of β-catenin were significantlyassociated with stages and stations of progression of colorectalcancer. Also we concur with the findings of Gina, et al. [(14)] who found that nuclear accumulation is correlated with increased cytoplasmic and reduced membranous expression. We also made agreement with Dan, et al. [(15)] who found that normal mucosa cells were uniformly negative for β-catenin nuclear staining, but all showed positive membrane staining while adenomas over-expressed nuclear β-catenin and all of them were large-sized adenomas (tumor diameter >8 mm), also Ougolkov, et al. [(16)] found that tumors of diffuse type (nuclear accumulation throughout the tumor) had more nuclear B-catenin than the invasion edge type (i.e., nuclear accumulation predominantly in the tumor cells that formed the invasion edge). In primary carcinoma, nuclear over expression of β-catenin was more frequently detected in serosa layer carcinomas than that in the muscularis layer. On the basis of colon cancer staging we found that the association between expression pattern and stages of colon cancer was significant (p= 0.019) indicating that obvious nuclear translocation of β-catenin is positively correlated to the advanced tumors and advanced stages of cancer; this assent opinion of Zhang et al. [(17)] who consider that nuclear beta-catenin accumulation is related to tumor stage and/or metastasis; and also enforced by observation of Hongxia, et al.[(18)] who suggested that nuclear beta-catenin over-expression is related to an advanced tumor stage and poor tumor cell

differentiation, conversely Takayama, et al.[19] found that; the reduced expression(±) of β-catenin was observed more frequently in advanced tumors and furthermore Gina, et al.[14] findings reported insignificant correlation between nuclear β-catenin and stages of colon cancer; we found no justification for these disagreements. This study also illustrated grading of cancer, which was negatively correlated to the expression pattern of β-catenin (p=0.103) this insignificance indicating absence of association between β-catenin expression pattern and tumor differentiation. This matched Gina, et al. [14] conclusion; that there is insignificant association between β-catenin expression and histologicalnuclear B-catenin than the invasion edge type (i.e., nuclear accumulation predominantly in the tumor cells that formed the invasion edge). In primary carcinoma, nuclear over expression of β-catenin was more frequently detected in serosa layer carcinomas than that in the muscularis layer. On the basis of colon cancer staging we found that the association between expression pattern and stages of colon cancer was significant (p= 0.019) indicating that obvious nuclear translocation of β-catenin is positively correlated to the advanced tumors and advanced stages of cancer; this assent opinion of Zhang et al.[17]who consider that nuclear beta-catenin accumulation is related to tumor stage and/or metastasis; and also enforced by observation of Hongxia, et al.[18] who suggested that nuclear beta-catenin over-expression is related to an advanced tumor stage and poor tumor cell differentiation, conversely Takayama, et al. [19] found that; the reduced expression (±) of β-catenin was observed more frequently in advanced tumors and furthermore Gina, et al.[14] findings reported insignificant correlation between nuclear β-catenin and stages of colon cancer; we found no justification for these disagreements. This study also illustrated grading of cancer, which was negatively correlated to the expression pattern of β-catenin (p=0.103) this insignificance indicating absence of association between β-catenin expression pattern and tumor differentiation. This matched Gina, et al.[14]conclusion; that there is insignificant association between β-catenin expression and histological grading; this unlike Takayama, et. al.[19] conclusion as he reported significant positive correlation between reduced β-catenin expression and dedifferentiation; this divergence may justified by unequal distribution of grades throughout our samples under study as the well differentiated colon cancer samples were 13 (65%), 5 (25%) moderate, 1 (5%) poor and 1 (5%) undifferentiated colorectal.

CONCLUSION

The study suggested the potential role of β-catenin localization in colon tumorigenesis and cancer transformation and showed the magnitude of it as diagnostic and prognostic marker.

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