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Expression of β-Catenin and Cyclooxygenase 2 in Colorectal Carcinoma: An Immunohistochemical Study

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Abstract The Wnt/β-catenin pathway plays an important role in the genesis of familial adenomatous polyposis, the most common form of inherited colorectal carcinoma (CRC). Also, the inflammatory bowel diseases (IBDs) predispose to cancer development; and cyclooxygenase 2 (COX-2) seems to be pivotal in their pathogenesis. This study aimed to investigate the relationship between the expression of COX-2 protein and β-catenin in colorectal cancer. The study enrolled 45 patients, all of whom underwent surgery and immunohistochemical staining of tissue specimens for COX-2 and β-catenin was done. Correlation between the two modulators and their relationship with clinicopathological features were examined. In 34 cases (75.56%) of the tumor samples; β-catenin immunoreactivity was found in the cytoplasm and/or membrane compartment. On the other hand, COX-2 immunoreactivity was weakly and/or strongly positive in 32 cases (71.11%) and negative in 13 (28.89%). Positivity was detected in the cytoplasm and in the perinuclear area. Increased expression of β-catenin was correlated to Duke stage (P=0.009). Furthermore, nuclear β-catenin localization showed a correlation to the Duke stage (P=0.029) and insignificant correlation with distant metastases (P = 0.336). Positive COX-2 expression showed a significant relation to, liver metastases (P = 0.042), and Duke stage (P = 0.011) and insignificant correlation to lymph node invasion (P=0.25). These data indicate that cytoplasmic/membrane β-catenin over-expressions as well as positive COX-2 expressions are associated with a more aggressive behavior of the disease.

Keywords β-catenin, Cyclooxygenase 2, Immunohistochemical Staining, Histology, Pathology, Colorectal Cancer

1. Introduction

Colorectal cancer (CRC) is one of the most frequent malignancies and is the third most common cause of cancer-related death worldwide [1,2]. It might be related to a number of predisposing factors including the prior existence of benign tumours (adenomas), the prior existence of ulcerative colitis, inherited syndromes such as familial adenomatous polyposis, dietary factors such as a low fiber diet and so on [3]. CRC occurs either in a sporadic (75 - 80%) or in an inherited form (20-25%) [4]. A significant number of patients who undergo apparently curative operation unfortunately develop local recurrences or distant metastases leading to shorter survival [5]. Identification of factors that affect tumor aggressiveness and allow a more accurate prognosis is required. The molecular mechanisms of CRC initiation and/or progression have been only partially elucidated. The Wnt/β-catenin pathway plays a pivotal role in the genesis of familial adenomatous polyposis, the most common form of inherited CRC [1,6]. In the presence of adenomatous polyposis coli (APC) or β-catenin mutations, β-catenin is no longer phosphorylated and degraded in an ubiquitin-dependent fashion. β-Catenin can translocate to the nucleus where it associates with T-cell factor/ lymphoid enhancing factor (TCF/LEF) transcription factors to stimulate the expression of genes involved in cell proliferation [4,5]. Loss-of-function mutations of the DNA repair genes coupled with genome instability are the leading causes of human non-polyposis colorectal cancer, the other inherited form of CRC [7]. Mutations of the Wnt/β-catenin pathway play a causative role also in 60- 80% of sporadic cases [1,6]. Growing evidence, however, supports the notion that other pathway alterations may be relevant in colon carcinogenesis [1,7]. Inflammatory bowel diseases (IBDs) predispose to cancer development [9,10]. Cyclooxygenase 2 (COX-2) seems to be pivotal in their pathogenesis, as it is involved in the biosynthesis of prostaglandins [11]. These compounds, in turn, activate pro-inflammatory genes through the nuclear factor κB (NFκB) signaling pathway [12]. Its sustained expression stimulates cell proliferation, thus linking inflammation to cancer.

2. Objectives

The aim of the present study was to clarify the correlation between β-catenin and COX-2 with CRC evolution, through investigation of their protein expression and sub-cellular localization in tissue specimens of CRCs.

3. Material and Methods

3.1. Patient Histories and Tissue Samples

Forty five systematic random samples from patients who had undergone surgical resection (starting from 2004 to 2009) at the Department of Surgery, Zagazig University hospitals, were investigated in this study. Informed consent was obtained from patients for their tissues to be used in research. The specimens were obtained immediately after surgical resection, fixed in 10% neutral buffered formalin and then embedded in paraffin blocks. Ten samples were obtained from the proximal colon; and thirty-five from the distal colon. Each sample was matched with the distant non-neoplastic mucosa removed during the same surgery, usually 15 to 20 cm away from the border of the main tumor lesion. Only in 11 cases was the so-called transitional mucosa, that is the mucosa closer (less than 3 cm) to the tumor mass, available for analysis. All patients were selected at their first diagnosis; and none had received chemotherapy or radiation therapy before resection, or referred associated with IBD. CRCs were classified according to International Union Against Cancer criteria and were recorded as adenocarcinoma with a variable degree of differentiation. Staging at the time of diagnosis was based on the tumor-nodes-metastasis system. A median follow-up for 10 months (2-24 months) was applied to all 45 patients who received conventional postoperative treatment.

3.2. Immunohistochemistry

Serial 4-μm sections were mounted on poly-L-lysine-coated slides, de-paraffinized, rehydrated, and microwaved for 15 minutes at high power in 10 mmol/L citrate buffer (pH 6.0) to unmask the epitopes. Endogenous peroxidase was quenched using 3% H_2O_2 for 20 minutes. Slides were then washed in phosphate-buffered saline (pH 7.5) and incubated with the corresponding antibodies containing 5% normal bovine blocking serum in phosphate-buffered saline. The primary antibodies used were anti–COX-2 antibody (Dako Cytomation, Glostrup, Denmark) for 2 hours at room temperature 1:200 and anti–β-catenin antibody (Dako Cytomation, Glostrup, Denmark) for 2 hours at 37°C 1:200. A standard labeled streptavidin-biotin-peroxidase complex (LSAB+ System-HRP kit; Dako Cytomation, Glostrup, Denmark) was used to amplify the immunoreaction. Tissues were stained for 5 minutes with DAB (3,3'-diaminobenzidine) chromogen and counterstained with Meyer's hematoxylin, dehydrated, and cover-slipped. Each experiment was performed in duplicate. Primary antibodies were omitted in negative controls. Sections from breast carcinoma immunostained with β-catenin; and sections from colon cancer with strong immunostaining for Cox-2 were used as positive controls.

3.3. Evaluation of Immunohistochemistry

Immunostaining for each marker was graded by a semi-quantitative method based on a scale that takes into account the intensity and distribution of the staining. The intensity was scored as follows: 1 (weak), 2 (moderate), or 3 (strong). The percentage of positive cells was scored as follows: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), or 4 (76-100%). The 2 scores were combined to obtain the final one: negative (0-2), weakly positive (3-5), or strongly positive (6-7). For statistical analysis, positive cases include both weakly and strongly positive. Membrane, cytoplasm, and/or nuclear signal localization was independently evaluated using light microscopes (Olympus, CX31 binuclear) and classified for each marker.

3.4. Statistical Analysis

Data were analyzed by using SPSS software. Association between the expression levels of the proteins investigated and the clinicopathological parameters was assessed using the $\chi 2$ test and Fisher exact test. The P-value < 0.05 was considered to be significant.

4. Results

Table 1. Clinico-pathological data

Item	No.	%
Age	35-80	Mean: 57.5 SD: 10.2
Sex: Male Female	 34 11	 75.55% 24.44%
Total	45	100%
Histopathological diagnosis: Well-differentiated Moderately-differentiated Poorly- differentiated	 13 30 2	 28.89% 66.66% 4.44%
Total	45	100%
Depth of invasion: Submucosa Muscularis propria Serosa	 6 37 2	 13.33% 82.22% 4.44%
Total	45	100%
Lymph node invasion	11	24.44%
Distant metastasis	10	22.22%
Dukes stages : A B C D	 4 20 11 10	 8.88% 44.44% 24.44% 22.22%
Total	45	100%

4.1. Clinicopathological Results

Patients' age ranged from 35 to 80 years, with a mean of 57.5 years. Sex composition was 34 men and 11 women. Thirteen cases were well-differentiated; 30 cases were moderately differentiated; and only 2 cases were poorly differentiated adenocarcinoma. Six tumors invaded the submucosa; 37 invaded the muscularis propria; and 2 invaded the serosa and the adjacent organs. Eleven patients had regional lymph node invasion, whereas 34 were negative. Distant metastases were found in 10 patients. According to Duke' s classification [13], stages A, B, C, and D were detected in 4, 20, 11, and 10 patients, respectively (Table 1).

4.2. Immunohistochemical Results

In the adjacent non-neoplastic colonic mucosa, β-catenin was distributed between the cell membrane and the cytoplasm. In 34 cases (75.56%) of the tumor samples; similar or increased protein level were predominantly found. In 27 cases of them (79.4 %), there were increased protein levels in the cytoplasm and/or membrane compartment (C/M) (Figs 1, 2). In 7 cases (15.56%), the protein was essentially localized in the nucleus (N) (Fig 3). In 11 cases (24.44%), the protein was not detected (Fig 4). In the non-neoplastic mucosa adjacent to the tumor mass, negative immunostaining reaction for COX-2 was observed. In tumor specimens, COX-2 immunoreactivity was weakly and/or strongly positive in 32 cases (71.11%) (Fig 5), and negative in 13 cases (28.89%). Positivity was detected in the cytoplasm and in the perinuclear area. Using the F test, (2-tailed); increased expression of β-catenin was correlated to Duke staging (P =0.009) (Table 2). Furthermore, nuclear β-catenin localization showed an association to the Duke stage (P =0.002) and insignificant association with distant metastases (P =0.33) compared with the group with cytoplasmic/membrane localization (Table 2). Positive COX-2 expression showed a significant relation to, liver metastases (P = 0.04), and Duke staging (P =0.011) and insignificant association to lymph node invasion (P =0.25) (Table 3). These data indicate that cytoplasm/membrane β-catenin over-expression and positive COX-2 expression are related to a more aggressive behavior of the disease; most patients passed the period of 10-months survival except 5 patients; 2 of whom with stage C, and 3 with cancer stage D who died at the end of the 10th month.

Table 2. Association between β-catenin expression, and some clinicopathological parameters

Parameters	β-catenin score						Localization					
	Positive		Negative		Total	P-value	C/M		N		Total	*P*-value
Depth of invasion	No.	%	No.	%			No.	%	No.	%		
pT1	2	33.3	4	66.7	6		2	50	2	50	4	
pT2	1	25	3	75	4		1	25	3	75	4	
pT3	29	87.9	4	12.1	33	0.002	23	92	2	8	25	0.007
pT4	2	100	0	0	2		1	100	0	0	1	
Total	34		11		45		27		7		34	
Lymph node metastasis												
Absent	28	82.4	6	17.6	34		18	78.3	5	21.7	23	
Present	6	54.5	5	45.5	11	0.103	9	81.8	2	18.2	11	0.83
Total	34		11		45		27		7		34	
Distant metastases												
Absent	30	78.9	8	21.1	38		21	77.8	6	22.2	27	
Present	4	57.1	3	42.9	7	0.336	6	85.7	1	14.3	7	0.95
Total	34		11		45		27		7		34	
Duke stage												
A	1	25	3	75	4		0	0.00	1	100	1	
B	13	65	7	35	20		10	80	3	20	13	
C	10	90.9	1	9.1	11	0.009	10	100	0	0.00	10	0.002
D	10	100	0	0.00	10		7	100	3	0.00	10	
Total	34		11		45		27		7		34	

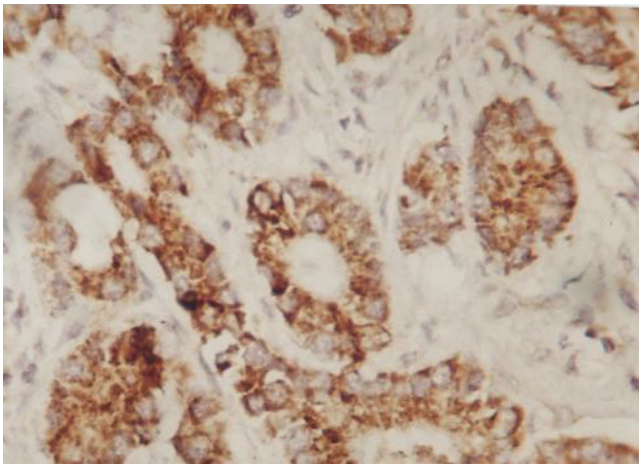

Figure 1. Photomicrograph of a case of moderately differentiated adenocarcinoma of the colon; stained with anti β-catenin immunohistochemical staining (strong cytoplasmic and membranous reaction) (Meyer`s hematoxylin counter stain, 400 X)

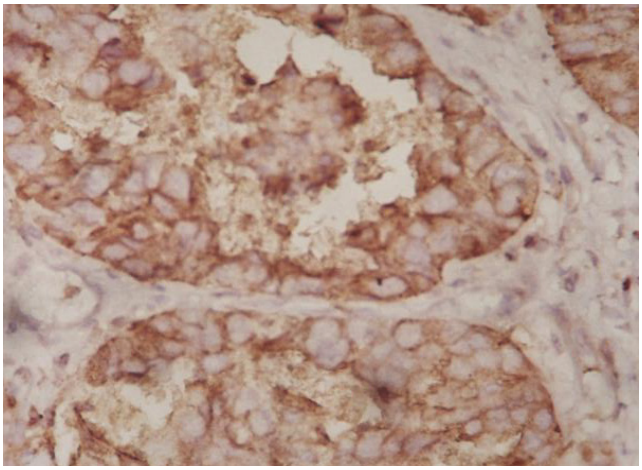

Figure 2. Photomicrograph of a case of moderately differentiated adenocarcinoma of the colon; stained with anti β-catenin immunohistochemical staining (strong membranous reaction) (Meyer`s hematoxylin counter stain, 400 X)

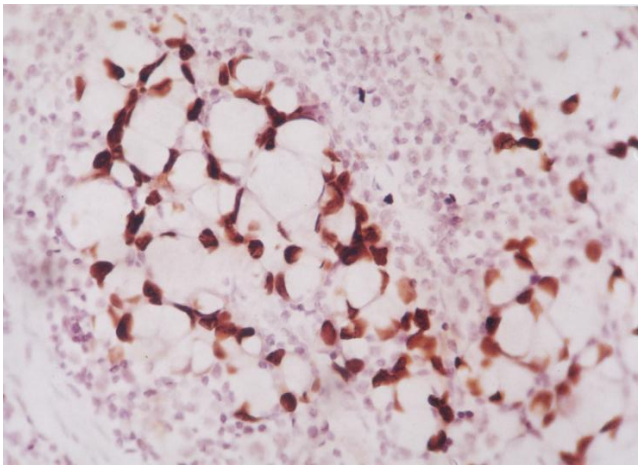

Figure 3. Photomicrograph of a case of moderately differentiated adenocarcinoma of the colon (with mucinous changes); stained with anti β-catenin immunohistochemical staining (strong nuclear reaction) (Meyer`s hematoxylin counter stain, 400 X)

Table 3. Association between COX-2 expression, and some clinico-pathologic parameters

Parameters	COX-2					
	Positive		Negative		Total	*P*-value
Depth of invasion	No.	%	No.	%		
pT1	2	33.3	4	66.7	6	0.011
pT2	1	25	3	75	4	
pT3	27	81.8	6	18.2	33	
pT4	2	100	0	0.00	2	
Total	32	71.1	13	28.9	45	
Lymph node metastasis						
Absent	26	76.5	8	23.5	34	0.251
Present	6	54.5	5	45.5	11	
Total	32	71.1	13	28.9	45	
Distant metastases						
Absent	22	57.9	13	34.2	38	0.042
Present	10	100	0	0.00	10	
Total	32	71.1	13	28.9	45	
Duke stage						
A	0	0.00	4	100	4	0.011
B	15	75	5	25	20	
C	9	81.8	2	18.2	11	
D	8	80	2	20.0	10	
Total	32	71.1	13	28.9	45	

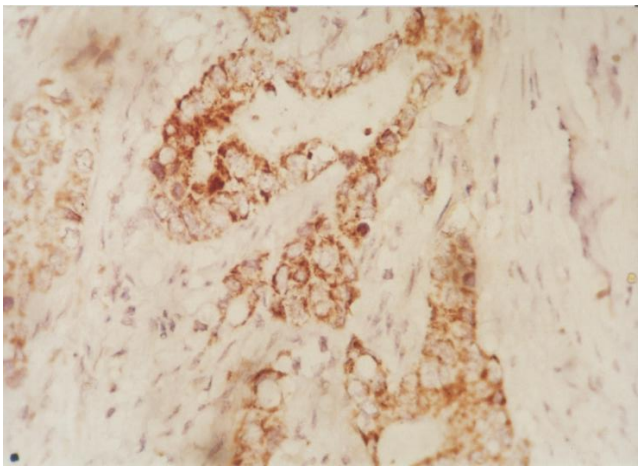

Figure 4. Photomicrograph of a case of moderately differentiated adenocarcinoma of the colon; stained with anti β-catenin immunohistochemical staining (weak cytoplasmic and membranous reaction) (Meyer`s hematoxylin counter stain, 400 X)

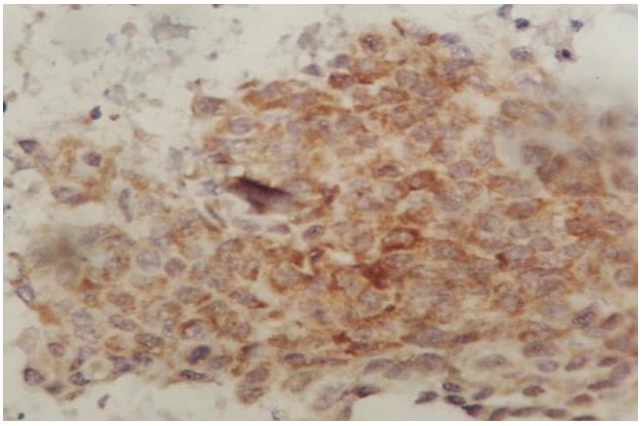

Figure 5. Photomicrograph of a case of poorly-differentiated adenocarcinoma of the colon; stained with anti-COX-2 immunohistochemical staining (weak cytoplasmic and membranous reaction) (Meyer`s hematoxylin counter stain, 400 X)

5. Discussion

This study investigated the expression profile of β-catenin and COX-2 in colonic cancer and tumor-genesis in a series of sporadic CRCs. We reported here a significant correlation between β-catenin and increased COX-2 levels, and tumor metastatic progression. β-catenin is a component of the Wnt signaling pathway which controls the specification, maintenance and activation of intestinal stem/progenitor cells. Deregulation of this pathway due to either genetic or epigenetic defects can potentially result in the development of familial and/or sporadic epithelial cancers [14]. The functional versatility of Wnt/β-catenin signaling are seen through its action in cancer stem cells. These cells require β-catenin in mediating the response to Wnt signaling for specification of different tissues [15]. The cellular β-catenin resides mainly at sites of intercellular (adherens) junctions where it interacts with E-cadherin and α- catenin. In the absence of Wnt signal, the cytoplasmic pool of β- catenin is limited and undergoes rapid degradation. Concomitantly, Wnt signaling promotes inactivation of glycogen synthase kinase-3 beta (GSK3β) causing β- catenin to accumulate in the cytoplasm. Stabilized β-catenin interacts with transcription factors of the T-cell factor/lymphoid enhancing factor (TCF/LEF) family to form a bipartite complex [16,17]. TCF/LEF provide the DNA-binding specificity, while β-catenin provides trans-activation domains. This bipartite complex then leads to the expression of Wnt target genes, such as c-myc and cyclin D1, well known for their role in cell proliferation and oncogenesis [12]. β-catenin participates in trans-activation by recruiting two other transcriptional co-factors, CBP/p300 acetyltransferase and the chromatin-remodeling protein Brg-1, to TCF target gene promoters [11,12]. Nuclear accumulation of β-catenin is considered a hallmark of activated canonical Wnt signaling. Recent studies in rodents illuminate the role of canonical Wnt signaling in proliferation of normal intestinal epithelium during development and adult stages of life [18]. β-catenin expression was elevated in about 79.4% of the cases; only in about 20 % of which was the protein predominantly located in the nucleus. This result mostly agreed with the studies reporting that mutations of the Wnt/β-catenin pathway and consequent accumulation of β-catenin in the nucleus were found in 60% - 80% of sporadic CRCs [1].

COX-2 expression was also monitored in the current study because it is a key enzyme in the production of prostaglandins, hence activating the inflammatory response. In IBDs, in addition, over-expression of the inducible isoform COX-2 has been shown to occur at multiple stages of colon carcinogenesis allowing for elevated prostaglandin synthesis to occur in the tumor microenvironment [19]. Negative COX-2 was noticed to be significantly associated with very low, if any, distant metastases. Those with high β-catenin expression and elevated COX-2 showed more frequent distant metastases and Duke C and D. A similar relationship was also observed in the cases with nuclear β-catenin accumulation. An increase of COX-2 in the distant non neoplastic mucosa, the transitional mucosa and primary tumor was found in patients with liver metastases. A key role in this process is thought to be played by Wnt-5a, a member of the Wnt family of secreted growth factors, which regulate tumor cell migration and macrophage proteolytic activity [20]. The role of Wnt-5a and other members of the non-canonical Wnt pathway in cancer progression is, however, still debated [21]. In summary, the data presented here indicate that both COX-2 and β-catenin play a pivotal role in the genesis of colorectal cancers. Nevertheless, a direct link between these two key pathways has remained elusive. Previous reports showed that one of the bioactive products of COX-2, prostaglandin E2, activates components of the canonical Wnt signaling system [22]. On the other hand, there is an observation that accumulation of β-catenin induces COX-2 expression in articular chondrocytes [23]. Kazem et al. suggested the use of both β-catenin and COX-2 as a marker of tumor progression and poor prognosis [24]. Some epidemiologic studies suggested that anti-inflammatory drugs (NSAIDs) have chemopreventive effects and reduce the incidence of mortality from GIT cancers [17].

One potential limitation of the study is the fact that we were not able to look for so large number of tissue samples, as the enrolled 45 patients were the available cases in our institute along the period of the study. This might reflect a relatively low incidence of CRCs in our region, that represent a leading cause of death in the Western world [25]. There are many predisposing factors for such cases [3]. Dietary habits among populations, such as high levels of saturated fats have been implicated in occurrence of CRCs [26]. Recently, it has been suggested that there is a potential value of phytic acid extracted from rice bran in reducing colonic cancer risk in rats [27]. Observations of the expression patterns of the oncogenic genes may only represent a starting point for a thorough investigation of the functions of these genes in pathogenesis of CRCs.

6. Conclusion

The study indicated that cytoplasmic/membrane β-catenin over-expressions as well as positive COX-2 expressions are associated with a more aggressive behavior of the disease. However, it is still controversial, regarding whether Wnt/ β-catenin is the target for expression of COX-2 or the reverse. More future studies including larger numbers of patients and in correlation with therapy are recommended.

Conflict of Interest

The authors declare that no conflicts of interest exist.

REFERENCES

[1] S. Narayan, D. Roy. Role of APC and DNA mismatch repair genes in the development of colorectal cancers. Mol Cancer, Vol. 2,41-55, 2003.

[2] J. D. Potter. Colorectal cancer: molecules and populations. J Natl Cancer Inst, Vol. 91, No. 11,916-932, 1999.

[3] B. Young, J. S. Lowe, A. Stevens, et al. Wheater' s Functional Histology: A Text and Colour Atlas. 5th ed., Churchill, Livingston. & Elsevier. 284-285, 2006.

[4] A. de la Chapelle. Genetic predisposition to colorectal cancer. Nat Rev Cancer, Vol. 4, No. 10,769-780, 2004.

[5] R. J. Feezor, E. M. Copeland, S. N. Hochwald. Significance of micrometastases in colorectal cancer. Ann Surg Oncol, Vol. 9, No. 10,944-953, 2002.

[6] K. W. Kinzler, B. Vogelstein. Lessons from hereditary colorectal cancer. Cell, Vol. 87, No. 2,159-170, 1996.

[7] E. Pikarsky, R. M. Porat, I. Stein, et al. NF-kB functions as a tumour promoter in inflammation-associated cancer. Nature, Vol. 431,461-466, 2004.

[8] M. Oshima, J. Dinchuk, S. L. Kargman, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell, Vol. 87, No. 5,803-809, 1996.

[9] C. Pohl, A. Hombach, W. Kruis. Chronic inflammatory bowel disease and cancer. Hepatogastroenterology, Vol. 47, No. 31,57-70, 2000.

[10] E. Shacter, S. A. Weitzman. Chronic inflammation and cancer. Oncology (Huntingt), Vol. 16, No. 2,217-226, 2002.

[11] J. Dimberg, A. Samuelsson, A. Hugander, et al. DiVerential expression of cyclooxygenase 2 in human colorectal cancer. Gut, Vol. 45,730–732, 1999.

[12] T. C. He, A. B. Sparks, C. Rago, et al. Identification of c-MYC as a target of the APC pathway. Science, Vol. 281, No. 5382,1509–1512, 1998.

[13] C. E. Dukes, H. J. R. Bussey. The spread of rectal cancer and its effect on prognosis. Br J Cancer, Vol. 12, No. 3,309-320, 1958. Online available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2073915/pdf/brjcancer00372-0007.pdf

[14] M. Peifer, P. Polakis. Wnt signaling in oncogenesis and embryogenesis. A look outside the nucleus. Science, Vol. 287, No. 5458,1606-1609, 2000.

[15] J. D. Holland, A. Klaus, A. N. Garratt, et al. Wnt signaling in stem and cancer stem cells. Current Opinion in Cell Biology, Vol. 25, No. 2,254–264, 2013.

[16] K. I. Takemaru, R. T. Moon. The transcriptional coactivator CBP interacts with beta-catenin to activate gene expression. J Cell Biol, Vol. 149, No. 2,249–254, 2000.

[17] S. S. Husain, I. L. Szabo, A. S. Tarnawski. NSAID inhibition of GI cancer growth: clinical implications and molecular mechanisms of action. Am J Gastroenterol, Vol. 97,542-553, 2002.

[18] L. G. Van der Flier, J. Sabates-Bellver, I. Oving, et al. The intestinal Wnt/TCF Signature. Gastroenterol, Vol. 132, No. 2, 628–632, 2007.

[19] G. Thyssen, T. H. Li, L. Lehmann, et al. LZTS2 is a novel β-catenin–interacting protein and regulates the nuclear export of β-catenin. Mol Cell Biol, Vol. 26, No. 23,8857-8867, 2006.

[20] D. Wang, J. R. Mann, R. N. Dubois. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. Gastroenterology, Vol. 128, No. 5,1445-1461, 2005.

[21] T. Pukrop, C. Binder. The complex pathway of Wnt-5a in cancer progression. J Mol Med, Vol. 86, No. 3,259-266, 2008.

[22] F. G. Buchanan, R. N. Dubois. Connecting COX-2 and Wnt in cancer. Cancer Cell, Vol. 9, No. 1,6-8, 2006.

[23] S. J. Kim, D. S. Im, S. H. Kim, et al. β-Catenin regulates expression of cyclooxygenase-2 in articular chondrocyte. Biochemical and Biophysical Research Communications, Vol. 296,221–226, 2002.

[24] A. Kazem, K. El Sayed, Y. El Kerm. Prognostic significance of COX-2 and β-Catenin in colorectal carcinoma. Alexandria Journal of Medicine, 2013, Online available from: http://ac.els-cdn.com/S2090506813000559/1-s2.0-S2090506813000559-main.pdf?_tid=0fed2a14-189f-11e3-a770-00000aacb362&acdnat=1378655901_b3ecbd81574b5a7e07a7075e8389f033

[25] R. S. Snell. Clinical Anatomy by Regions, 9th ed., Lippincott Williams & Wilkins, 180-206. 2012.

[26] S. S. Mader. Understanding human anatomy and physiology, 5th ed., The McGraw-Hill Companions, 16-30. 2004.

[27] N. Saad, N. M. Esa, H. Ithnin. Suppression of β-catenin and Cyclooxygenase-2 Expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA). Asian Pac J Cancer Prev, Vol. 14,No 5,:3093-9, 2013.