Clinics of Oncology

Research Article ISSN: 2640-1037 | Volume 7

The Role of Crypto-1 in Progression of Inflammatory Bowel Disease to Colorectal Cancer

Abgir AH[1], Tavassoli A[2], Miladpour B[3*], Hosseini SV[4] and Goodarzi A[5]

[1]Department of Clinical Biochemistry, Fasa University of Medical Sciences, Fasa, Iran

[2]Department of Pathology, Fasa University of Medical Sciences, Fasa, Iran

[3]Clinical Biochemistry, Department of Clinical Biochemistry, Fasa University of Medical Sciences, Fasa, Iran

[4]General Surgery, Colorectal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[5]Department of Tissue Engineering, School of Advanced Technologies in Medical Sciences, Fasa University of Medical Sciences, Fasa, Iran

[*]Corresponding author:

Behnoosh Miladpour,
Clinical Biochemistry, Department of Clinical Biochemistry, Fasa University of Medical Sciences, Fasa, Iran

Received: 02 Jan 2024
Accepted: 06 Feb 2024
Published: 12 Feb 2024
J Short Name: COO

Keywords:

Colorectal cancer; Crypto-1 (Cr-1); Inflammation bowel disease (IBD); β-catenin

Citation:

Miladpour B, The Role of Crypto-1 in Progression of Inflammatory Bowel Disease to Colorectal Cancer. Clin Onco. 2024; 7(9): 1-10

1. Abstract

1.1. Background: Chronic inflammation leads to the progression of many diseases, including colorectal cancer (CRC). Crypto-1 (Cr-1) and β-catenin markers are involved in the Wnt signaling pathway. Increasing the concentration of NOS-2 and FGF-2 plays an important role in regulating the cell cycle and causing tumors. We aimed to determine the importance of Cr-1, β-catenin, NOS-2, FGF-2, and PGE-2 biomarkers in the process of inflammatory bowel disease progressing to colorectal cancer.

1.2. Materials and Methods: We had three groups: 30 patients with IBD dysplasia (UC), 25 patients with CAC, and 30 patients with CRC. Immunohistochemistry (IHC) was used to determine the expression of Cr-1, β-catenin and E-cadherin. PGE-2, NOS-2 and FGF-2, and Cr-1 gene expressions were analyzed with Real-time PCR.

1.3. Results: The outcomes in IHC showed that in IBD, Cr-1 was not present in the membrane, but it was present in the cytoplasm. While in CAC and CRC, Cr-1 presents in both membrane and cytoplasm. Also, β-catenin was not present in the nucleus of IBD tissue, but it was present in the nucleus of CAC and CRC tissues. Real-time – PCR confirms the results from IHC for Cr-1. FGF-2 and PGE-2 had higher expression in CAC and CRC than IBD, and NOS-2 had higher expression in CRC than IBD and CAC.

1.4. Conclusion In progressing from IBD to CAC, Cr-1 appears in the membrane in addition to the cytoplasm. Also, β-catenin presents in the nucleus in CAC and CRC while it is not seen in the nucleus in IBD.

2. Introduction

Chronic inflammation can lead to the development of many diseases, including colorectal cancer (CRC) [1, 2]. CRC is the third most common cancer in the world and the fourth leading cause of death. It is also the third leading cause of cancer in men and the second leading cause of cancer in women [3]. Ulcerative colitis (UC) and Crohn's disease (CD) are the two main types of inflammatory bowel disease (IBD) that can spread to the entire gastrointestinal tract [4]. Intermediate between IBD and CRC, colitis-associated cancer (CAC), was first discovered by Herman Rosenberg and Burrill Crohn in 1925 [5]. The molecular mechanisms that exist between these two diseases remain unknown [6]. In a study, Cr-1 was introduced as a new diagnostic marker and therapeutic target for CRC [7] . Cr-1 is present in lipid rafts in the plasma membrane and is attached to the glycosylphosphatidylinositol (GPI) anchor [8]. This protein is one of the main members of the epidermal growth factor-Cripto-1/FRL1/Cryptic (EGF-CFC) family of proteins that is essential for the early development of vertebrates [9]. TadahiroNagaoka et al. reported that Cr-1 increases the canonical Wn-

t/β-catenin signaling pathway through binding to LRP5 and LRP6 receptors [10].Also, its high expression level was associated with lung cancer, liver cancer, glioblastoma, and breast cancer [11-14]. Another study discussed the role of the Wnt/ β-catenin signaling pathway in the process of inflammatory bowel disease [15]. It has been shown that mutated β-catenin or Wnt activation leads to the accumulation of β-catenin in the cytoplasm, thereby translocating into the nucleus and interacting with TCF/LEF, leading to tumor progression [16, 17]. In addition, β-catenin is essential for cell adhesion and can be essential for the adhesion function of E-cadherin (epithelial markers), tight adhesion between epithelial cells, and inhibition of cell mobility [18, 19]. So that the disruption of E-cadherin and β-catenin may cause the nuclear transfer of β-catenin and induce the transcription of Epithelial-mesenchymal transition (EMT) stimulating gene which play an effective role in cancer progression.[20] Therefore, in the process of EMT, the expression and function of E-cadherin (epithelial markers) is disturbed. [21]. Also, in a study on IBD, it was reported that changes in E-cadherin expression lead to increased cell migration during epithelial regeneration of the gastrointestinal mucosa [22]. Lai Y et al. reported that increased COX-2 is associated with β-catenin accumulation [23]. Furthermore, activation of β-catenin induces the expression of cyclooxygenase-2 (COX-2) [24]. COX-2 converts arachidonic acid into prostaglandins, among which prostaglandin E2 (PGE-2) leads to tumor growth by binding to its own receptor [25]. Prostaglandin E2 (PGE-2) increases the invasion of CRC cells by increasing β-catenin tyrosine phosphorylation [26]. Also, COX-2/PGE-2 controls β-catenin by regulating GSK3β activity [27]. In a study, it was reported that β-catenin leads to the increase of nitric oxide synthase-2 (NOS-2), which can be a target gene for the Wnt signaling pathway[28] .It was also investigated in two studies that NOS-2 expression was increased in both human colon tissue and IBD tissues [29, 30]. Also, Silvio et al. showed that FGF-2 can be a target gene for β-catenin and play a role in the development of CRC tumor cells [31]. In this study, our aim is to investigate the expression of Cr-1, COX-2/PGE-2, β-catenin, E-cadherin, FGF2 and NOS-2 genes in the progression of inflammatory bowel disease towards colitis-associated cancer (CAC).

3. Material and Method

The study was performed at Fasa University of Medical Sciences from June 2021 to November 2022. We collected paraffin block tissues from patients with IBD, CAC, CRC, and healthy people as control, demographic information is presented in table 1. The research committee approved all experimental procedures Code 99227, Ethical code: IR.FUMS.REC.1400.051.one hundred people participated in the study; all participants signed the consent form. Healthy subjects with diarrhea and abdominal pain who presented to the clinic with no history of cancer and no pathology reports as inflammation were considered as controls. Including criteria were individuals with IBD, CAC, and CRC. Excluding criteria was any history of other kinds of cancers, immunodeficiency, or inflammatory diseases. The samples were divided into four groups: control, IBD (UC), CAC, and CRC.

Table 1: Clinic pathologic features of the IBD (UC), CAC and CRC cases studied.

		Control	IBD (UC)	CAC	CRC
Age	<50	14 (93.4%)	25(83.4%)	23 (92.0%)	26 (86.6%)
	>50	1 (6.6%)	5 (16.6%)	2 (8.0%)	4 (13.4%)
Sex	Male	9 (60%)	16 (53.3%)	12 (48.0%)	14 (46.6%)
	Female	6 (40%)	14 (46.7%)	13 (52.0%)	16 (53.4%)
Site	Rectum	0 (0%)	5 (16.6%)	2 (8.0%)	6 (20.0%)
	Colon	12 (80%)	23 (76.7%)	10 (40.0%)	16 (53.4%)
	Colon and rectum	3 (20%)	2 (6.07%)	13 (52.0%)	8 (26.6%)
Type	Adenocarcinoma	----	----	25 (100%)	30 (100%)
Grade	Well	----	----	20 (80.0%)	26 (86.6%)
	moderate	----	----	5 (20.0%)	4 (13.4%)
Stage	IIA	----	Moderate:9 (30.0%)	5 (20.0%)	7 (23.4%)
	IIIC	----	Severe:21 (70.0%)	6 (24.0%)	11 (36.6%)
	IIIB	----		9 (36.0%)	8 (26.6%)
	IVA	----		5 (20.0%)	4 (13.4%)

3.1. Assessment of Protein Expression

In tumor tissues and inflammatory tissues, Cr-1, E-cadherin as a cytoplasmic and membrane marker and B-catenin as a cytoplasmic, membrane and nuclear marker were stained. To evaluate the immunohistochemical staining of Cr-1, β-catenin and E-cadherin, we evaluated the staining intensity of positive cells visible in the microscopic field, while the intensity score indicated the average staining intensity (0 – negative, 1 – weak, 2 – intermediate, and 3 – strong)

3.2. RNA Extraction and Real-time PCR Analysis

To analyze the gene expression levels of Cr-1, Cox-2, PGE-2, MMP-2, NOS-2, FGF-2 in IBD (UC), CAC and CRC tissues, total RNA were extracted using TRI Reagentm Sigma Aldtichm, Switzerland. cDNA synthesis was performed by cDNA synthesis kit (addbio, Korea). Expression levels of the genes were calculated according to ΔΔCt-2, which were normalized by GAPDH. The sequences of primers were used are shown in table 2.

Table 2: The sequences of primers used in Real-time PCR.

Gene name	Forward primer	Reverse primer
Cr-1	5'- GATACAGCACAGTAAGGAGC -3'	5'- TAGTTCTGGAGTCCTGGAAG-3'
NOS-2	5'- TCCGAGGCAAACAGCACATTC -3'	5'- GGGTTGGGGGTGTGGTGATGT-3'
PGE-2	5'- CTTACCTGCAGCTGTACG-3'	5'- GATGGCAAAGACCCAAGG-3'
FGF-2	5'- AGGAGAGCGACCCACACATCAA-3'	5'- AGCCAGCAGTCTTCCATCTTCC-3'
GAPDH	5'- CCAGGTGGTCTCCTCTGACTT-3'	5'- GTTGCTGTAGCCAAATTCGTTGT-3'

3.3. Immunohistochemistry (IHC)

Paraffin tissues were obtained from the Pathology Department of Faqihi Hospital, Shiraz University of Medical Sciences. Three-micron slices were taken from paraffin blocks and mounted on glass slides using 3-aminopropyltrimethoxysilane. Before immunohistochemically staining, the sections were deparaffinized by the standard method explained in the procedure of the favor gene kit. Briefly, tissues were fixed with absolute ethanol (40 s), then Tris buffer was used for 20 s, primary antibodies: anti-Ecadherin (ZYTOMED 3ML, German), anti-β-catenin (zeta 3ML, USA), Vimentin, clone V 9 (ZYTOMED 3ML, German) and anti-Cripto-1(TDGF-1, 0/5mg/ml), Secondary antibody (20 min), DAB was used as chromogen and hematoxylin for 1 min. PBS was used as a washing solution, according to the master polymer plus Detection System (Peroxidase), (lncl. DAB chromogen, LOT.237QK100042) kit.

3.4. Statistical Analysis

Data were presented as frequency and percentage for categorical variables and mean and median and standard deviation for quantitative variables. Comparison of gene expression between study groups was done using a nonparametric Mann-/Whitney test because of not normal distribution. Statistical analysis was performed via IBM SPSS version 26 (IBM SPSS co, Redmond, WA). A P-value less than 0.05 were considered a statistically significant level. *30 patients with IBD (UC), 25 patients with CAC, 30 patients with CRC and 15 with control were included in the present study. Histopathological examination of tissue samples showed in table 3.*

4. Results

4.1. Immunohistochemistry (IHC) Analysis

4.1.1. Cr-1: The presence of Cr-1 in the tissue samples of patients with IBD (UC) (n=30), CRC (n=30), CAC (n=25) and control (n=15) was investigated by immunohistochemical method. The results showed that Cr-1 in IBD, 53.4% is present in cytoplasm with grade +3 and 76.7% in membrane with grade 0. in CAC, 68% in cytoplasm and 64% in membrane with grade +3. in CRC 73.4% in cytoplasm and 76.6% in membrane with grade +3 and in control samples observed 93.4% in membrane with grade 0 and 46.6% in cytoplasm with grade +2 .showed that presentation of Cr-1 increased in membrane from IBD to CAC (Table 3).

4.1.2. B-catenin: Immunohistochemical analysis showed the presence of β-catenin in the cytoplasm of IBD (UC) tissues, 90% with grade +2, in CAC and CRC 92% and 80% with grade +2 respectively. in the membrane of IBD tissues, 80% with grade +3, in CAC and CRC tissues, 48% and 66.6% with grade +3 were present respectively. The nuclear level of β-catenin was present in 56.6% and 33.4% of CRC tissues with grade +2 and +3 respectively. In CAC nuclei, B-catenin present in 32% and 48% with grades +2 and +3 respectively. In IBD tissues, 96.6% with grade 0 and absent in IBD (UC) nuclei. in control, 100% in nucleus with grade 0 ,86.6% in membrane with grade +3 and 93.4% with grade 0 in cytoplasm (Table 3).

Table 3: Immunohistochemistry of different markers presentation in membrane, cytoplasm and nucleus in IBD (UC), CAC and CRC. The scores of 0, 1, 2, 3 shows the degrees of the markers presentation.

CRC	CAC	IBD (UC)	Control	Score		
N (%)	N (%)	N (%)	N (%)			
0 (0%)	0 (0%)	0 (0%)	3 (20.0%)	0	**Cytoplasmic**	Cr-1
2 (6.6%)	2 (8.0%)	5 (16.6%)	5 (33.4%)	1+		
6 (20.0%)	6 (24.0%)	9 (30.0%)	7 (46.6%)	2+		
22 (73.4%)	17 (68.0%)	16 (53.4%)	0 (0%)	3+		
0 (0%)	1 (4.0%)	23 (76.7%)	14 (93.4%)	0	**Membrane**	
0 (0%)	0 (0%)	5 (16.6%)	1 (6.6%)	1+		
7 (23.4%)	8 (32.0%)	2 (6.7%)	0 (0%)	2+		
23 (76.6%)	16 (64.0%)	0 (0%)	0 (0%)	3+		
1 (3.4%)	3 (12.0%)	29 (96.6%)	15 (100%)	0	**Nucleus**	B-catenin
2 (6.6%)	2 (8.0%)	1 (3.4%)	0 (0%)	1+		
17 (56.6%)	8 (32.0%)	0 (0%)	0 (0%)	2+		
10 (33.4%)	12 (48.0%)	0 (0%)	0 (0%)	3+		
0 (0%)	0 (0%)	0 (0%)	14 (93.4%)	0	**Cytoplasmic**	
6 (20.0%)	2 (8.0%)	3 (10.0%)	1 (6.6%)	1+		
24 (80.0%)	23 (92.0%)	27 (90.0%)	0 (0%)	2+		
0 (0%)	0 (0%)	0 (0%)	0 (0%)	3+		
0 (0%)	0 (0.0%)	1 (3.3%)	0 (0%)	0	**Membrane**	
3 (10.0%)	4 (16.0%)	4 (13.4%)	0 (0%)	1+		
7 (23.4%)	9 (36.0%)	2 (6.6%)	2 (13.4%)	2+		
20 (66.6%)	12 (48.0%)	23 (76.6%)	13 (86.6%)	3+		
0 (0%)	0 (0%)	0 (0%)	0 (0%)	0	**Cytoplasmic**	E-cadherin
2 (6.6%)	0 (0%)	0 (0%)	13 (86.6%)	1+		
28 (93.4%)	25 (100%)	30 (100%)	2 (13.4%)	2+		
0 (0%)	0 (0%)	0 (0%)	1 (6.6%)	3+		
0 (0%)	0 (0%)	0 (0%)	0 (0%)	0	**Membrane**	
0 (0%)	0 (0%)	0 (0%)	0 (0%)	1+		
3 (10.0%)	0 (0%)	0 (0%)	1 (6.6%)	2+		
27 (90.0%)	25 (100%)	30 (100%)	14 (93.4%)	3+		

4.2. Analysis of E-cadherin immunoreactivity

E-cadherin protein was present in the membrane of IBD (UC) and CAC tissues, 100% with grade +3 and in the cytoplasm of IBD and CAC tissues, 100% with grade +2. In the cytoplasm of CRC tissues, 93.4% with grade +2 were presented .in the membrane of CRC, 86.6% were present with grade +3. In the E-cadherin samples control, 93.4% in membrane with grade +3 and 93.4% with grade +2 (Table 3).

4.3. Real-time PCR

Total RNA samples were extracted from IBD (UC) (n=20), CAC (n=18) and CRC (n=22) tissue samples to evaluate the expression level of Cr-1, PGE-2, FGF-2, and NOS-2 genes using Real- Time PCR method. The mRNA levels of the mentioned genes were measured in triplicates; the expression was normalized according to the GAPDH, Figure 1-3.

The expression level of Cr-1 was significantly lower in IBD (UC) patients compared with CRC (P=0.043), despite the level of Cr-1 gene expression was higher in CRC than CAC, the difference was not significant. The expression level of FGF-2 gene was significantly lower in IBD (UC) samples than CAC (P=0.002), also insignificant lower in IBD (UC) than CRC samples (P=0.202). An increase in PGE-2 gene expression level was observed in CRC (P<0.001) and CAC (P<0.001) than IBD (UC) samples. While NOS-2 gene expression was higher expression in IBD (UC) than CAC (P<0.001) also in CRC was higher than IBD (UC) and CAC (P<0.001 for both).

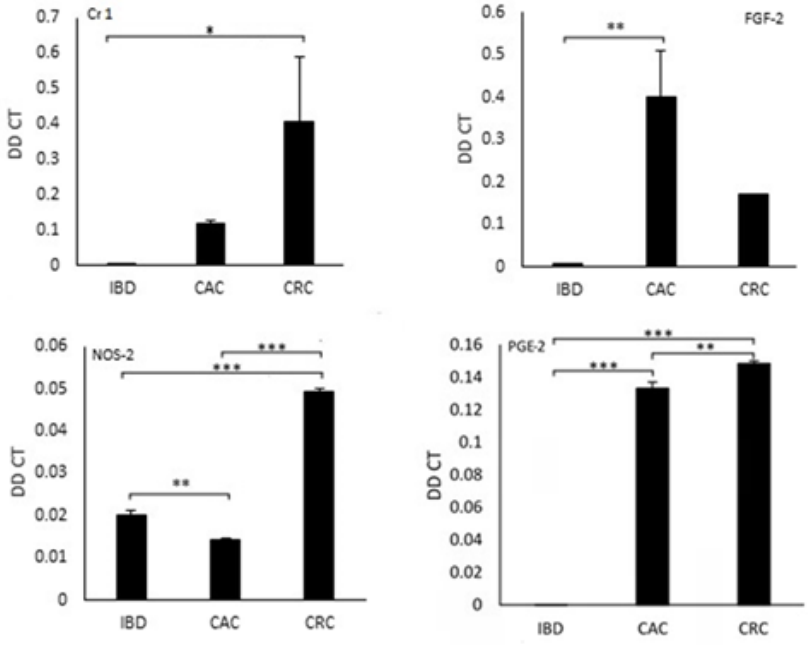

Figure 1: Gene expression of Cr-1, FGF-2, NOS-2 and PGE-2 in inflammatory bowel disease (UC, colitis associated cancer (CAC) and colorectal carcinoma (CRC). Levels of Cr-1, PGE-2, FGF-2 and NOS-2 mRNA in IBD, CAC CRC was determined by Real Time PCR method. GAPDH mRNA levels were used to normalize the expression of Cr-1, PGE-2, FGF-2 and NOS-2 mRNA. Statistical analysis using nonparametric Mann-/Whitney test was performed ***$p < 0.00$

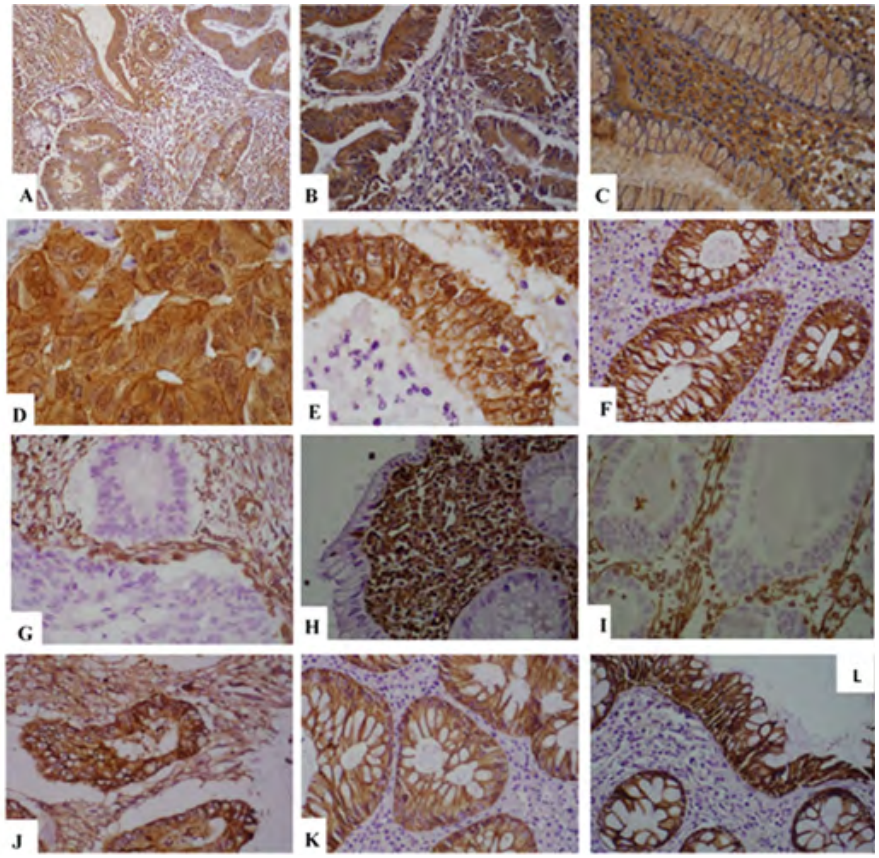

Figure 2: Immunohistochemical staining for Cr-1, β-catenin and E-cadherin in IBD, CAC and CRC tissues. (A):CR-1(X200) in CRC and (B) Cr-1(X400) in CAC ,Well-differentiated adenocarcinoma showing moderate to strong membrane and cytoplasmic reactivity in the epithelium of malignant glands.(C):Cr-1 (X 400) in IBD, Moderate cytoplasmic immunoreactivity in glandular epithelium.(D,E): β-catenin (X1000) in CAC and CRC , Well-differentiated adenocarcinoma showing Positive nuclear as well as cytoplasmic & membranous staining in tumoral cells.(F): β-catenin (X400) in IBD , strong membranous & moderate cytoplasmic staining.(G, H): E-cadherin (X 400) in IBD and CRC, strong membranous & moderate cytoplasmic staining. (I): E-cadherin (X 400) in CAC, E-cadherin, strong membranous & cytoplasmic staining.

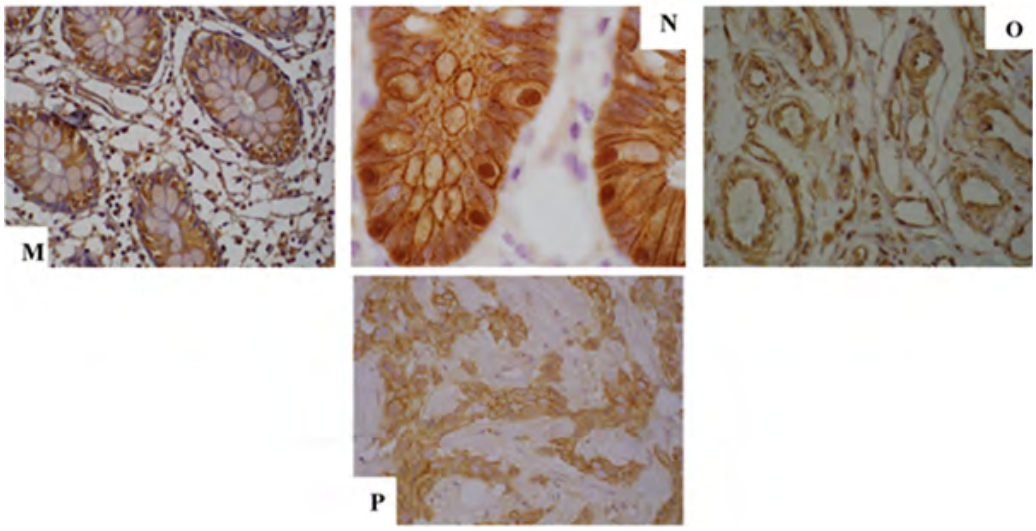

Figure 3: (M): Cr-1(X400) in control samples showing moderate cytoplasmic immunoreactivity in glandular epithelium, with cytoplasmic reactivity in lymphocytes infiltrating the mucosa. (N(:The nuclei of the normal epithelial cells of the normal glands of the large intestine are not stained, but the cells of the basal generative layer are stained like tumor cells, which is a normal phenomenon in the normal tissue of adults. (O): E-Cadherin (X400) in control showing moderate to strong membrane & mild cytoplasmic staining.

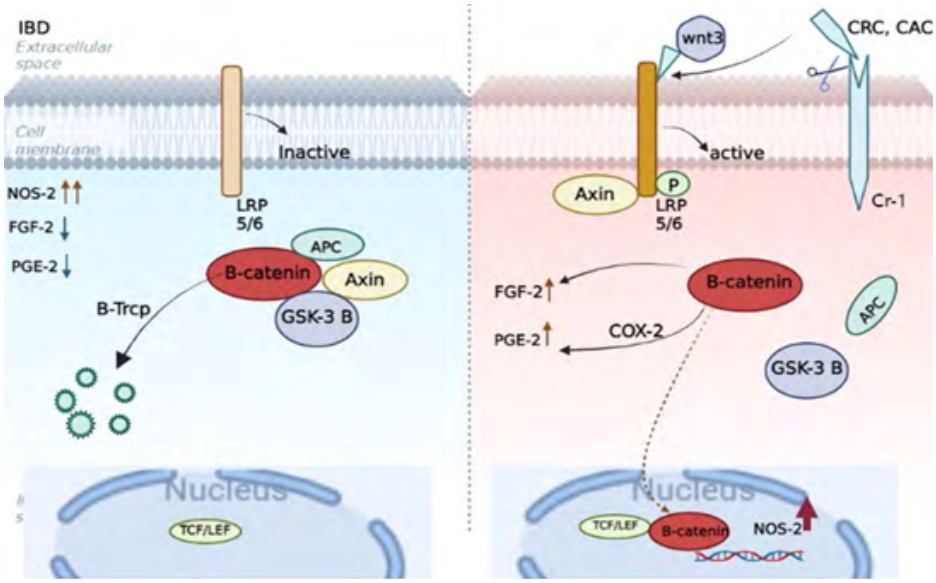

Figure 4: Schematic of Wnt/ β-catenin /Cr-1/NOS-2 signaling pathway in IBD, CAC and CRC. Cr-1 acts as a ligand for the LRP 5/6 receptor in CAC and CRC membranes and leads to the destruction of the β-catenin degradation complex, the activation of the Wnt/β-catenin signaling pathway, and finally, β-catenin is transferred to the nucleus and The TCF/LEF factor has interacted and led to the increase of NOS-2 gene expression. Also, the accumulation of β-catenin in the cytoplasm of CAC and CRC tissues has increased the PGE-2 gene. While the absence of Cr-1 in the IBD membrane leads to the inactivation of the Wnt/ β-catenin signaling pathway and finally β-catenin is not transferred to the nucleus. Therefore, it is possible that the increased expression of NOS-2 is related to the NF-κB pathway in IBD tissues. It is also possible that the inactivation of the β-catenin in cytoplasm has led to a decrease in the expression of PGE-2 in IBD tissues. It is also seen that FGF-2 gene expression increases from IBD to CAC.

5. Discussion

In this study, we investigated the molecular changes from IBD (UC) to CAC in the Cr-1, PGE-2, β-catenin, E-cadherin, FGF2, and NOS-2 gene expressions.We observed high expression levels of Cr-1 in the membrane and cytoplasm of CRC and CAC which is consistent previous report in CRC [7].Cr-1 was found to be increased due to EGF-CFC family members [32, 33] . Various studies have shown that CR-1 plays an effective role in the metastasis of many human cancer tumors.[34, 35]Bianco et al reported that Cr-1 leads to ras/raf/MAPK activation and suppresses epithelial cells [36]. Also, Tadahiro et al. reported that Cr-1 can lead to an increase in the Wnt/ β-catenin signaling pathway through binding to LRP 5/6 (Wnt receptors) [10]. A De Luca et al found that Cr-1 leads to invasion and proliferation of melanoma cells [37]. Also, in two other studies, they reported that the serum level of Cr-1 was very high in patients with lung cancer and mouse mammary gland

[38, 39].Regarding the role of Cr-1 in tumor progression, which occurs through the signaling pathway in tumor cells, some studies have investigated the role of Cr-1 in colon cancer, liver cancer, breast cancer, and bladder cancer [12, 40-43]. The results of IHC showed that Cr-1 is expressed in both membrane and cytoplasm of CAC and CRC tissues, which was in accordance with Yan Liu's report in prostate cancer [33]. Although it was not seen in the membrane of IBD (UC) patients, it was only seen in the cytoplasm of these patients. Strizzi et al showed that the Wnt/B-catenin/LEF-1 signaling pathway may interact with the Cr-1 signaling pathway to regulate cell adhesion and migration[44] .Regarding the role of Cr-1 in the Wnt signaling pathway, Our findings in IHC and Real-time PCR tests also suggest that the high expression level of Cr-1 may increase the metastatic ability and tumor progression stages through the Wnt/β-catenin pathway in CAC and CRC patients. And it seems that Cr-1 does not play a role in the process of inflammatory bowel disease progressing to cancer, it shows the role of Cr-1 as a potential therapeutic target in CAC and CRC samples but not in IBD. Berna Savas et al reported that the expression of β-catenin in CRC was significantly increased in the nucleus[45]. Alexander et al reported that both cytoplasmic and nuclear β-catenin are associated with poor survival outcomes[46] . In the present study, the increase in the expression level of Cr-1 in CAC and CRC membranes coincides with the increase in the level of β-catenin in the cytoplasm and nucleus, so there may be a relationship between Cr-1 and the increase in the accumulation of β-catenin in the nucleus and cytoplasm in CAC and CRC patients. While the absence of Cr-1 marker in the membrane of IBD (UC) patients was observed simultaneously with the absence of β-catenin in the nucleus of IBD (UC) patients. It may be argued that the absence of the Cr-1 marker in the membrane of IBD (UC) patients did not lead to the activation of the Wnt/β-catenin signaling pathway and therefore the accumulation of β-catenin in the nucleus is not seen. This evidence can be partly related to the reports of Strizzi et al about the relationship between Cr 1 and the Wnt/B-catenin signaling pathway [44]. Immunohistochemistry is a common method to detect the decrease in membrane expression level of adhesion molecules, aberrant expression of E-cadherin and expression of B-catenin and activation of Wnt pathway. In the present study, we found a high percentage of abnormal immunoexpression of E-cadherin and B-catenin in the membrane and cytoplasm of CAC, CRC and IBD tissues. Other authors have also reported high aberrant expression of E-cadherin and B-catenin in colon cancer cells and inflammatory bowel disease [47, 48] [49]. In our study, the expression changes of E-cadherin marker were not observed in all three tissues of IBD (UC), CAC and CRC. Amani Kazem et al reported that both β-catenin and COX-2 can lead to the progression of CRC and can be considered as effective diagnostic markers in CRC [50].SJ Kim and colleagues reported that IL-1β can lead to an increase in the expression of cyclooxygenase-2 (COX-2), that β-catenin can lead to the induction of COX-2 expression through the regulation of IL-1B expression [24]. As a result, COX-2 Through the conversion of arachidonic acid to prostaglandins, it leads to the production of PGE-2 [51]. which leads to tumor progression and metastasis of cancer cells through binding to its receptor [52]. R Pai et al investigated that PGE-2 can increase the invasion of CRC cells through tyrosine phosphorylation of β-catenin [26]. It was also reported that the expression of cytoplasmic β-catenin was associated with the increased expression of COX-2, supporting the role of cytoplasmic β-catenin in the stabilization of PGE-2 (COX-2) mRNA [53]. In the present study, PGE-2 expression was significantly increased from IBD (UC) to CAC and CRC. Increased expression of PGE-2 was seen in CAC and CRC patients, while it was less expressed in IBD (UC) patients, It is possible that the cytoplasmic β-catenin in IBD (UC) patients is not related to the increase in PGE-2 expression, while the increase in cytoplasmic β-catenin expression in CRC and CAC patients may be related to the increase in PGE-2 expression. Qiang Du et al. reported that β-catenin upregulates NOS-2 (nitric oxide synthase-2) through interaction with TCF-4 in the nucleus, which suggests a novel mechanism by which the Wnt/β-catenin signaling pathway may increase production of NO and progression of cancer [28] .On the other hand, NOS-2 can lead to the increase of Wnt/ β-catenin through the negative regulation of DKK-1 [54]. Other research has shown that inactivation of GSK-3β induce β-catenin, leading to NF-κB-dependent iNOS upregulation in hepatocytes [55]. Also, Taylor et al showed that the hiNOS gene is regulated and targeted by NF-kB [56]. However, it was seen that β-catenin also exerts an inhibitory effect on NF-κB-mediated transcriptional activation, including iNOS [57].It was also reported in two studies that iNOS (NOS-2) increases in both IBD (UC) and CRC [29, 58]. In the present study, the presence of β-catenin was observed in the nucleus of CAC and CRC cells in IHC, Real-time PCR report also shows that NOS-2 is expressed in CAC and CRC patients. According to the available information and the presence of β-catenin in the nucleus of CAC and CRC cells, it is possible that nuclear β-catenin increases the level of NOS-2 expression through interaction with the TCF-4 factor, but the presence of β-catenin in the cell nucleus IBD (UC) was not observed. Real-time PCR reports showed that NOS-2 was overexpressed in IBD (UC) patients compared with CAC patients, which may indicate that β-catenin does not affect NOS-2 expression and that NOS expression -2 may be regulated by NF-kB signaling. In a study of pediatric IBD (UC) patients, mean serum FGF-2 levels in patients with CD or UC were similar to patients with functional abdominal pain or other conditions, and there was a significant association between serum FGF-2 and disease activity in both CD and UC [59].Also, Silvio et al hypothesized that endogenous FGF-2 may be part of a ß-catenin regulated mechanism to maintain cancer stem cell (CSC) properties in cultured colorectal tumor cell lines [31]. In our study, the expression

of FGF-2 increased significantly from IBD (UC) to CAC. There was also the expression of β-catenin in the cytoplasm of IBD (UC) and CAC patients. It is possible that cytoplasmic β-catenin has no effect on the expression of FGF-2 in IBD.

6. Statements and Declarations

6.1. Funding: This work was supported by Fasa University of Medical Sciences (Grant numbers [99227]). Author Behnoosh Miladpour has received research support from Fasa University of Medical Sciences.

6.2. Competing Interest: The authors have no relevant financial or non-financial interests to disclose

6.3. Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Behnoosh Miladpour], [Alireza Hosseini Abgir], [Alireza Tavassoli], [Arash Goodarzi], and [Seyed Vahid Hosseini]. The first draft of the manuscript was written by [Alireza Hosseini Abgir], [Behnoosh Miladpour] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

6.4. Data Availability: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

6.5. Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Fasa University of Medical Sciences, IR.FUMS.REC.1400.051.

References

1. Stidham, RW, Higgins PD, Colorectal cancer in inflammatory bowel disease. Clinics in colon and rectal surgery. 2018; 31(03): 168-78.
2. Eaden J, Abrams K, Mayberry J, The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001; 48(4): 526-535.
3. Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi RE, Corcione Fet, Worldwide burden of colorectal cancer: a review. Updates in surgery. 2016; 68(1): 7-11.
4. Keller D, Windsor A, Cohen R, Chand M, Colorectal cancer in inflammatory bowel disease: review of the evidence. Techniques in coloproctology. 2019; 23(1): 3-13.
5. Crohn, B., The sigmoidoscopic picture of chronic ulcerative colitis (non-specific). Am J Med Sci, 1925. 170: 220-228.
6. Itzkowitz SH. and X Yio, Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol, 2004; 287(1): G7-G17.
7. Sato J, Karasawa H, Suzuki T, Nakayama S, Katagiri M, Maeda S, et al. The function and prognostic significance of cripto-1 in colorectal cancer. Cancer Investigation. 2020; 38(4): 214-27.
8. Minchiotti G, Parisi S, Liguori G, Signore M, Lania G, Adamson ED, et al. Membrane-anchorage of Cripto protein by glycosylphosphatidylinositol and its distribution during early mouse development. Mechanisms of development, 2000. 90(2): p. 133-142.
9. Xu C, Liguori G, Persico MG, Adamson ED, Abrogation of the Cripto gene in mouse leads to failure of postgastrulation morphogenesis and lack of differentiation of cardiomyocytes. Development, 1999; 126(3): 483-94.
10. Nagaoka T, Karasawa H, Turbyville T, Rangel MC, Castro NP, Gonzales M, et al. Cripto-1 enhances the canonical Wnt/β-catenin signaling pathway by binding to LRP5 and LRP6 co-receptors. Cellular signalling, 2013; 25(1): 178-89.
11. Xu C, Zou J, Li L, Yuan Q, Wang W, Elevated serum Cripto-1 and VEGF levels in patients with non-small cell lung cancer. FASEB BioAdvances. 2022; 4(8): 539.
12. Wang Jh, Wei W, Xu J, Guo Zx, Xiao Cz, Zhang Yf, et al. Elevated expression of Cripto-1 correlates with poor prognosis in hepatocellular carcinoma. Oncotarget. 2015; 6(33): 35116.
13. Tysnes BB, Satran HA, Mork SJ, Margaryan NV, Eide GE, Petersen K, et al. Age-dependent association between protein expression of the embryonic stem cell marker cripto-1 and survival of glioblastoma patients. Translational oncology, 2013; 6(6): 732-IN33.
14. Ragab MA, Sallam AAM, Amer EA, Mesallamy HOE, Evaluation of KCTD12 and Cripto-1 as novel upregulated proteins participating in breast cancer development and drug resistance. Egyptian Journal of Chemistry, 2022; 65(1): p. 1-2.
15. Moparthi L, Koch S, Wnt signaling in intestinal inflammation. Differentiation, 2019; 108: 24-32.
16. Brabletz T, Herrmann K, Jung A, Faller G, Kirchner T, Expression of nuclear β-catenin and c-myc is correlated with tumor size but not with proliferative activity of colorectal adenomas. The American journal of pathology, 2000; 156(3): 865-70.
17. Yuan S, Tao F, Zhang X, Zhang Y, Sun X, Wu D, Role of Wnt/β-catenin signaling in the chemoresistance modulation of colorectal cancer. BioMed research international, 2020.
18. Cheng X, Xu X, Chen D, Zhao F, Wang W, Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. Biomedicine & Pharmacotherapy, 2019; 110: 473-81.
19. Ma CT, Luo HS, Feng G, Tang QC, Chen W, Fusobacterium nucleatum promotes the progression of colorectal cancer by interacting with E-cadherin. Oncology letters, 2018; 16(2): 2606-12.
20. Ghahhari NM, Babashah S, Interplay between microRNAs and WNT/β-catenin signalling pathway regulates epithelial–mesenchymal transition in cancer. European journal of cancer, 2015; 51(12): 1638-49.
21. Ye Z, Zhou M, Tian B, Wu B, Li J, Expression of lncRNA-CCAT1, E-cadherin and N-cadherin in colorectal cancer and its clinical significance. International journal of clinical and experimental medicine, 2015; 8(3): 3707.
22. Karayiannakis AJ, Syrigos KN, Efstathiou J, Valizadeh A, Noda M, Playford RJ, et al. Expression of catenins and E-cadherin during epithelial restitution in inflammatory bowel disease. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 1998; 185(4): 413-18.
23. Lai Y, Zhong W, Yu T, Zhong-Sheng X, Li JY, Hui O, et al. Rebam-

ipide promotes the regeneration of aspirin-induced small-intestine mucosal injury through accumulation of β-Catenin. PLoS One, 2015; 10(7): p. e0132031.

24. Kim SJ, Im DS, Kim SH, Ryu JH, Hwang SG, Seong JK, et al. β-Catenin regulates expression of cyclooxygenase-2 in articular chondrocytes. Biochemical and biophysical research communications, 2002; 296(1): p. 221-226.
25. Myung SJ, Kim IH, Role of prostaglandins in colon cancer. The Korean Journal of Gastroenterology= Taehan Sohwagi Hakhoe chi, 2008; 51(5): p. 274-279.
26. Pai R, Nakamura T, Moon WS, Tarnawski AS, Prostaglandins promote colon cancer cell invasion; signaling by cross-talk between two distinct growth factor receptors. The FASEB Journal, 2003; 17(12): p. 1640-1647.
27. Zhang L, Tai Y, Zhao C, Ma X, Tang S, Tong H, et al. Inhibition of cyclooxygenase-2 enhanced intestinal epithelial homeostasis via suppressing β-catenin signalling pathway in experimental liver fibrosis. Journal of cellular and molecular medicine, 2021; 25(16): 7993-8005.
28. Du Q, Park KS, Guo Z, He P, Nagashima M, Shao L, et al. Regulation of human nitric oxide synthase 2 expression by Wnt β-catenin signaling. Cancer research, 2006; 66(14): 7024-31.
29. Gochman E, Mahajna J, Shenzer P, Dahan A, Blatt A, Elyakim R, et al. The expression of iNOS and nitrotyrosine in colitis and colon cancer in humans. Acta histochemica, 2012; 114(8): 827-35.
30. Dhillon SS, Mastropaolo LA, Murchie R, Griffiths C, Thöni C, Elkadri A, et al. Higher activity of the inducible nitric oxide synthase contributes to very early onset inflammatory bowel disease. Clinical and translational gastroenterology, 2014. 5(1): p. e46.
31. Scheel SK, Das B, Kriegi L, Reza BM, Micky T, Yeger H, et al. Abstract LB-252: Expression of FGF-2 is essential for the maintenance of cancer stem cell characteristics in human colorectal tumor cell lines. Cancer Research, 2010; 70(8): LB-252-LB-252.
32. Adamson ED, Minchiotti G, Salomon DS, Cripto: a tumor growth factor and more. Journal of cellular physiology, 2002; 190(3): 267-78.
33. Liu Y, Qin Z, Yang K, Liu R, Xu Y, Cripto-1 promotes epithelial-mesenchymal transition in prostate cancer via Wnt/β-catenin signaling. Oncology reports, 2017; 37(3): 1521-28.
34. Zhang JG, Zhao J, Xin Y, Significance and relationship between Cripto-1 and p-STAT3 expression in gastric cancer and precancerous lesions. World journal of gastroenterology: WJG, 2010; 16(5): 571.
35. Das AB, Loying P, Bose B, Human recombinant Cripto-1 increases doubling time and reduces proliferation of HeLa cells independent of pro-proliferation pathways. Cancer letters, 2012; 318(2): 189-198.
36. Bianco C, Castro NP, Baraty C, Rollman K, Held N, Rangel MC, et al. Regulation of human Cripto-1 expression by nuclear receptors and DNA promoter methylation in human embryonal and breast cancer cells. Journal of cellular physiology, 2013; 228(6): 1174-88.
37. De Luca A, Strizzi L, Lamura L, Roma C, D'Antonio A, Margaryan N, et al. Expression and functional role of CRIPTO-1 in cutaneous melanoma. British journal of cancer, 2011; 105(7): 1030-38.
38. Wechselberger C, Strizzi L, Kenney N, Hirota M, Sun Y, Ebert A, et al. Human Cripto-1 overexpression in the mouse mammary gland results in the development of hyperplasia and adenocarcinoma. Oncogene, 2005; 24(25): 4094-4105.
39. Xu CH, Wang Y, Qian LH, Yu LK, Zhang XW, Wang QB, et al. Serum Cripto-1 is a novel biomarker for non-small cell lung cancer diagnosis and prognosis. The Clinical Respiratory Journal, 2017; 11(6): 765-71.
40. Bianco C, Normanno N, De Luca A, Maiello MR, Wechselberger C, Sun Y, et al. Detection and localization of Cripto-1 binding in mouse mammary epithelial cells and in the mouse mammary gland using an immunoglobulin–cripto-1 fusion protein. Journal of cellular physiology, 2002; 190(1): 74-82.
41. Wei B, Jin W, Ruan J, Xu Z, Zhou Y, Liang J, et al. Cripto-1 expression and its prognostic value in human bladder cancer patients. Tumor Biology. 2015; 36(2): 1105-13.
42. Castro NP, Fedorova-Abrams ND, Merchant AS, Rangel MC, Nagaoka T, Karasawa H, et al. Cripto-1 as a novel therapeutic target for triple negative breast cancer. Oncotarget. 2015; 6(14): 11910.
43. Igder S, Mohammadiasl J, Azadpour S, Mansouri E, Ashktorab H, Mokarram P. KRAS mutation and abnormal expression of Cripto-1 as two potential candidate biomarkers for detection of colorectal cancer development. Journal of cellular biochemistry. 2020; 121(4): 2901-08.
44. Strizzi L, Postovit LM, Margaryan NV, Seftor EA, Abbott DE, Seftor REB, et al. Emerging roles of nodal and Cripto-1: from embryogenesis to breast cancer progression. Breast disease. 2008; 29(1): 91-103.
45. Savas B, Ensari A, Percinel S, Kuzu I, Kuzu MA, Bektas M, et al. The significance of beta-catenin, E-cadherin, and P-cadherin expressions in neoplastic progression of colorectal mucosa: an immunohistochemical study. Carcinogenesis. 2007; 10: 11.
46. Alexander PG, Matly AAM, Jirapongwattana N, Pennel KAF, van Wyk HC, McMillan DC, et al. The relationship between the Glasgow Microenvironment Score and Markers of Epithelial-to-Mesenchymal Transition in TNM II-III Colorectal Cancer. Human Pathology. 2022.
47. Mehta S, Nijhuis A, Kumagai T, Lindsay J, Silver Al. Defects in the adherens junction complex (E-cadherin/β-catenin) in inflammatory bowel disease. Cell and tissue research. 2015; 360: 749-60.
48. Tsanou E, Peschos D, Batistatou A, Charalabopoulos A, Charalabopoulos K. The E-cadherin adhesion molecule and colorectal cancer. A global literature approach. Anticancer research. 2008; 28(6A): 3815-26.
49. Bruun J, Kolberg M, Nesland JM, Svindland A, Nesbakken A, Lothe RA. Prognostic significance of β-catenin, E-cadherin, and SOX9 in colorectal cancer: results from a large population-representative series. Frontiers in oncology. 2014; 4: 118.

50. Kazem A, K. El Sayed, El Kerm Y. Prognostic significance of COX-2 and b-catenin in colorectal carcinoma. Alexandria Journal of Medicine. 2014; 50(3): 211-20.
51. Sheng J, Sun H, Yu FB, Li B, Zhang Y, Zhu YT. The role of cyclo-oxygenase-2 in colorectal cancer. International journal of medical sciences. 2020; 17(8): 1095.
52. Fujino H, Seira N, Kurata N, Araki Y, Nakamura H, Regan JW, et al. Prostaglandin E2-stimulated prostanoid EP4 receptors induce prolonged de novo prostaglandin E2 synthesis through biphasic phosphorylation of extracellular signal-regulated kinases mediated by activation of protein kinase A in HCA-7 human colon cancer cells. European journal of pharmacology. 2015; 768: 149-159.
53. Kawasaki T, Nosho K, Ohnishi M, Suemoto Y, Kirkner GJ, Dehari R, et al. Correlation of β-catenin localization with cyclooxygenase-2 expression and CpG island methylator phenotype (CIMP) in colorectal cancer. Neoplasia. 2007; 9(7): 569-77.
54. Du Q, Zhang X, Liu Q, Zhang X, Bartels CE, Geller DA. Nitric Oxide Production Upregulates Wnt/β-Catenin Signaling by Inhibiting Dickkopf-1iNOS/NO Activates Canonical Wnt Signaling. Cancer research. 2013; 73(21): 6526-37.
55. Bandino A, Compagnone A, Bravoco V, Cravanzola C, Lomartire A, Rossetto C, et al. ß-Catenin triggers nuclear factor? B-dependent up-regulation of hepatocyte inducible nitric oxide synthase. The international journal of biochemistry & cell biology. 2008; 40(9): 1861-71.
56. Taylor BS, et al. Multiple NF-κB enhancer elements regulate cytokine induction of the human inducible nitric oxide synthase gene. Journal of biological chemistry. 1998; 273(24): 15148-56.
57. Du Q, Geller D. Cross-regulation between Wnt and NF-κB signaling pathways. Onco Therapeutics. 2010; 1(3).
58. Cross RK, Wilson KT. Nitric oxide in inflammatory bowel disease. Inflammatory bowel diseases. 2003; 9(3): 179-89.
59. Bousvaros A, Zurakowski D, Fishman SJ, Keough K, Law T, Sun C, Leichtner AM. Serum Basic Fibroblast Growth Factor in Pediatric Crohn's Disease (Implications for Wound Healing). Digestive diseases and sciences. 1997; 42(2): 378-86.