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# The association of p21, inhibin, and Tob-1 expression with the clinicopathological characteristics of benign and malignant colorectal lesions

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## Abstract

**Background** Worldwide, colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer-related deaths. p21, inhibin, and Tob-1 are tumor suppressors that play a role in the development and progression of several cancers, however, their role in CRC is not well-established. This study aims to evaluate the expression of these proteins by immunohistochemistry and correlate their expression with the clinicopathological characteristics of CRCs and preneoplastic lesions [adenomas and ulcerative colitis] to study the potential for their use as targeted therapies. The study was performed on sections of 30 CRCs, 30 adenomas, 30 UC, 30 chronic colitis, and 20 controls.

**Results** p21 expression was lower in CRCs and adenomas compared to inflammatory lesions (chronic colitis and UC). High-grade CRCs, adenomas with high-grade dysplasia, and UC with dysplasia showed insignificantly lower expression compared to their counterparts. Inhibin expression was absent in CRCs; however, its expression was higher in chronic colitis than in UC and adenomas. Adenomas with high-grade dysplasia and UC with dysplasia showed insignificantly higher expression than their counterparts. Tob-1 expression increased significantly from chronic colitis to UC to adenomas to CRCs. High-grade CRCs, adenomas with high-grade dysplasia, and UC with dysplasia showed higher expression compared to their counterparts.

**Conclusions** Decreased p21 and increased inhibin and Tob-1 expressions are associated with the progression of adenomas and UC to more dysplastic lesions, then possibly to CRC. Despite being tumor suppressors, the studied proteins may potentially have tumor-promoting properties. They can be useful targets for therapeutic intervention.

**Keywords** p21, Inhibin, Tob-1, Colon cancer, Colon adenoma, Ulcerative colitis, Chronic colitis, Risk assessment

## 1 Background

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of cancer-related mortality despite effective screening methods. It mostly affects elderly persons who are 50 years and beyond [1]. Globally, the prevalence of colorectal cancer (CRC) has been gradually rising, especially in developing countries that have embraced the "Western" way of life, which includes consuming a diet high in saturated fats and

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refined carbohydrates with minimal intake of plant-based foods [2].

In Egypt, CRC constitutes 2.7% of the diagnosed cancer patients ranking as the ninth most prevalent malignancy in both sexes, and is the eleventh leading cause of cancer-related mortality [3]. Over one-third of CRC cases occur in individuals under the age of 40 years and are discovered at an advanced stage. These cases usually arise on top of polyposis and ulcerative colitis [4]. Egyptian patients with CRC before the age of 30 had a threefold higher risk of death within five years than patients with CRC over the age of 50 [5].

Most CRCs are incidental in nature and are mostly caused by controllable environmental factors including smoking, obesity, inactivity, poor dietary habits, and alcohol use. Several genetic disorders are responsible for modifying signal pathways, including PIK3CA, Wnt, and transforming growth factor-beta (TGF-β). This is caused by somatic or hereditary mutations over a period of 10 to 15 years [6–8].

Long-standing inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), predispose to the development of CRC [9]. According to Terzic et al. [10] study; more than 20% of IBD patients proceed to cancer-associated colitis (CAC) within 30 years of disease onset, and 50% of them die from CAC.

p21 Waf1/Cip1 is a 21-kDa protein encoded by wild-type p53-activated fragment gene 1 (Waf1) that is a cyclin-dependent kinase (CDK) inhibitor belonging to the Cip/Kip family of CDK inhibitors. It primarily represses the course of the cell cycle and suppresses growth by acting as a tumor suppressor [11, 12]. Additionally, it is crucial for triggering apoptosis and cell senescence, promoting DNA repair, and maintaining genome stability. Meanwhile, p21 has also an oncogenic activity as it inhibits apoptosis via activation of cell cycle arrest and DNA repair [13]. Therefore, it is challenging to recognize targeted therapy that specifically blocks p21 oncogenic action rather than its tumor suppressor function. Some cancers have p21 overexpression such as breast cancer [14], while others have low expression like bladder cancer [15]. However, the significance of p21 in CRC was not clearly established by previous studies, thus this work attempts to evaluate p21 expression in CRCs.

Inhibin is a dimeric glycoprotein hormone belonging to the TGF-β superfamily that prevents the release or synthesis of follicle-stimulating hormone (FSH). It is secreted by the ovarian granulosa cells and the testicular Sertoli cells [16]. Inhibin is up-regulated in some cancer types, such as pancreatic, adrenal, and prostate tumors. It is engaged in cell signaling pathways that are crucial for controlling the development and proliferation of cells [17], whereas other studies reported its down-regulation such as in hepatocellular carcinoma and gastric cancer [18, 19]. To date, few studies have concerned the role of inhibin in CRCs, thus our study aims to emphasize this role.

Tob-1 is a tumor-suppressor protein that is involved in the regulation of cell division, proliferation, and apoptosis. It maintains the quiescent state of cells and, its overexpression causes cell cycle arrest in the G1 phase [20]. Tob-1 role in malignancy is controversial, being down-regulated in some malignancies such as pancreatic and gastric cancers [21, 22], while upregulated in others such as colon cancer [23]. Furthermore, Tob-1 plays a critical function in controlling the immune response through the T-helper cells 17 (Th17) pathway. While low expression of Tob-1 is necessary for T-cell activation and growth, Tob-1 overexpression prevents T-cell multiplication [24]. Our study aims to demonstrate the differences in Tob-1 expression between benign and malignant colorectal tumors.

This work aims to evaluate the expression of p21, inhibin, and Tob-1 proteins in a variety of chronic colonic lesions, including neoplastic and non-neoplastic lesions to assess the relationship between the expression of these proteins and risk of CRC development. This could guide the benefit of using antagonists to these proteins in patients with pre-neoplastic changes to hinder transformation into cancer.

## 2 Methods

This retrospective cross-sectional study was conducted on 140 paraffin blocks of neoplastic and non-neoplastic colonic lesions obtained from the Surgical Pathology Department from January 2020 to October 2021. The samples were obtained as either resection colectomy specimens (21 specimens) or endoscopic biopsies (119 specimens). None of the studied cases had received chemotherapy or radiotherapy.

Samples categorized as [1] 30 CRCs specimens (21 colorectal resection specimens and 9 endoscopic biopsies), divided histopathologically into 21 conventional adenocarcinomas (13 of low-grade and 8 of high-grade) and 9 mucinous adenocarcinomas (all of high-grade dysplasia); [2] 30 adenoma specimens, divided histopathologically into adenomas with low-grade dysplasia (LGD) (13 specimens) and adenomas with high-grade dysplasia (HGD) (17 specimens); [3] 30 ulcerative colitis specimens, divided histopathologically into UC without dysplasia (23 specimens) and UC with dysplasia (7 specimens); [4] 30 chronic non-specific colitis specimens, and [5] 20 control specimens of colonic tissue with unremarkable changes, obtained from surgical margins of colectomy specimens.

The inclusion criteria were cases of non-specific colitis, UC, primary conventional or mucinous CRC, and properly recorded clinical, radiological, and operative data in their files. Exclusion criteria were cases of specific types of colitis, other variants of CRC, or those with missing data in their files.

### 2.1 Ethic approval

The study protocol was approved by the Institutional Review Board (IRB) for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland (2013). IRB approval number FWA00010609. This retrospective study was performed using archival specimens that were anonymized before the study. There is no need to obtain patient consent.

### 2.2 Immunohistochemical (IHC) technique

One paraffin-embedded block was selected from each case and cut into 4 μm sections. The sections were put in the oven at 60 °C for 4 h, deparaffinized in xylene, and rehydrated in a graded ethanol series. Antigen retrieval was performed with 10 ml sodium citrate buffer, pH 6.0 at 90 °C for 30 min. Sections were incubated in 0.03% hydrogen peroxide (EnVision/HRP, Dako, Denmark) for 10 min at room temperature, then were rinsed in buffer. The sections were incubated with p21 (clone SX118, code M7202, Dako, Denmark) at a dilution of 1:50, inhibin (clone R1, code IR058, Dako, Denmark), ready-to-use, and Tob-1 (code HPA047839, Atlas antibodies, Sweden) at a dilution of 1:100 for overnight at 4°C. Sections were then washed three times for 5 min in buffer. The bound antibodies were detected using Envision Detection System (Dako, Denmark). Finally, slides were counterstained with hematoxylin and eosin, dehydrated in alcohol, and mounted. For each setting; positive and negative controls were routinely used. Positive controls were skeletal muscle tissues for p21, granulosa cell tumor of the ovary for inhibin, and adrenal tissue for Tob-1. Negative controls were carried out in which phosphate-buffered saline was used instead of the primary antibody.

### 2.3 Scoring and data analysis

Scoring of p21, inhibin, and Tob-1 immunostaining was performed blindly to the patients' clinicopathological data by examining randomly selected 10 high-power fields. Percentage scores were graded as 1 = positive cells ≤ 10%; 2 = positive cells from 11–50%; 3 = positive cells from 51%-75%; 4 = positive cells from 76%-100%. Intensity scores were graded as 1 = no staining; 2 = weak staining; 3 = moderate staining; and 4 = strong staining. The final scores ranged from 0–12; scores ≥ 3 are considered positive expressions [25]. P21 and Tob-1 expression were detected as cytoplasmic/nuclear staining. Inhibin expression was detected as cytoplasmic staining.

### 2.4 Statistical analysis

Analyses were performed using SPSS version 26 (IBM Corp., Armonk, New York, USA). The significance of differences in means was calculated using the Student's t-test. Spearman rho test was used to assess the significance of differences between different clinicopathological variables. Differences were considered statistically significant whenever $p < 0.05$.

## 3 Results

### 3.1 Immunohistochemical expression of p21 in colonic lesions (Table 1) (Fig. 1)

The highest expression of p21 was found in UC specimens (63.3%), while CRC specimens showed the least expression (40%).

We did not find a significant relationship regarding p21 expression in adenomas versus UC ($p = 0.22$), chronic

**Table 1** The association between p21 expression and clinicopathological characteristics of studied colonic lesions

| Variables | P21 expression | | |
|---|---|---|---|
| | Positive no. (%) | Negative no. (%) | *p*-value |
| Age (in years) | 51.43 ± 16.14 | 54.62 ± 16.45 | 0.25 |
| Age categories | | | |
| ≤ 50 years (45) | 22 (49) | 23 (51) | 0.41 |
| > 50 years (95) | 50 (52.6) | 45 (47.4) | |
| Sex | | | |
| Female (61) | 38 (62.3) | 23 (37.7) | 0.018 |
| Male (79) | 34 (43) | 45 (57) | |
| CRC (30) | 12 (40) | 18 (60) | |
| Type of cancer | | | |
| Conventional (21) | 10 (47.6) | 11 (52.4) | 0.19 |
| Mucinous (9) | 2 (22.2) | 7 (77.8) | |
| Grade | | | |
| Low-grade CRC (13) | 6 (46.2) | 7 (53.8) | 0.41 |
| High-grade CRC (17) | 6 (35.3) | 11 (64.7) | |
| Adenomas (30) | 15 (50) | 15 (50) | 0.30* |
| LGD (14) | 11 (78.6) | 3 (21.4) | 0.005 |
| HGD (16) | 4 (25) | 12 (75) | |
| UC (30) | 19 (63.3) | 11 (36.7) | 0.06* |
| No dysplasia (23) | 16 (63.3) | 7 (30.4) | 0.20 |
| With dysplasia (7) | 3 (43) | 4 (57) | |
| Chronic colitis (30) | 16 (53.3) | 14 (46.7) | 0.22* |
| Control (20) | 10 (50) | 10 (50) | 0.34* |
| Total = 140 | 72 (51.4) | 68 (48.6) | |

*LGD* low-grade dysplasia, *HGD* high-grade dysplasia, *UC* ulcerative colitis, *CRC* Colorectal cancer, *no.* number of cases, %; percentage

* *p*-value compared to CRCs

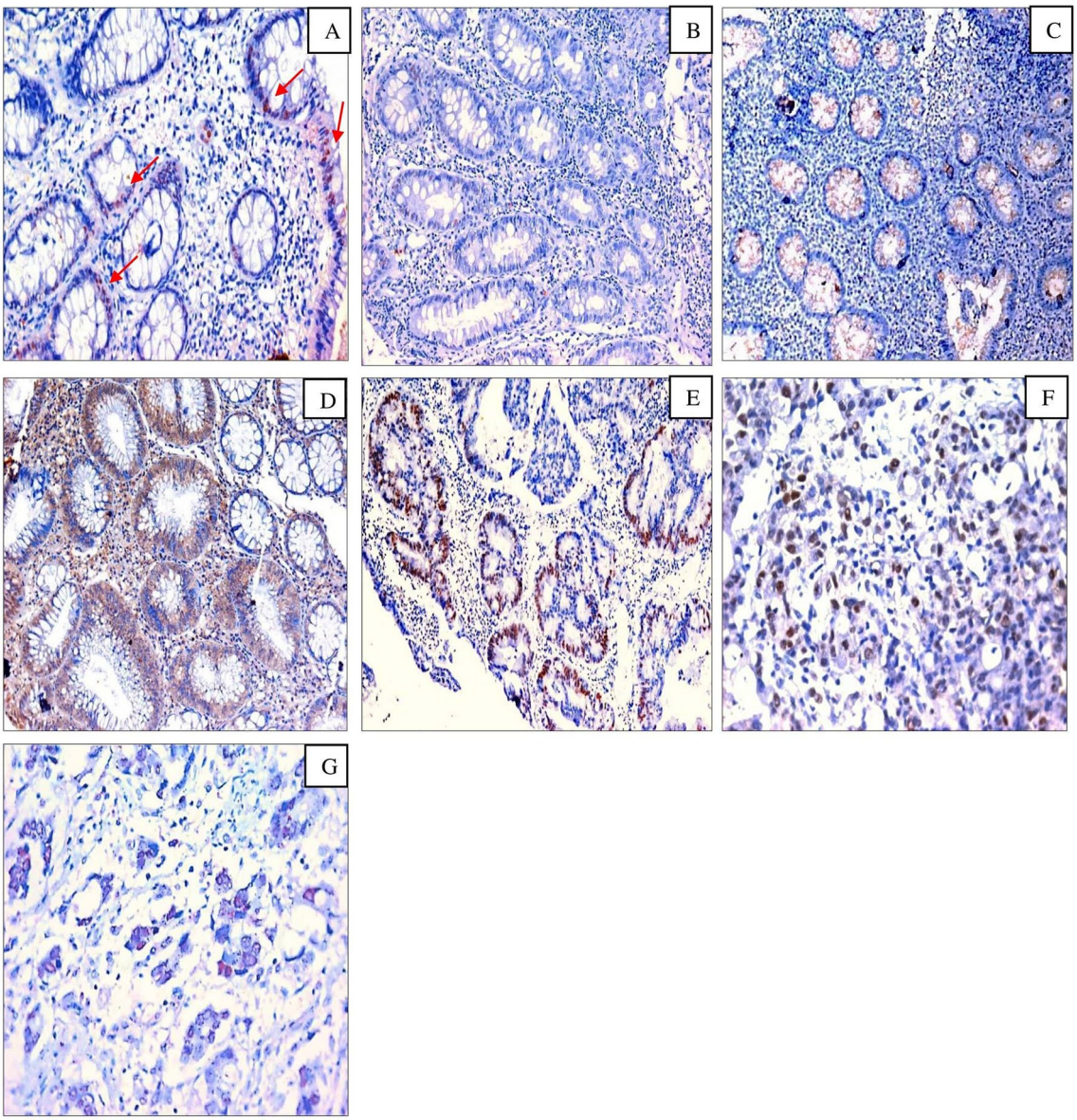


**Fig. 1** p21 expression **a** positive expression in control (arrows) (×200), **b** negative expression in chronic colitis (arrows) (×200), **c** positive expression in UC without dysplasia (×200), **d** positive expression in tubular adenoma with LGD (×200), **e** positive expression in low-grade CRC (×200), **f** positive expression in high-grade CRC (×400), **g** negative expression in mucinous carcinoma (×200). UC; ulcerative colitis, LGD; low-grade dysplasia, HGD; high-grade dysplasia, CRC; Colorectal cancer

colitis ($p=0.5$), or controls ($p=0.61$). Also, there was no significant relationship between UC versus chronic colitis ($p=0.3$), or controls ($p=0.26$).

Regarding CRCs, we detected p21 expression in 47.6% of conventional CRCs compared to 22.2% of mucinous carcinomas, without statistical significance ($p=0.19$). Moreover, although p21 expression was higher in low-grade CRCs (46.2%) compared to high-grade tumors (35.3%), this relation did not achieve a significant value ($p=0.41$).

Adenomas with LGD showed significantly higher p21 expression (78.6%) than HGD (25) ($p=0.005$).

P21 expression was higher in UC without dysplasia (69.6%) compared to UC with dysplasia (43%), without statistical significance ($p=0.20$).

Although there was significant overexpression of p21 in females (62.3%) compared to males (43%), however, no significant relation was found between p21 expression and age (Table 4).

### 3.2 Immunohistochemical expression of inhibin in colonic lesions (Table 2) (Fig. 2)

None of the controls or CRCs were positive for inhibin, while 40% of chronic colitis specimens were positive. This percentage decreased to 26.7% in UC and 13.3% in adenomas.

There is a significant relationship regarding inhibin expression in adenomas versus chronic colitis ($p=0.02$) and UC versus controls ($p=0.01$), meanwhile, we did not find a significant relationship regarding inhibin expression in either adenomas versus UC ($p=0.17$), nor controls ($p=0.12$), or UC versus chronic colitis ($p=0.21$).

Four out of 16 (25%) adenomas with HGD showed expression for inhibin compared to none in adenomas with LGD. Moreover, UC specimens with dysplasia showed higher expression (43%) compared to UC without dysplasia (21.7%), yet without statistical significance ($p=0.26$).

We found no significant relationship between inhibin expression and both age and sex (Table 4).

**Table 2** The association between inhibin expression and clinicopathological characteristics of studied colonic lesions

| Variables | Inhibin expression | | |
|---|---|---|---|
| | Positive no. (%) | Negative no. (%) | *p*-value |
| Age (in years) | 54.04 ± 17.05 | 52.76 ± 16.23 | 0.73 |
| Age categories | | | |
| ≤ 50 years (45) | 8 (17.8) | 37 (82.2) | 0.53 |
| > 50 years (95) | 16 (16.8) | 79 (83.2) | |
| Sex | | | |
| Female (61) | 13 (21.3) | 48 (78.7) | 0.18 |
| Male (79) | 11 (14) | 68 (86)) | |
| CRC (30) | 0 | 30 (100) | |
| Adenomas (30) | 4 (13.3) | 26 (86.7) | 0.06* |
| LGD (14) | 0 | 14 (100) | |
| HGD (16) | 4 (25) | 12 (75) | |
| UC (30) | 8 (26.7) | 22 (73.3) | 0.002* |
| No dysplasia (23) | 5 (21.7) | 18 (78.3) | 0.26 |
| With dysplasia (7) | 3 (43) | 4 (57) | |
| Chronic colitis (30) | 12 (40) | 18 (60) | 0.001* |
| Control (20) | 0 | 20 (100) | |
| Total = 140 | 24 (17) | 116 (83) | |

*LGD* low-grade dysplasia, *HGD* high-grade dysplasia, *UC* ulcerative colitis, *CRC* Colorectal cancer, *no.* number of cases, %; percentage

* *p*-value compared to CRCs

### 3.3 Immunohistochemical expression of Tob-1 in colonic lesions (Table 3) (Fig. 3)

Tob-1 immunopositivity was mainly located in the cytoplasm or cytoplasmic/nuclear location.

We found a significant increase of Tob-1 expression from controls (no expression) to chronic colitis (10%), to UC (30%), to adenomas (60%), and finally to carcinomas (83.3%).

We found a significant relationship regarding Tob-1 expression in adenomas versus UC ($p=0.02$), chronic colitis ($p=0.001$), and controls ($p=0.001$), as well as between UC versus chronic colitis ($p=0.05$), and controls ($p=0.006$), Meanwhile, no significant relation between chronic colitis versus control ($p=0.21$).

Regarding CRCs, we detected Tob-1 expression in all mucinous carcinomas compared to 76.2% of conventional CRCs, without statistical significance ($p=0.14$). Moreover, its expression was insignificantly higher in high-grade CRCs (88.2%) compared to low-grade tumors (77%) ($p=0.37$).

In adenomas with HGD, Tob-1 was expressed in 75% of specimens compared to 43% in adenomas with LGD, without statistical significance ($p=0.78$).

It was significantly higher in UC with dysplasia (71.4%) compared to ones without dysplasia (17.4%) ($p=0.014$), as cases with dysplasia have more than 2 times the risk of Tob-1 positive expression.

There is a significant relation between Tob-1 expression and age but not with sex (Table 4).

In all positive specimens, we noticed stromal positive Tob-1 expression in fibroblasts and lymphocytes (Fig. 4).

## 4 Discussion

CRC is characterized by complex genetic alterations in both oncogenes and tumor-suppressor genes that drive the initiation and promotion of malignancy [7].

The main treatment strategies for CRC include surgery, chemotherapy, radiation, immunotherapy, and/or targeted therapy, meanwhile, surgery and chemotherapy are of limited value for advanced CRC patients [26]. Therefore, besides early diagnosis, the efficient targeted therapies that rely on understanding the molecular characteristics of CRC are cornerstones for the successful treatment of CRC [27]. Thus, currently, a lot of research is being done to get better knowledge about targeted and personalized therapy.

p21, inhibin, and Tob-1 are examples of tumor suppressors that have variable expression in cancers and chronic tissue lesions. The current study was conducted on

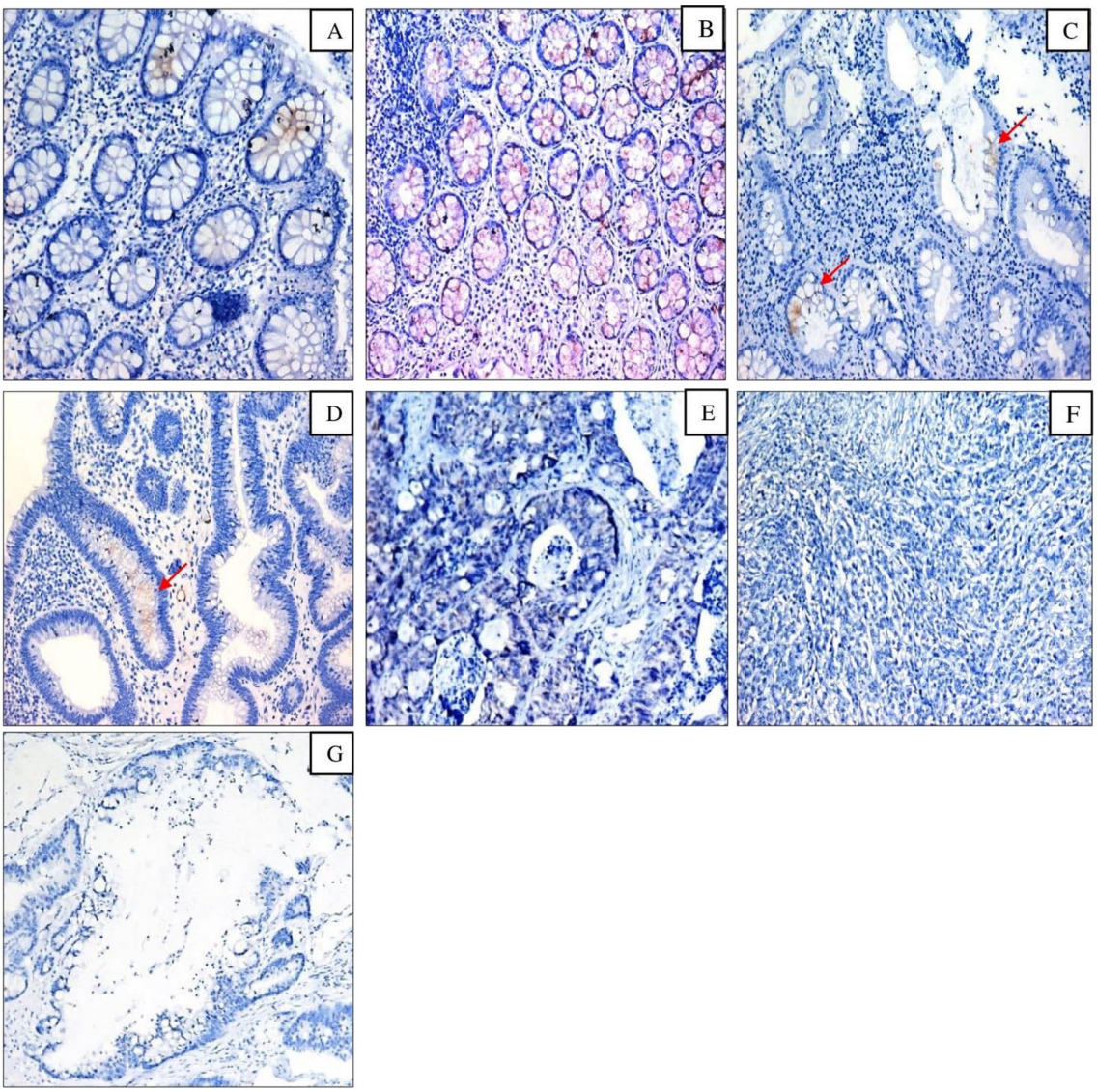


**Fig. 2** Inhibin expression **a** negative expression in control (arrows) (×200), **b** positive expression in chronic colitis (×200), **c** positive expression in UC (arrows) (×200), **d** positive expression in tubular adenoma with HGD (arrows) (×200), **e** negative expression in low-grade CRC (×200), **f** negative expression in high-grade CRC (arrows)(×200), **g** negative expression in mucinous carcinoma (×200). UC; ulcerative colitis, LGD; low-grade dysplasia, HGD; high-grade dysplasia, CRC; Colorectal cancer

archival 140 paraffin blocks of different colonic lesions to assess by immunohistochemistry the expression of p21, inhibin, and Tob1 antibodies in CRC, adenoma, UC, and chronic colitis.

### 4.1 p21 expression

P21 is one of the tumor suppressor proteins that can regulate the cell cycle, induce apoptosis, and promote DNA repair and genome stability. Alternations in p21 expression have been linked to several cancers, including CRC [13].

In our study, CRCs showed a significantly lower p21 expression (40%) than adenomas (66.7%). This is consistent with the results of Abdulamir et al. [28] who detected p21 expression in 34% and 64% of their studied CRC and adenoma cases, respectively and they concluded that p21 expression decreases with the progression of the cytopathological changes from adenoma to carcinoma. However, Zirbes et al. [29] reported a higher percentage of positive CRC cases (67%).

There was no statistically significant correlation between p21 expression with CRC types or differentiation grades. This was the same finding of Pasz-Walczak

**Table 3** The association between Tob-1 expression and clinicopathological characteristics of studied colonic lesions

| Variables | Inhibin expression | | |
|---|---|---|---|
| | Positive no. (%) | Negative no. (%) | *p*-value |
| Age (in years) | 56.84 ± 12.19 | 50.48 ± 18.148 | 0.024 |
| Age categories | | | |
| ≤ 50 years (45) | 12 (26.7) | 33 (73.3) | 0.026 |
| > 50 years (95) | 43 (45.3) | 52 (54.7) | |
| Sex | | | |
| Female (61) | 23 (37.7) | 38 (62.3) | 0.44 |
| Male (79) | 32 (40.5) | 47 (59.5) | |
| CRC (30) | 25 (83.3) | 5 (16.7) | |
| Type of cancer | | | |
| Conventional (21) | 16 (76.2) | 5 (23.8) | 0.14 |
| Mucinous (9) | 9 (100) | 0 | |
| Grade | | | |
| Low-grade CRC (13) | 10 (77) | 3 (23) | 0.37 |
| High-grade CRC (17) | 15 (88.2) | 2 (11.8) | |
| Adenomas (30) | 18 (60) | 12 (40) | 0.04* |
| LGD (14) | 6 (43) | 8 (57) | 0.78 |
| HGD (16) | 12 (75) | 4 (25) | |
| UC (30) | 9 (30) | 21 (70) | 0.001* |
| No dysplasia (23) | 4 (17.4) | 19 (82.6) | 0.01 |
| With dysplasia (7) | 5 (71.4) | 2 (28.6) | |
| Chronic colitis (30) | 3 (10) | 27 (90) | 0.001* |
| Control (20) | 0 | 20 (100) | 0.001* |
| Total = 140 | 55 (39.3%) | 85 (60.7) | |

*LGD* low-grade dysplasia, *HGD* high-grade dysplasia, *UC* ulcerative colitis, *CRC* Colorectal cancer, *no.* number of cases, %; percentage

* *p*-value compared to CRCs

et al. [30]. On the contrary, Abdulamir et al. [28] found a significant relationship between p21 and both the staging and grading of CRC.

Our studied adenomas with LGD showed significantly higher expression of p21 than adenomas with HGD. However, Doglion et al. [31] reported no significance between p21 expression and the degree of dysplasia in adenoma cases. The examined UC specimens showed insignificantly higher expression (63.3%) than CRCs (40%). These results are similar to that of Popp et al. [32] who found p21 expression in 63% of their UC cases. Furthermore, Ioachim et al. [33] reported higher p21 expression in UC than in CRCs and they supposed that p21 expression may implicate colorectal carcinogenesis in IBD-related carcinomas. Moreover, we found more p21 expression in UC without dysplasia than with dysplasia.

Expression of p21 increased from controls to chronic colitis to UC, yet expression decreased in adenomas and CRCs. Moreover, there is decreased p21 expression in dysplastic changes of adenomas and UC compared to those without dysplasia. This paradox can be attributed to the dual role played by p21. First, it functions as a tumor suppressor protein that represses the cell cycle and prevents uncontrollable cell proliferation in dysplastic and cancer cells. Second, it functions as a tumor-promoting protein by inhibiting apoptosis and allowing the promotion of tumorigenesis [34].

### 4.2 Inhibin expression

Inhibin is a tumor suppressor that shows lower expression in testicular and ovarian cancers than in normal counterparts and lower expression in malignant adrenal tumors versus benign forms [35]. To our knowledge, this study is one of the first studies that analyzed the expression of inhibin in both benign and malignant colonic lesions.

We found no expression in control or CRC specimens, meanwhile, Wildi et al. [36] reported absence of inhibin expression in normal tissues obtained from stage IV CRCs and expression in the normal tissues obtained from stage I disease.

Contrary to our results, Yoon et al. [35] and He et al. [37] found higher inhibin expression in CRC tissues than in para-carcinoma and normal tissues. Wildi et al. [35] demonstrated weak to moderate expression in stage I CRCs but marked expression in stage IV cancers. This controversy can be attributed to the random selection of our CRC samples and different genetic and other possible predisposing factors triggering colorectal carcinogenesis. A similar finding to ours was reported by Mylonas et al. [38] who found the highest inhibin expression in secretory endometria and endometrial hyperplasia, with a continuous decrease of expression in atypical hyperplasia; then the lowest expression in uterine adenocarcinomas.

We detected inhibin expression in 40% of chronic colitis and 26.7% of UC cases. De la Fuente-Granada et al. [39] supposed a role of inhibin in inflammation and immune system regulation. They proposed that inhibin controls the recruitment, maintenance, and de novo production of regulatory T lymphocytes (Tregs) in the gut mucosa. The constitutive presence of Tregs is required for the prevention of colitis. Also, a study by El-Gendi et al. [40] revealed that inhibin serum levels correlated positively with the activity of chronic inflammatory diseases.

Dysplastic changes associated with UC and high-grade dysplasia associated with adenomas were associated with higher inhibin expression than their counterparts without dysplasia. This finding may indicate a possible role of inhibin in the initiation of dysplastic or neoplastic changes on top of chronic inflammatory conditions or

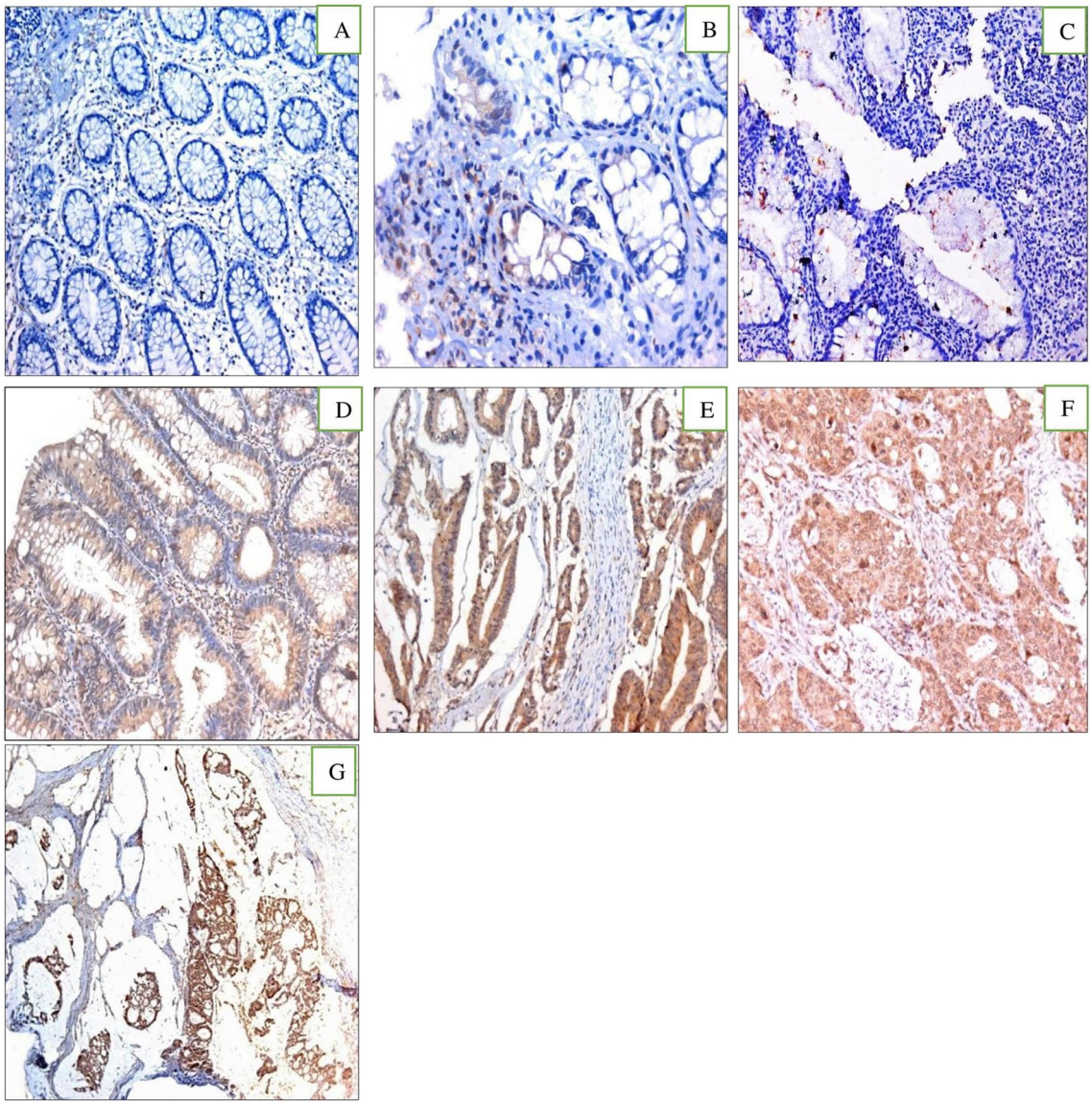


**Fig. 3** Tob-1 expression **a** negative expression in control (×200), **b** positive expression in chronic colitis(×200), **c** positive expression in UC, **d** positive expression in adenoma (×200), **e** positive expression in low-grade CRC (×200), **f** positive expression in high-grade CRC (×200), **g** positive expression in mucinous carcinoma (×50). UC; ulcerative colitis, LGD; low-grade dysplasia, HGD; high-grade dysplasia, CRC; Colorectal cancer

prior dysplastic alterations, respectively. To resolve this controversy about the role of inhibin in carcinogenesis, Ball et al. [41] proposed the hypothesis that inhibin can have a dual role in carcinogenesis as both a tumor suppressor and a pro-oncogenic factor.

### 4.3 Tob-1 expression

Tob–1 is a tumor suppressor protein that is downregulated in certain types of tumors such as breast, pancreatic, lung, thyroid, and stomach cancers [42], however, its role in CRC is not well-defined.

We detected Tob-1 expression in 83.3% of CRCs that was expressed as cytoplasmic and/or nuclear immunopositivity. Our finding agreed with Li et al. [23] who stated that Tob-1 expression was significantly higher in CRCs than in normal tissues and was mainly of cytoplasmic location. Contrary to our findings, studies regarding cancers other than CRC reported decreased expression of Tob-1 in cancer tissues compared to normal tissues in gastric cancer [43], thyroid cancer [21], and pancreatic cancer [22]. These expressions were mainly nuclear. This controversy is explained by Li et al. [23] who suggested that nuclear Tob-1 has an

**Table 4** Correlation between p21, inhibin, and Tob-1 expression with clinicopathological features

| | p21 expression | Inhibin expression | Tob-1 expression | |
|---|---|---|---|---|
| Sex | Correlation Coefficient | 0.191* | − 0.017 | − 0.028 |
| | Sig. (2-tailed) | 0.024 | 0.838 | 0.739 |
| Age category | Correlation Coefficient | 0.035 | 0.110 | 0.178* |
| | Sig. (2-tailed) | 0.682 | 0.195 | 0.036 |
| CRC differentiation | Correlation Coefficient | 0.110 | – | − 0.150 |
| | Sig. (2-tailed) | 0.563 | – | 0.428 |
| CRC type | Correlation Coefficient | − 0.238 | – | 0.293 |
| | Sig. (2-tailed) | 0.206 | – | 0.116 |
| Adenoma | Correlation Coefficient | 0.535** | − 0.367* | − 0.327 |
| | Sig. (2-tailed) | 0.002 | 0.046 | 0.077 |
| Ulcerative colitis | Correlation Coefficient | 0.234 | − 0.202 | − 0.499** |
| | Sig. (2-tailed) | 0.212 | 0.284 | 0.005 |
| p21 expression | Correlation Coefficient | 1.000 | 0.139 | − 0.038 |
| | Sig. (2-tailed) | – | 0.102 | 0.659 |
| Inhibin expression | Correlation Coefficient | 0.139 | 1.000 | 0.177* |
| | Sig. (2-tailed) | 0.102 | | 0.036 |
| Tob-1 expression | Correlation Coefficient | − 0.038 | 0.177* | 1.000 |
| | Sig. (2-tailed) | 0.659 | 0.036 | – |

*CRC* Colorectal cancer

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

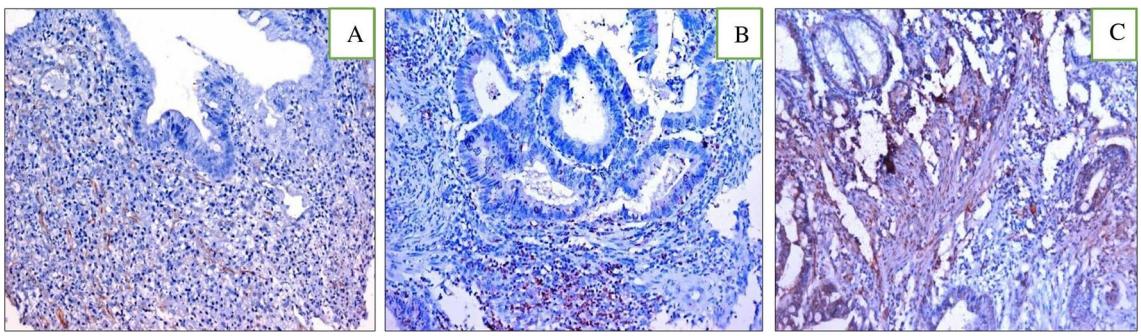


**Fig. 4** Tob-1 positive expression in stromal cells of colonic lesions **a** UC, **b** colonic adenoma, **c** CRC. UC; ulcerative colitis, CRC; Colorectal cancer

antiproliferative effect and anti-cancer activity, meanwhile, Tob-1 is mainly located in the cytoplasm of colon cancer cells where it binds to β-catenin and activates Wnt/β-catenin signaling, which in turn increases Tob-1 expression, thus forming a positive feedback loop to promote cell proliferation.

High-grade CRCs showed significantly higher expression than low-grade ones. This goes with Li et al. [23] who stated that Tob-1 expression is increased with poor differentiated CRCs. Moreover, all examined mucinous tumors were positive for Tob-1. This can be related to the fact that mucinous CRCs are frequently associated with poor differentiation, increased cell proliferation, and resistance to apoptosis, which according to Li et al. [23] is a character of cytoplasmic Tob-1 expression.

Tob-1 expression was insignificantly higher in adenomas with HGD compared to LGD. This can be explained by the role played by Tob-1 in cell proliferation and the promotion of cancer development.

In our study, no Tob-1 expression was detected in controls, while expression was significantly higher in UC than in chronic colitis, and in UC with dysplasia than without dysplasia. This is in contrast to the findings of Fonseca-Camarillo et al. [24] who found a significantly higher expression of Tob-1 in control patients compared to UC.

We noticed Tob-1 expression in stromal tissue and inflammatory cells of the studied chronic colitis, UC, adenoma, and CRC specimens. Baranzini et al. [44] stated that Tob-1 has a key role in T-cell activation and T-helper 17 (Th17) cell function, and is linked to immune-related disorders. The role of Tob-1 in inflammatory conditions needs to be well-studied in ongoing research.

In our study, no statistical relationship was detected between demographic parameters (age and sex) with p21 and inhibin positivity. This agrees with Pasz-Walczak et al. [30] and He et al. [37], respectively. Tob-1 positivity was correlated with age, but not sex, a finding that differs from Fonseca-Camarillo et al. [24] who showed no statistical significance in Tob-1 expression with both age and sex.

In summary, high-grade CRCs, adenomas with HGD, and UC with dysplasia showed lower p21 expression and higher Tob-1 expression than their counterparts (low-grade CRCs, adenomas with LGD, UC without dysplasia). Therefore, decreased p21 expression and increased Tob-1 expression may be an indicator of the progression to more aggressive forms. In our examined specimens cytoplasmic localization of Tob-1 can be related to its oncogenic role.

Furthermore, adenomas with HGD, and UC with dysplasia showed higher inhibin expression than their counterparts (adenomas with LGD, UC without dysplasia). Therefore, increased inhibin expression in these lesions may be an indicator of progression of the disease. Meanwhile, no inhibin expression was detected in CRCs.

This study is one of the first studies that analyzed the expression of inhibin in both benign and malignant colorectal lesions and found that expression of inhibin in CRC is down-regulated as reported in many cancer types. Higher expression of inhibin in inflammatory colonic lesions compared to adenoma and cancer suggests a role for inhibin in immune system modulation and inflammation. More research is required to further understand the role of inhibin in cancer pathogenesis.

Our work has the advantage of demonstrating p21, inhibin, and Tob-1 expressions in both benign and malignant colonic lesions in the same patients which might lead to more significant results; nevertheless, the study was limited by the small number of cases investigated.

## 5 Conclusions

Estimating the expression of p21, inhibin, and Tob-1 can be used to diagnose and predict the likelihood of carcinogenesis in premalignant colonic lesions and identify patients at increased risk of developing CRC and they might provide targets for therapeutic intervention. To date, no targeted therapies have been identified against these proteins. More research is required to understand the biology of these proteins, their interactions, and their roles in the pathogenesis of cancer.

### Abbreviations

| | |
|---|---|
| CRC | Colorectal cancer |
| UC | Ulcerative colitis |
| C | Crohn's disease |
| IBD | Inflammatory bowel disease |
| CAC | Cancer-associated colitis |
| CDK | Cyclin-dependent kinase |
| LGD | Low-grade dysplasia |
| HGD | High-grade dysplasia |
| IRB | Institutional review board |

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### Author contributions

N.S.H. conception, study design, writing the manuscript, performing IHC procedure, acquisition, analysis, and interpretation of IHC data. M.M. conception, study design, reviewing the manuscript, and providing p21 and inhibin antibodies. D.E. conception, study design, and reviewing the manuscript. A.M.A. drafting the manuscript and collecting data and material. M.M.Y. collecting data and providing Tob-1 antibody. All authors approved the version to be published; and agreed on the journal to which the article has been submitted.

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### Availability of data and materials

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

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (IRB) for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland (2013). IRB approval number FWA00010609. This retrospective study was performed using archival specimens that were anonymized before the study. There is no need to obtain patient consent.

### Consent for publication

Non-applicable.

### Competing interests

The authors declare that they have no conflict of interest.

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