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Expression of *Interleukin 23* and *Interleukin 28* in Mucinous & Non-Mucinous Colorectal Carcinoma and Their Relation to Clinicopathological Features and Prognosis

Abd Al-Rahman Mohammad Foda[1,2], Rania Rifat Abdel-Maqsoud[3], Doaa Elsayed Abdelaziz Salama[3,4]*, Amira Nasr Ismail Elsokary[3], Azza Kamal Taha[3]

Abstract

Introduction: Interleukin *(IL)-28*, a type III interferon related to the *IL-10* family, shares multiple structural and functional characteristics with *IL-22*, another member of the *IL-10* cytokine family. Previous research identified *IL-22* as a downstream effector of *IL-23* in colorectal tumorigenesis. However, data on *IL-28* expression in colorectal cancer (CRC) and its relation to clinicopathological features, prognosis, and *IL-23* expression remain limited. **Methods:** *IL-23* and *IL-28* immunohistochemical reactivity was evaluated in 75 specimens of colorectal mucinous adenocarcinoma (MA) and 75 specimens of non-mucinous adenocarcinoma (NMA) using the high-density manual tissue microarray method. Clinicopathological features and survival data were statistically analyzed. **Results:** MA exhibited higher *IL-28* and *IL-23* expression than NMA; however, this was statistically significant only for *IL-23*. *IL-23* and *IL-28* positivity rates showed a highly significant interrelation in MA only. Within the NMA group, no significant association was observed between *IL-23* or *IL-28* expression and clinicopathological features or survival. In the MA group, high *IL-23* expression was significantly associated with positive lymphovascular invasion, advanced pathological tumor (pT) stage, late TNM stage, and decreased disease-free survival and overall survival. High *IL-28* expression was significantly associated with advanced pT stage, lymph node spread, advanced TNM stage, and decreased disease-free survival and overall survival. **Conclusion:** For the first time, the expression of *IL-23* and *IL-28* has shown a highly significant interrelation in MA patients, suggesting a possible interplay between them. High *IL-23* and *IL-28* expressions may have adverse prognostic effects on survival in MA. Molecular studies are necessary to further investigate the interaction between *IL-23* and *IL-28* in CRC.

Keywords: *IL-23- IL-28-* Mucinous- Non-mucinous- Colorectal adenocarcinoma

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Introduction

Colorectal adenocarcinoma is the third most common malignancy worldwide, with limited treatment options [1, 2]. Mucinous adenocarcinoma (MA) is a distinct subtype of colorectal adenocarcinoma in which at least half of the neoplasm consists of extracellular mucinous pools (colloid carcinoma) or signet ring cells with intracellular mucin (signet ring carcinoma). It accounts for 5-20% of CRC cases [3]. MA differs from non-mucinous adenocarcinoma (NMA) in clinical, morphological, and molecular features and has been long associated with a poor therapeutic response and an unfavorable prognosis [4-6]. Previous research has demonstrated that inflammatory mediators regulate multiple critical aspects of carcinogenesis, including tumor cell proliferation, survival, and dissemination [2].

IL-23, a member of the interleukin-12 superfamily [7], consists of a unique p19 subunit and a common p40 subunit, which is shared with its relative IL-12. It is primarily secreted by activated dendritic cells and macrophages, playing a role in mucosal immunity [8, 9]. *IL-23* has been implicated in the progression of chronic inflammation through the activation of T helper 17 (Th17) cells [10] and has been associated with several autoimmune disorders, including inflammatory bowel disease (IBD) [8], multiple sclerosis [11], and rheumatoid arthritis [12]. Recent studies suggest that *IL-23*-mediated effects contribute to tumor dissemination in various tissues. For example, *IL-23* promoted liver cancer cell dissemination through the upregulation of matrix metalloproteinase 9 (MMP-9) [13]. Moreover, an

*[1]Anatomic Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. [2]Department of Pathology, General Medicine Practice Program, Batterjee Medical College, Jeddah 21442, Saudi Arabia. [3]Pathology Department, Faculty of Medicine for Girls (Cairo), Al-Azhar University, Egypt. [4]Pathology Department, School of Medicine, Badr University in Cairo (BUC), Egypt. *For Correspondence: dr.doaa.salama2007@gmail.com*

anti-*IL-23*p19 monoclonal antibody significantly inhibited the spread of lung carcinoma cells in a mouse model, indicating a pro-metastatic role of *IL-23* [14].

IL-28, known as IFN-λ, belongs to the recently identified type III IFN family [15, 16]. It is primarily secreted by epithelial cells following viral infection or by plasmacytoid dendritic cells. Other sources include antigen-presenting cells, Th17 cells, and neutrophils [17, 18]. IFN-λ has been suggested to exert dual effects in malignancy, with its anti-cancer properties demonstrated in specific tumor models, such as melanoma, lung cancer, and hepatocellular carcinoma [19, 20]. Conversely, IFN-λ has been shown to promote the dissemination of bladder carcinoma cells by upregulating matrix metalloproteinase 9 (MMP-9) [21]. Furthermore, IFN-λ induces angiogenesis, epithelial-mesenchymal transition, and cancer metastasis through a STAT3-dependent mechanism [22]. IFN-λ signals through its receptor, composed of IFNLR1 and *IL-10*R2. *IL-10*R2 is a receptor subunit shared by members of the *IL-10* family [23, 24]. Therefore, IFN-λ is considered part of the *IL-10* superfamily [25-27].

In CRC studies, *IL-22* has been identified as a downstream effector of *IL-23* in the development of colon carcinogenesis [28-31]. *IL-22*, a member of the *IL-10* cytokine superfamily, signals through the *IL-10*R2 chain, which is overexpressed in CRC tissues and utilized by tumor cells to activate STAT3, promoting survival and proliferation. Among the ligands for *IL-10*R2, in addition to *IL-10* and *IL-22*, IFN-λ shares this receptor chain [32]. Data regarding the role of IFN-λ in CRC are limited and warrant further investigation [2]. Moreover, the relationship between *IL-23* and *IL-28* in CRC has not yet been described. The present study aimed to investigate the relationship between *IL-23* and *IL-28* in CRC and their association with the clinicopathological features and prognosis of colorectal adenocarcinoma by evaluating their immunohistochemical expression in colorectal MA and NMA using the manual tissue microarray (TMA) method.

Materials and Methods

Cases

This retrospective study was conducted on 150 resected CRC specimens obtained from the Gastroenterology Center in Mansoura, Egypt, between 2007 and 2011. These included 75 specimens of MA (56 mucoid adenocarcinomas and 19 signet ring cell carcinomas) and 75 specimens of NMA (47 ordinary adenocarcinomas and 28 adenocarcinomas with mucoid differentiation < 50%). Clinico-pathological data were reviewed from patients' files and included age, sex, tumor site, size, gross appearance, histological type, microscopic tumor borders, lymphovascular emboli, perineural invasion, pathological T stage, lymph node (LN) spread, distant metastasis (M), and TNM stage. All slides were re-evaluated. During case selection, cases with insufficient clinicopathological information, pre-operative therapy, or those composed entirely of hypocellular mucin lakes were excluded.

Tissue microarray design

Three TMA paraffin blocks were constructed using the mechanical pencil tip method and its modification, as previously described by Shebl et al. [33] and Foda [34]. For each specimen, three cores, each 0.8 mm in diameter, were obtained. Fifty cores of non-neoplastic colorectal mucosa were included as controls. Sections of 4 μm thickness were prepared for hematoxylin and eosin staining and immunostaining.

Immunostaining

Sections were deparaffinized and then incubated with 0.3% hydrogen peroxide in methanol for 30 minutes. This was followed by heating in an EDTA buffer solution (pH = 8.0) for 30 minutes using a microwave. Subsequently, the indirect immunoperoxidase method was performed using anti-*IL-23* antibody (ab45420, rabbit polyclonal, Abcam; 1:100 dilution) and anti-*IL-28* antibody (BZ16031, rabbit polyclonal, Bioworld Technology, Inc.; 1:100 dilution). Primary antibodies were applied for 30 minutes at room temperature. The immunoperoxidase technique was then carried out using the ImmunoPure Ultra-Sensitive ABC Peroxidase (catalog no. 32052; Thermo Scientific, UK), with diaminobenzidine as the chromogen.

Assessment of Immunostaining

The immunoreactivity of *IL-23* and *IL-28* was semi-quantitatively evaluated for each specimen. For both *IL-23* and *IL-28*, the intensity and extent of positive tumor cells were scored for each core. Cytoplasmic and/or nuclear immunoreactivity was considered positive. The authors independently assessed immunostaining. Immunoreactivity intensity was scored as follows: 0 = no staining, 1 = faint staining, 2 = moderate, and 3 = strong. The proportion of positive neoplastic cells was graded as follows: 0 = 0% positive tumor cells, 1 = 1-10%, 2 = 11-50%, 3 = 51-80%, and 4 = > 80%. The intensity score was multiplied by the proportion score to calculate a final immunohistochemical score (IHS) ranging from 0 to 12. IHS scores were interpreted as follows: 9-12 = strong immunoreactivity, 5-8 = moderate, 1-4 = weak, and 0 = negative [35]. The mean score for each core of each specimen was calculated. For data interpretation, cases were classified into a low expression group (score = 0-4) and a high expression group (score = 5-12).

Statistical methodology

Data were analyzed using the SPSS statistical package version 25 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range, as appropriate. Qualitative data were expressed as frequency and percentage. The chi-square test (Fisher's exact test) was used to assess significant differences between MA and NMA cases in clinicopathological and histological features, as well as the relationship of *IL-23* and *IL-28* expression with these features within each group. The Kaplan–Meier method was used to estimate survival probabilities, and the log-rank test was applied to compare survival probabilities between the two groups. All tests were two-tailed, and a p-value ≤ 0.05 was considered significant. Last seen or death was considered the endpoint

for cases. Disease-free survival (DFS) was defined as the period between diagnosis and the occurrence of disease recurrence (or the date last seen disease-free). Overall survival (OS) was defined as the period between diagnosis and the date last seen or death.

Results

The current study included 150 specimens of colonic adenocarcinoma. Ages ranged from 20 to 80 years (mean = 52.7 years). The study comprised 93 males and 57 females. The clinicopathological and histopathological features of 75 MA specimens and 75 NMA specimens are presented in Table 1. MA was significantly associated with older age (P = 0.017), advanced pT stage (P = 0.008), advanced pN stage (P = 0.008), and decreased tumoral neutrophils (P < 0.001) compared to NMA. All cases were stained for *IL-23* and *IL-28*. Immunohistochemical expression of *IL-23* and *IL-28* was detected as cytoplasmic and/or nuclear brown staining in tumor cells (Figures 1 and 2). MA exhibited higher expression of *IL-23* (68%) and *IL-28* (65.3%) compared to NMA (50.7% and 52%, respectively); however, this difference was statistically significant only for *IL-23* (P = 0.030) (Table 2). A highly significant association between *IL-23* and *IL-28* positivity rates was observed in MA cases (P < 0.001), but not in NMA cases (P = 0.16). Approximately 82.4% of MA cases with high *IL-23* expression showed high *IL-28* expression (Table 3). The relationship between *IL-23* and *IL-28* expression and clinicopathological and histological features was examined within each group. In the NMA group, no significant association was found between *IL-23* or *IL-28* expression and any clinicopathological characteristics. In contrast, in the MA group, high *IL-23* expression was significantly associated with positive lymphovascular emboli (P = 0.05), advanced pT stage (P = 0.01), and advanced TNM stage (P = 0.03). High *IL-28* expression was significantly associated with advanced pT stage (P = 0.001), nodal spread (P = 0.008), and advanced TNM stage (P = 0.02) (Tables 3 and 4). The remaining parameters showed no significant relationship with *IL-23* or *IL-28* expression within either group (data not shown).

To investigate the effect of *IL-23* and *IL-28* expression on prognosis, we analyzed differences in DFS and OS between high and low *IL-23* and *IL-28* expression within each group. In the non-mucinous group, the Kaplan–Meier test revealed no significant impact of *IL-23* or *IL-28* expression on DFS or OS (p = 0.39, log-rank [Mantel-

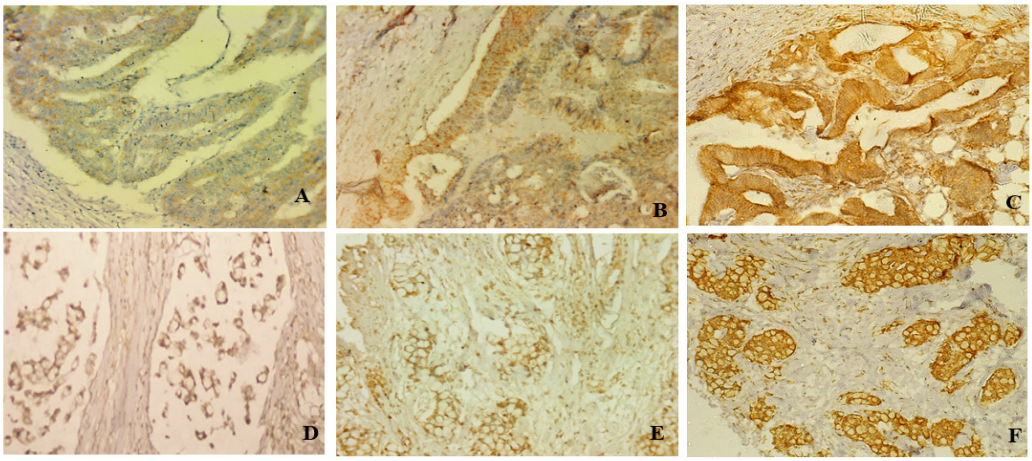

Figure 1. *IL-23* Expression in CRC (×200). (A) Weak staining in NMA. (B) Moderate staining in NMA (C) Strong staining in NMA. (D) Weak staining in MA. (E) Moderate staining in MA (F) Strong staining in MA.

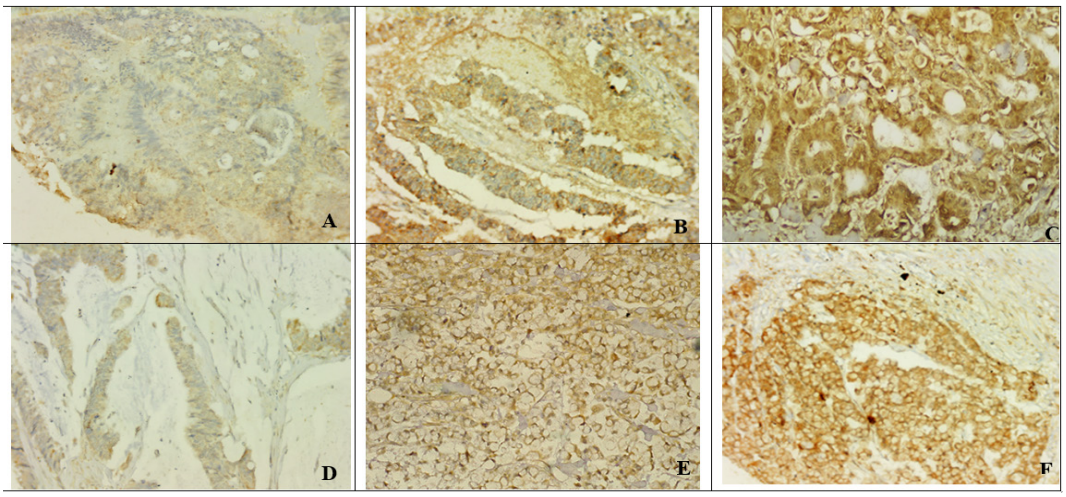

Figure 2. *IL-28* Expression in CRC (×200). (A) Weak staining in NMA. (B) Moderate staining in NMA (C) Strong staining in NMA. (D) Weak staining in MA. (E) Moderate staining in MA (F) Strong staining in MA.

Table 1. Clinicopathological Features of 150 Cases of Mucinous & Non-Mucinous Colorectal Carcinomas

	Non- mucinous [(a)]		mucinous [(b)]		chi-square	P Value
	No.	(%)	No.	(%)		
Age						
<40	10	13.3%	22	29.3%	5.720	0.017*
≥40	65	86.7%	53	70.7%		
Gender						
Male	48	64.0%	45	60.0%	0.255	0.614
Female	27	36.0%	30	40.0%		
Tumor size						
<6	46	61.3%	36	48.0%	2.690	0.101
≥6	29	38.7%	39	52.0%		
Depth of invasion						
T1/T2	18	24%	6	8%	7.143	0.008*
T3/T4	57	76%	69	92%		
LN						
N0	38	50.7%	27	36.0%	9.729	0.008*
N1	25	33.3%	19	25.3%		
N2	12	16.0%	29	38.7%		
TNMstage						
I/II	37	49.3%	27	36%	2.725	0.099
III/IV	38	50.7%	48	64%		
Location						
Right	18	24%	23	30.7%	0.887	0.829
Left	6	8%	6	8%		
Recto-sigmoid	45	60%	41	54.7%		
Transverse	6	8%	5	6.7%		
Gross picture						
Fungating	34	45.3%	27	36%	2.218	0.33
Ulcerating	19	25.3%	27	36%		
Annular	22	29.3%	21	28%		
Lymphovascular emboli						
Negative	30	40%	23	30.7%	1.430	0.232
Positive	45	60%	52	69.3%		
Perineural invasion						
Negative	54	72%	48	64%	1.103	0.294
Positive	21	28%	27	36%		
Tumor margins						
Budding	68	90.7%	69	92%	0.084	0.772
Pushing	7	9.3%	6	8%		

LN, lymph node; TNM, tumor-node-metastases; *P value ≤0.05 is significant; **P <0.001 is highly significant; [a], Non-mucinous group includes 47 cases of ordinary adenocarcinoma & 28 cases of adenocarcinoma with mucinous component <50 %; [b], Mucinous group includes 56 cases of mucoid adenocarcinoma & 19 cases of signet ring carcinoma

Table 2. *IL-23 & IL-28* Expressions in NMA & MA

	NMA No. (%)	MA No. (%)	Chi-square (χ^2)	P value
Il-23 expression (150 cases)				
Low	37 (49.3%)	24 (32%)	4.669	0.030*
High	38 (50.7%)	51 (68%)		
IL-28 expression (150 cases)				
Low	36 (48%)	26 (34.7%)	2.749	0.097
High	39 (52%)	49 (65.3%)		

NMA, non-mucinous adenocarcinoma; MA, mucinous adenocarcinoma; *P value ≤0.05 is significant

Table 3. Relation of *IL-23* Expression to Selected Clinicopathological & Histological Parameters within NMA & MA Groups

	NMA		MA	
	IL-23 Low expression (N=37) No. (%)	*IL-23* High expression (N=38) No. (%)	*IL-23* Low expression (N=24) No. (%)	*IL-23* High expression (N=51) No. (%)
Lymphovascular emboli				
Negative	14 (37.8 %)	16 (42.1 %)	11 (45.8 %)	12 (23.5 %)
Positive	23 (62.2 %)	22 (57.9 %)	13 (54.2 %)	39 (76.5 %)
P value	0.82		0.05*	
Chi-square (χ^2)	0.14		3.81	
Depth of invasion				
T1/T2	11 (29.7 %)	7 (18.4 %)	5 (20.8 %)	1 (2.0 %)
T3/T4	26 (70.3 %)	31 (81.6 %)	19 (79.2 %)	50 (98.0 %)
P value	0.28		FE 0.01*	
Chi-square (χ^2)	131		7.89	
LN				
N0	19 (51.4 %)	19 (50.0 %)	13 (54.2 %)	14 (27.5 %)
N1	11 (29.7 %)	14 (36.8 %)	5 (20.8 %)	14 (27.5 %)
N2	7 (18.9 %)	5 (13.2 %)	6 (25.0 %)	23 (45.0 %)
P value	0.74		0.07	
Chi-square (χ^2)	0.68		5.22	
TNM stage				
I/II	19 (51.4 %)	18 (47.4 %)	13 (54.2 %)	14 (27.5 %)
III/IV	18 (48.6 %)	20 (52.6 %)	11 (45.8 %)	37 (72.5 %)
P value	0.81		0.03*	
Chi-square (χ^2)	0.12		5.05	
IL-28 expression				
Low	21 (56.8 %)	15 (39.5 %)	17 (70.8 %)	9 (17.6 %)
High	16 (43.2 %)	23 (60.5 %)	7 (29.2 %)	42 (82.4 %)
P value	0.16		<0.001**	
Chi-square (χ^2)	2.24		20.38	

NMA, non-mucinous adenocarcinoma; MA, mucinous adenocarcinoma; *P≤0.05 is significant **P <0.001 is highly significant; FE, Fisher's Exact test

Cox] = 0.73; p = 0.38, log-rank [Mantel-Cox] = 0.76; p = 0.88, log-rank [Mantel-Cox] = 0.023; and p = 0.78, log-rank [Mantel-Cox] = 0.024, respectively). In contrast, within the mucinous group, cases with high *IL-23* and *IL-28* expression showed significantly shorter DFS than those with low expression (p = 0.018, log-rank [Mantel-Cox] = 5.57 and p = 0.009, log-rank [Mantel-Cox] = 6.91, respectively). Additionally, cases with high *IL-23* and *IL-28* expression exhibited significantly shorter OS compared to those with low expression (p = 0.009, log-rank [Mantel-Cox] = 6.89 and p = 0.021, log-rank [Mantel-Cox] = 5.36, respectively) (Figures 3-6).

Discussion

Preclinical studies using mice and human cell lines in models of sporadic and inflammation-related CRC have demonstrated a critical role of *IL-23* and its downstream interactor *IL-22* in CRC development and dissemination [28-31]. One study examining *IL-10* signaling genes in matched neoplastic and non-neoplastic tissue pairs from CRC patients reported upregulation of *IL-10*R2 and STAT3 in CRC tissue. *IL-22* signals through the upregulated *IL-10*R2 to activate STAT3, thereby contributing to the development of colorectal tumors. In addition to *IL-10* and *IL-22*, *IL-28* shares this receptor subunit [32]. Furthermore, *IL-28* exhibits a high degree of structural similarity to *IL-22* [36]. Given the close relationship between *IL-22* and *IL-28* and their shared use of the *IL-10*R2 subunit, we speculated that *IL-23* and *IL-28* may be interrelated in colorectal tumorigenesis. Due to the distinct molecular characteristics of MA and NMA, the present study hypothesized that this interrelation may differ between the two types.

The results of the present investigation demonstrated, for the first time, that high expression of *IL-23* and *IL-28* is more frequent in MA than in NMA; however, this difference was statistically significant only for *IL-23* expression. Moreover, the current findings revealed a highly significant association between high expression of *IL-23* and *IL-28* within the MA group. These observations may be explained by the higher prevalence of KRAS

Table 4. Relation of *IL-28* Expression to Selected Clinicopathological & Histological Parameters within NMA & MA Groups

	NMA		MA	
	IL-28 Low expression (N=36) No. (%)	*IL-28* High expression (N=39) No. (%)	*IL-28* Low expression (N=26) No. (%)	*IL-28* High expression (N=49) No. (%)
Lymphovascular emboli				
Negative	15 (41.7 %)	15 (38.5 %)	11 (42.3 %)	12 (24.5 %)
Positive	21 (58.3 %)	24 (61.5 %)	15 (57.7 %)	37 (75.5 %)
P value	0.82		0.12	
Chi-square (χ^2)	0.08		2.5	
Depth of invasion				
T1/T2	8 (22.2 %)	10 (25.6 %)	6 (23.1 %)	0 (0.0 %)
T3/T4	28 (77.8 %)	29 (74.4 %)	20 (76.9 %)	49 (100 %)
P value	0.79		FE 0.001*	
Chi-square (χ^2)	0.12		12.29	
LN				
N0	17 (47.2 %)	21 (53.8 %)	14 (53.8 %)	13 (26.5 %)
N1	13 (36.1 %)	12 (30.8 %)	8 (30.8 %)	11 (22.4 %)
N2	6 (16.7 %)	6 (15.4 %)	4 (15.4 %)	25 (51.0 %)
P value	0.86		0.008*	
Chi-square (χ^2)	0.34		9.56	
TNM stage				
I/II	16 (44.4 %)	21 (53.8 %)	14 (53.8 %)	13 (26.5 %)
III/IV	20 (55.6 %)	18 (46.2 %)	12 (46.2 %)	36 (73.5 %)
P value	0.49		0.02*	
Chi-square (χ^2)	0.66		5.5	

NMA, non-mucinous adenocarcinoma; MA, mucinous adenocarcinoma; *P≤0.05 is significant; FE, Fisher's Exact test

mutations in MA [37] and the role of oncogenic KRAS in activating NF-κB signaling, which, in turn, enhances the transcription of various cytokines and chemokines essential for the initiation and progression of inflammation-induced cancer [38-40]. It has been observed that most CRC cases with a high frequency of KRAS mutations are associated with chronic inflammatory diseases [41] and that MA is more frequently diagnosed in patients with IBD [42]. This hypothesis is supported by a study conducted by Petanidis et al. [43], which demonstrated a significant correlation between high *IL-23* expression and KRAS mutation in CRC patients.

When evaluating *IL-23* expression with clinicopathological parameters and prognosis within each group, none of the examined parameters showed a significant association with *IL-23* expression in the NMA group. In contrast, within the MA group, a significant association was observed between high *IL-*

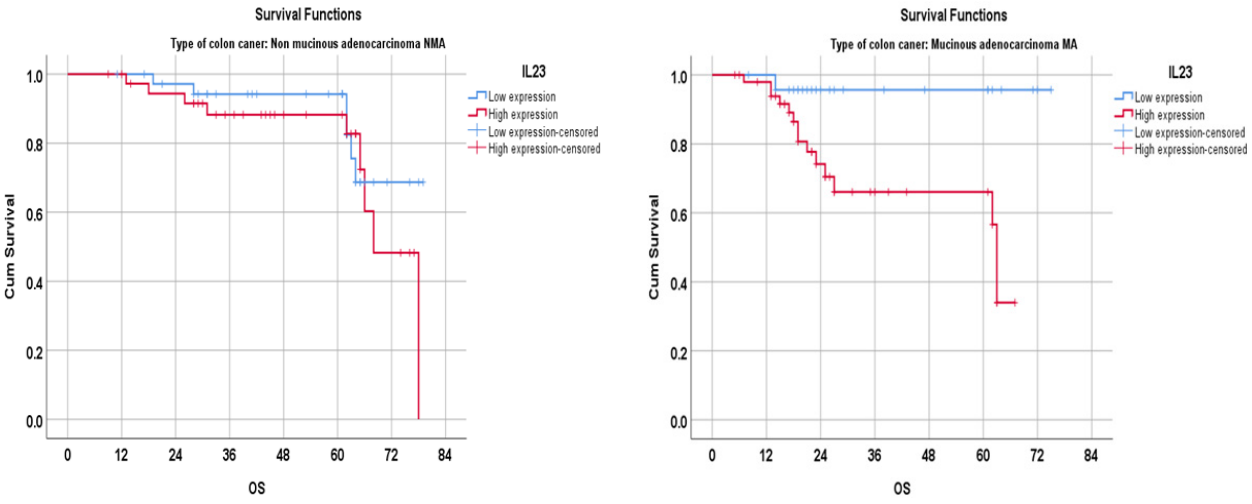

Figure 3. The Overall Survival Function in Patients from NMA & MA Groups in Relation to *IL-23* Expression in Tumor Cells

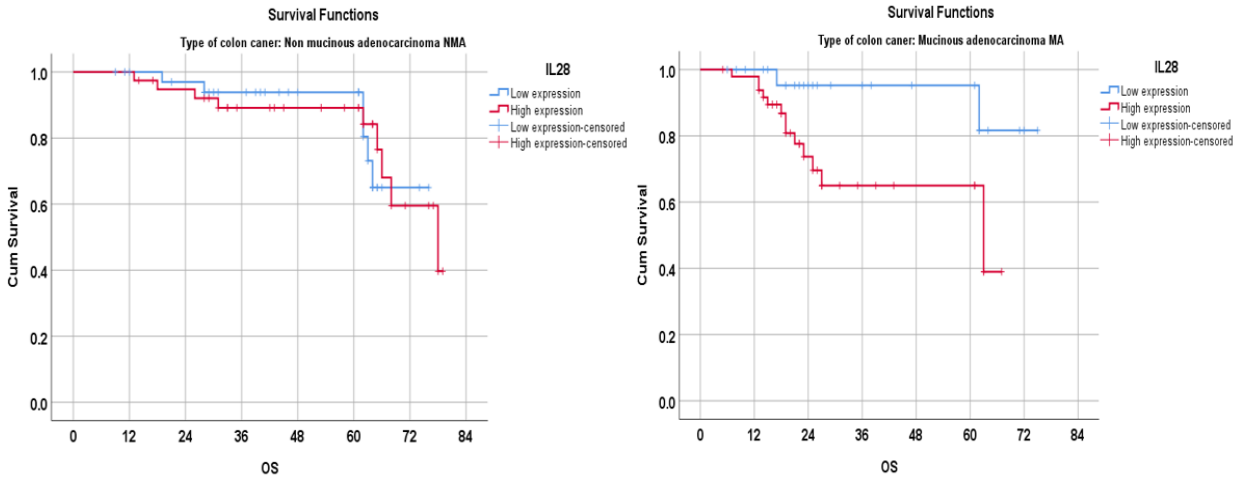

Figure 4. The Overall Survival Function in Patients from NMA & MA Groups in Relation to *IL-28* Expression in Tumor Cells

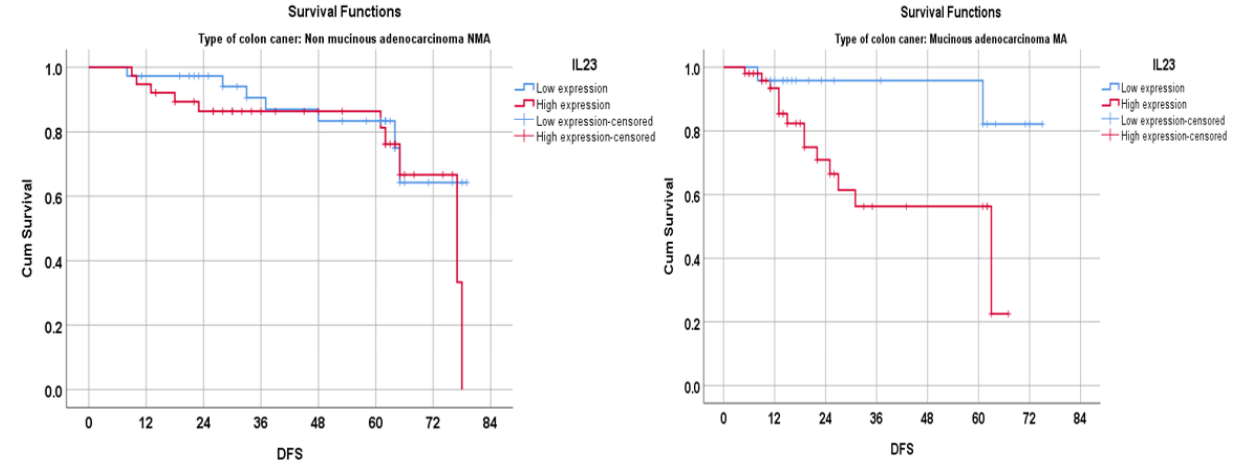

Figure 5. The Disease-Free Survival Function in Patient from NMA and MA Groups in Relation to *IL-23* Expression in Tumor Cells

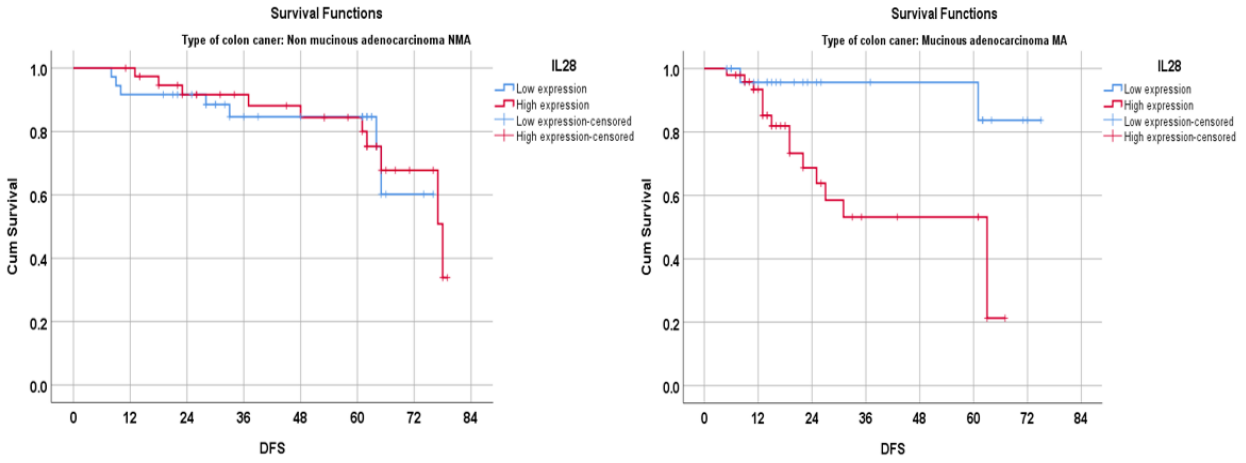

Figure 6. The Disease-Free Survival Function in Patients from NMA and MA Groups in Relation to *IL-28* Expression in Tumor Cells

23 expression and lymphovascular invasion, advanced depth of invasion, late TNM stage, and shorter DFS and OS. This observation is consistent with previously reported progressive increases in *IL-23* expression from non-neoplastic to neoplastic tissues [28, 44] and the established pro-oncogenic role of *IL-23*, which is mediated by the activation of STAT3 and leads to the upregulation of MMPs, VEGF, and Bcl-x, thereby promoting tumor survival and metastasis [45]. Similarly, Hu et al. [46] reported a significant association between high *IL-23* expression in CRC and increased depth of invasion, advanced TNM stage, and shorter DFS and OS. Furthermore, the current findings align with those of Petanidis et al. [43], who found a significant relationship between *IL-23* expression and KRAS-linked, stage-specific overexpression in CRC .

Conversely, Helbling et al. [47] found no significant association between *IL-23* expression in CRC and clinicopathological characteristics or outcomes. They classified cases into negative and positive groups based on a threshold value for the average percentage of positive neoplastic cells [47]. In contrast, the present study incorporated both the intensity and percentage of stained tumor cells in assessing *IL-23* expression. Variations in *IL-23* staining intensity may help distinguish between different stages of CRC and their outcomes.

Regarding the relationship between *IL-28* expression and the clinicopathological aspects and prognosis within each group, the results of the current study showed no significant association between *IL-28* expression and any of the examined parameters within the NMA group. In contrast, within the MA group, our results revealed a significant association between high *IL-28* expression and advanced pT stage, nodal spread, late TNM stage, and decreased DFS and OS. This finding is consistent with that of Sakahara et al. (2019), who demonstrated that activating mutations of KRAS contribute to tumorigenesis and drug resistance in CRC through upregulation of IFN/STAT signaling [48]. Additionally, it has been shown that the tumor suppressor gene BMP5 exerts its inhibitory effect in CRC by suppressing *IL-28*A expression, thereby blocking the JAK-STAT pathway [49]. Moreover, the present results align with those of Wang et al. (2023), who conducted a comprehensive analysis of IFN-λ genes across different malignancies, including colorectal adenocarcinoma, and concluded that endogenous IFN-λ expression is associated with poor prognosis, with IFN-λ2 and IFN-λ3 serving as independent prognostic factors. They further demonstrated that IFN-λ cooperates with STAT1, STAT2, and STAT3 to activate the JAK-STAT signaling pathway by upregulating the expression of IL24, IL10, IL26, and IL10RB, thereby promoting tumor progression [50].

In contrast to our findings, anti-proliferative effects of IFN-λ have been reported in colorectal adenocarcinoma models [51]. Moreover, Cheng et al. [52] demonstrated that *IL-28*B significantly inhibits CRC progression by suppressing the polarization of M2 macrophages .

The demonstrated pro- and anti-tumorigenic effects of IFN-λ in various CRC studies can be attributed to its emerging dual role in carcinogenesis. Several factors have been identified as regulators of IFN-λ function in cancer. One such factor is the level and duration of IFN-λ signaling in the tumor microenvironment. While low levels and short duration induce acute inflammation and tumor cell eradication, high levels and prolonged signaling result in chronic inflammation and inflammation-associated cancer [53]. Another factor involves the differential effects of IFN-λ on various immune cell types; IFN-λ can modulate the activity of both immune-stimulating and immune-suppressive cells [54]. Additionally, the type of interferon-stimulated genes (ISGs) activated may influence the effect of IFN-λ on cancer immunity. Some ISGs encode molecules that enhance immune responses, whereas others encode molecules that contribute to immune checkpoint blockade and immune suppression [53].

In conclusion, expressions of both *IL-23* and *IL-28* showed a highly significant interrelation in MA patients, suggesting a potential interplay between them. High expressions of *IL-23* and *IL-28* may have adverse prognostic implications for survival in MA. Further molecular studies are necessary to elucidate the interaction between *IL-23* and *IL-28* in colorectal tumorigenesis.

Author Contribution Statement

Study design: Abd Al-rahman Mohammad Foda; Data interpretation: Abd Al-rahman Mohammad Foda, Azza kamal Taha & Rania Refat Abdel Maqsoud; Drafting of the manuscript: Azza kamal Taha, Doaa E.A. Salama, Amira Nasr Ismail Elsokary & Rania Refat Abdel Maqsoud; Final revision of the manuscript: Abd Al-rahman Mohammad Foda, Doaa E.A. Salama, Amira Nasr Ismail Elsokary, Azza kamal Taha & Rania Refat Abdel Maqsoud.

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Data Availability Statement

The data that support the findings of this study are included in this published article & available on reasonable request from the corresponding author

Ethical Approval Statement

This research was conducted in accordance with the ethical guidelines of the Research Ethics Committee of the Faculty of Medicine for Girls, Al-Azhar University, and in adherence to the 1964 Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine for Girls, Cairo, Al-Azhar University, under approval number (IRB: 2023112161), dated 12-11-2024.

Disclosure Statement

The authors declare that they have no conflict of interest.

Abbreviations

Interleukin (IL), Colorectal Cancer (CRC), Mucinous Adenocarcinoma (MA), Non-Mucinous Adenocarcinoma (NMA), pathological tumor (pT), pathological node (pN), Tumor-Node-Metastasis (TNM), T helper 17 (Th17), Inflammatory Bowel Disease (IBD), Matrix Metalloproteinase 9 (MMP 9), Interferon (IFN), Tissue microarray (TMA), Lymph Node (LN), Ethylenediaminetetraacetic acid (EDTA), Immunohistochemical Score (IHS), Disease-free survival (DFS), Overall survival (OS), Signal Transducer & Activator of Transcription (STAT), Kirsten Rat Sarcoma (KRAS), Nuclear Factor Kappa Beta (NF-kB), Bone Morphogenetic Protein 5 (BMP5), Janus kinase (JAk), Interferon stimulated genes (ISGs).

References

1. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174. https://doi.org/10.1016/j.tranon.2021.101174.
2. Braumüller H, Mauerer B, Andris J, Berlin C, Wieder T, Kesselring R. The cytokine network in colorectal cancer: Implications for new treatment strategies. Cells. 2022;12(1):138-73. https://doi.org/10.3390/cells12010138.
3. Nagtegaal ID, Arends MJ, Odze RD, Lam AK. Tumors of the colon and rectum. In: WHO Classification of Tumors Editorial Board. Digestive System Tumors 5th Ed. Lyon: IARC Press; 2019. p. 157-91.
4. Yamaguchi T, Taniguchi H, Fujita S, Sekine S, Yamamoto S, Akasu T, et al. Clinicopathological characteristics and prognostic factors of advanced colorectal mucinous adenocarcinoma. Histopathology. 2012;61(2):162-9. https://doi.org/10.1111/j.1365-2559.2012.04235.x.
5. Foda AA-RM, El-Hawary AK, Abdel-Aziz A. Differential expression of matrix metalloproteinase-13 in mucinous and nonmucinous colorectal carcinomas. Ann Diagn Pathol. 2013;17(4):347-51. https://doi.org/10.1016/j.anndiagpath.2013.04.003.
6. Foda AA, El-Hawary AK, Abdel Aziz A, Hosni A, Zalata KR, GadoAI. Rare Mucinous colorectal adenocarcinoma: analysis of the epidemiological factors in relation to survival in Egyptian patients. Am J Cancer Epidemiol Prev. 2014;2(1):10–9
7. Xu M, Mizoguchi I, Morishima N, Chiba Y, Mizuguchi J, Yoshimoto T. Regulation of antitumor immune responses by the il-12 family cytokines, il-12, il-23, and il-27. Clin Dev Immunol. 2010;2010. https://doi.org/10.1155/2010/832454.
8. Hue S, Ahern P, Buonocore S, Kullberg MC, Cua DJ, McKenzie BS, et al. Interleukin-23 drives innate and t cell-mediated intestinal inflammation. J Exp Med. 2006;203(11):2473-83. https://doi.org/10.1084/jem.20061099.
9. Yen D, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, et al. Il-23 is essential for t cell-mediated colitis and promotes inflammation via il-17 and il-6. J Clin Invest. 2006;116(5):1310-6. https://doi.org/10.1172/jci21404.
10. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. Il-23 drives a pathogenic t cell population that induces autoimmune inflammation. J Exp Med. 2005;201(2):233-40. https://doi.org/10.1084/jem.20041257.
11. Li Y, Chu N, Hu A, Gran B, Rostami A, Zhang GX. Increased il-23p19 expression in multiple sclerosis lesions and its induction in microglia. Brain. 2007;130(Pt 2):490-501. https://doi.org/10.1093/brain/awl273.
12. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for il-23 and il-12 in joint autoimmune inflammation. J Exp Med. 2003;198(12):1951-7. https://doi.org/10.1084/jem.20030896.
13. Li J, Lau G, Chen L, Yuan YF, Huang J, Luk JM, et al. Interleukin 23 promotes hepatocellular carcinoma metastasis via nf-kappa b induced matrix metalloproteinase 9 expression. PLoS One. 2012;7(9):e46264. https://doi.org/10.1371/journal.pone.0046264.
14. Teng MW, von Scheidt B, Duret H, Towne JE, Smyth MJ. Anti-il-23 monoclonal antibody synergizes in combination with targeted therapies or il-2 to suppress tumor growth and metastases. Cancer Res. 2011;71(6):2077-86. https://doi.org/10.1158/0008-5472.Can-10-3994.
15. Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, et al. Ifn-lambdas mediate antiviral protection through a distinct class ii cytokine receptor complex. Nat Immunol. 2003;4(1):69-77. https://doi.org/10.1038/ni875.
16. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, et al. Il-28, il-29 and their class ii cytokine receptor il-28r. Nat Immunol. 2003;4(1):63-8. https://doi.org/10.1038/ni873.
17. Yin Z, Dai J, Deng J, Sheikh F, Natalia M, Shih T, et al. Type iii ifns are produced by and stimulate human plasmacytoid dendritic cells. J Immunol. 2012;189(6):2735-45. https://doi.org/10.4049/jimmunol.1102038.
18. Wolk K, Witte K, Witte E, Raftery M, Kokolakis G, Philipp S, et al. Il-29 is produced by t(h)17 cells and mediates the cutaneous antiviral competence in psoriasis. Sci Transl Med. 2013;5(204):204ra129. https://doi.org/10.1126/scitranslmed.3006245.
19. Steen HC, Gamero AM. Interferon-lambda as a potential therapeutic agent in cancer treatment. J Interferon Cytokine Res. 2010;30(8):597-602. https://doi.org/10.1089/jir.2010.0058.
20. Lasfar A, Gogas H, Zloza A, Kaufman HL, Kirkwood JM. Ifn-λ cancer immunotherapy: New kid on the block. Immunotherapy. 2016;8(8):877-88. https://doi.org/10.2217/imt-2015-0021.
21. Lee SJ, Lee EJ, Kim SK, Jeong P, Cho YH, Yun SJ, et al. Identification of pro-inflammatory cytokines associated with muscle invasive bladder cancer; the roles of il-5, il-20, and il-28a. PLoS One. 2012;7(9):e40267. https://doi.org/10.1371/journal.pone.0040267.
22. Mucha J, Majchrzak K, Taciak B, Hellmén E, Król M. Mdscs mediate angiogenesis and predispose canine mammary tumor cells for metastasis via il-28/il-28ra (ifn-λ) signaling. PLoS One. 2014;9(7):e103249. https://doi.org/10.1371/journal.pone.0103249.
23. Kotenko SV, Langer JA. Full house: 12 receptors for 27 cytokines. Int Immunopharmacol. 2004;4(5):593-608. https://doi.org/10.1016/j.intimp.2004.01.003.
24. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the il-10 family of cytokines in inflammation and disease. Annu Rev Immunol. 2011;29:71-109. https://doi.org/10.1146/annurev-immunol-031210-101312.
25. Uzé G, Monneron D. Il-28 and il-29: Newcomers to the interferon family. Biochimie. 2007;89(6-7):729-34. https://doi.org/10.1016/j.biochi.2007.01.008.
26. Witte K, Witte E, Sabat R, Wolk K. Il-28a, il-28b, and il-29: Promising cytokines with type i interferon-like properties. Cytokine Growth Factor Rev. 2010;21(4):237-51. https://doi.org/10.1016/j.cytogfr.2010.04.002.
27. O'Brien TR, Prokunina-Olsson L, Donnelly RP. Ifn-λ4: The paradoxical new member of the interferon lambda family.

J Interferon Cytokine Res. 2014;34(11):829-38. https://doi.org/10.1089/jir.2013.0136.
28. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, et al. Adenoma-linked barrier defects and microbial products drive il-23/il-17-mediated tumour growth. Nature. 2012;491(7423):254-8. https://doi.org/10.1038/nature11465.
29. Huber S, Gagliani N, Zenewicz LA, Huber FJ, Bosurgi L, Hu B, et al. Il-22bp is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature. 2012;491(7423):259-63. https://doi.org/10.1038/nature11535.
30. Kirchberger S, Royston DJ, Boulard O, Thornton E, Franchini F, Szabady RL, et al. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. J Exp Med. 2013;210(5):917-31. https://doi.org/10.1084/jem.20122308.
31. Wang C, Gong G, Sheh A, Muthupalani S, Bryant EM, Puglisi DA, et al. Interleukin-22 drives nitric oxide-dependent DNA damage and dysplasia in a murine model of colitis-associated cancer. Mucosal Immunol. 2017;10(6):1504-17. https://doi.org/10.1038/mi.2017.9.
32. Khare V, Paul G, Movadat O, Frick A, Jambrich M, Krnjic A, et al. Il10r2 overexpression promotes il22/stat3 signaling in colorectal carcinogenesis. Cancer Immunol Res. 2015;3(11):1227-35. https://doi.org/10.1158/2326-6066.Cir-15-0031.
33. Shebl AM, Zalata KR, Amin MM, El-Hawary AK. An inexpensive method of small paraffin tissue microarrays using mechanical pencil tips. Diagn Pathol. 2011;6:117. https://doi.org/10.1186/1746-1596-6-117.
34. Foda AA. No-cost manual method for preparation of tissue microarrays having high quality comparable to semiautomated methods. Appl Immunohistochem Mol Morphol. 2013;21(3):271-4. https://doi.org/10.1097/PAI.0b013e318268a93f.
35. El-Aswad BEW, Ammar AI, Mahmoud SF, Soliman SS, Abd El-Atty AF. Immunohistochemical evaluation of interleukin-23 and cyclooxygenase-2 in the muscles of mice infected with trichinella spiralis. Trop Biomed. 2020;37(1):75-88.
36. Gad HH, Dellgren C, Hamming OJ, Vends S, Paludan SR, Hartmann R. Interferon-lambda is functionally an interferon but structurally related to the interleukin-10 family. J Biol Chem. 2009;284(31):20869-75. https://doi.org/10.1074/jbc.M109.002923.
37. Luo C, Cen S, Ding G, Wu W. Mucinous colorectal adenocarcinoma: Clinical pathology and treatment options. Cancer Commun (Lond). 2019;39(1):13. https://doi.org/10.1186/s40880-019-0361-0.
38. Hamarsheh Sa, Groß O, Brummer T, Zeiser R. Immune modulatory effects of oncogenic kras in cancer. Nature Communications. 2020;11(1):5439. https://doi.org/10.1038/s41467-020-19288-6.
39. Cullis J, Das S, Bar-Sagi D. Kras and tumor immunity: Friend or foe? Cold Spring Harbor perspectives in medicine. 2018;8(9):a031849. https://doi.org/10.1101/cshperspect.a031849.
40. Karin M, Greten FR. Nf-kappab: Linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005;5(10):749-59. https://doi.org/10.1038/nri1703.
41. Fu X, Wang X, Duanmu J, Li T, Jiang Q. Kras mutations are negatively correlated with immunity in colon cancer. Aging. 2020;13(1):750-68. https://doi.org/10.18632/aging.202182.
42. Hugen N, van Beek JJ, de Wilt JH, Nagtegaal ID. Insight into mucinous colorectal carcinoma: Clues from etiology. Ann Surg Oncol. 2014;21(9):2963-70. https://doi.org/10.1245/s10434-014-3706-6.
43. Petanidis S, Anestakis D, Argyraki M, Hadzopoulou-Cladaras M, Salifoglou A. Differential expression of il-17, 22 and 23 in the progression of colorectal cancer in patients with k-ras mutation: Ras signal inhibition and crosstalk with gm-csf and ifn-γ. PLoS One. 2013;8(9):e73616. https://doi.org/10.1371/journal.pone.0073616.
44. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, et al. Il-23 promotes tumour incidence and growth. Nature. 2006;442(7101):461-5. https://doi.org/10.1038/nature04808.
45. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: Role of stat3 in the tumour microenvironment. Nat Rev Immunol. 2007;7(1):41-51. https://doi.org/10.1038/nri1995.
46. Hu WH, Chen HH, Yen SL, Huang HY, Hsiao CC, Chuang JH. Increased expression of interleukin-23 associated with progression of colorectal cancer. J Surg Oncol. 2017;115(2):208-12. https://doi.org/10.1002/jso.24505.
47. Helbling M, Lukesch A, Haimovici A, Karamitopoulou E, Berger MD, Hädrich M, et al. Investigation of il-23 (p19, p40) and il-23r identifies nuclear expression of il-23 p19 as a favorable prognostic factor in colorectal cancer: A retrospective multicenter study of 675 patients. Oncotarget. 2014;5(13):4671-82. https://doi.org/10.18632/oncotarget.2069.
48. Sakahara M, Okamoto T, Oyanagi J, Takano H, Natsume Y, Yamanaka H, et al. Ifn/stat signaling controls tumorigenesis and the drug response in colorectal cancer. Cancer Sci. 2019;110(4):1293-305. https://doi.org/10.1111/cas.13964.
49. Chen E, Yang F, He H, Li Q, Zhang W, Xing J, et al. Alteration of tumor suppressor bmp5 in sporadic colorectal cancer: A genomic and transcriptomic profiling based study. Mol Cancer. 2018;17(1):176. https://doi.org/10.1186/s12943-018-0925-7.
50. Wang ZZ, Wen XL, Wang N, Li XH, Guo Y, Zhu X, et al. Portraying the dark side of endogenous ifn-λ for promoting cancer progression and immunoevasion in pan-cancer. J Transl Med. 2023;21(1):615. https://doi.org/10.1186/s12967-023-04453-4.
51. Li W, Lewis-Antes A, Huang J, Balan M, Kotenko SV. Regulation of apoptosis by type iii interferons. Cell Prolif. 2008;41(6):960-79. https://doi.org/10.1111/j.1365-2184.2008.00558.x.
52. Cheng D, Wang H, Cheng H, Zhang H, Dong G, Yan F, et al. Il-28b reprograms tumor-associated macrophages to promote anti-tumor effects in colon cancer. Int Immunopharmacol. 2022;109:108799. https://doi.org/10.1016/j.intimp.2022.108799.
53. Zhang X, Wang S, Zhu Y, Zhang M, Zhao Y, Yan Z, et al. Double-edged effects of interferons on the regulation of cancer-immunity cycle. Oncoimmunology. 2021;10(1):1929005. https://doi.org/10.1080/2162402x.2021.1929005.
54. Parker BS, Rautela J, Hertzog PJ. Antitumour actions of interferons: Implications for cancer therapy. Nat Rev Cancer. 2016;16(3):131-44. https://doi.org/10.1038/nrc.2016.14.