




Associations Between *Fusobacterium nucleatum* and *msh2*, *mlh1*, and *msh6* Gene Expression in Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) is a major global health concern, and the link with *Fusobacterium nucleatum* has received considerable attention.

Objectives: This study aimed to explore the prevalence of *F. nucleatum* and to assess the expression of the *msh2*, *mlh1*, and *msh6* genes in CRC patients compared to a control group using real-time PCR.

Methods: Forty CRC patients and twenty individuals from a control group participated in this study. Gastroenterologists collected biopsy specimens from which DNA and RNA were extracted using a specialized tissue extraction kit. Complementary DNA (cDNA) was then synthesized. Real-time PCR was employed to evaluate the expression levels of the *msh2*, *mlh1*, and *msh6* genes and the presence of the *F. nucleatum*-specific 16srRNA gene to determine the relative prevalence of this bacterium in each group.

Results: Results indicated a higher prevalence of the *F. nucleatum*-specific 16srRNA gene in CRC patients than in the control group. Additionally, expression levels of the *msh2*, *mlh1*, and *msh6* genes were significantly higher in the cancer group, suggesting their role in CRC pathogenesis. The distribution of *F. nucleatum* was particularly high in the sigmoid and rectum areas of the colon.

Conclusions: This study underscores the significance of *F. nucleatum* in CRC and provides insights into its association with altered gene expression patterns. Understanding the prevalence of *F. nucleatum* and its impact on *msh2*, *mlh1*, and *msh6* genes may aid in developing improved diagnostic and therapeutic strategies for CRC. Further research is necessary to elucidate these relationships more comprehensively.

Keywords: *Fusobacterium nucleatum*, Mismatch Repair, Colorectal Cancer

1. Background

Colorectal cancer (CRC) is a highly prevalent malignancy worldwide, ranking as the third most common cancer after breast and lung cancers, with a higher incidence observed in men. Various risk factors, including diet, lifestyle, obesity, heredity, and inflammation, contribute to CRC development (1, 2). Microbiota dysbiosis is a common feature in the pathogenesis of certain cancers (3, 4). Notably, an increased presence of *Fusobacterium nucleatum* in CRC

patients compared to healthy individuals has emerged as a significant marker for CRC diagnosis (5). Studies have shown that *F. nucleatum* promotes CRC progression by activating signaling pathways such as E-cadherin/ β -catenin and TLR4/MyD88, and modulating autophagy and immune responses (6). Moreover, *F. nucleatum* is implicated in various molecular events, including microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and tumorigenic mutations in genes such as TP53, BRAF, CHD7, and CHD8,

which may contribute to tumor initiation and progression (7).

Key factors in *F. nucleatum* pathogenesis include Fusobacterium adhesin A (FadA), which facilitates cell attachment, lipopolysaccharides (LPS) that activate proinflammatory genes and promote CRC progression through the release of inflammatory cytokines, and Fusobacterium autotransporter protein 2 (Fap2), another virulence factor enhancing CRC receptor cell binding. Additionally, *F. nucleatum* has been shown to induce epigenetic alterations in CRC (8-10). Reactive oxygen species (ROS) and inflammatory cytokines produced by *F. nucleatum* contribute to the silencing of the mismatch repair protein (*mlh1*) gene, resulting in MSI tumors (11). Dysfunction in this repair system, often arising from epigenetic silencing, leads to an accumulation of mutations in the genome (12). Given the variations in *F. nucleatum* prevalence across different ethnicities and geographical regions, MMR status may differ accordingly (13). Understanding the interplay between *F. nucleatum* and MMR genes may provide valuable insights into the molecular mechanisms of CRC development.

2. Objectives

In this study, we aim to investigate the effects of *F. nucleatum* on the relative expression of *mlh1*, *msh2*, and *msh6* genes in Iranian CRC patients.

3. Methods

3.1. Study Design and Sample Collection

A case-control study design was employed to compare the relative prevalence of *F. nucleatum* and the expression of *mlh1*, *msh2*, and *msh6* genes in CRC patients versus a control group. This research was conducted at the Microbiology Department of Alborz University of Medical Sciences from January 2022 to February 2023. Biopsy specimens were collected from 40 CRC patients and 20 individuals with suspected CRC who underwent colonoscopies. The specimens were obtained by a gastroenterologist from the colon and rectum. Eligibility for the study required CRC patients to be aged 18 or older and to provide written informed consent. Patients who had received systemic chemotherapy were excluded. Specimens were transported to the Microbiology Department in

Transystem tubes containing normal saline and RNA-later and stored at -20°C until further processing.

3.2. DNA and RNA Extraction, and cDNA Synthesis

DNA extraction from the tissue samples was carried out using an extraction kit (Iran ROJE Co.), following the manufacturer's specifications. Bacterial cells were concentrated by centrifugation, and their DNA was extracted. The concentration and purity of the DNA samples were assessed using a spectrophotometer (NanoDrop 2000). RNA was extracted from the specimens using an RNA extraction kit (Iran ROJE Co.) per the manufacturer's instructions. The quality of the extracted RNA was also evaluated using a spectrophotometer (NanoDrop 2000). Complementary DNA was then synthesized from the RNA samples using a cDNA synthesis kit (Iran ROJE Co.).

3.3. Fusobacterium nucleatum Detection

The presence of *F. nucleatum* in the samples was determined using real-time PCR targeting the specific 16srRNA gene of *F. nucleatum* with the following program: Initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. Real-time PCR using universal 16srRNA primers assessed the relative abundance of *F. nucleatum* in CRC and control groups.

3.4. Expression Analysis of *mlh1*, *msh2*, and *msh6* Genes

Real-time PCR was conducted to measure the relative expression levels of the *mlh1*, *msh2*, and *msh6* genes in both CRC and control groups. The expression levels were normalized against a reference gene (*gapdh*) for each sample. Real-time PCR was carried out using the Applied Biosystems 7900 system with SYBR® Select Master Mix (Bioneer, Korea) in 20 µL reactions. The cycle conditions for the *mlh1*, *msh2*, and *msh6* genes included an initial denaturation step of 10 minutes at 95°C, followed by 40 cycles of 20 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C. All reactions were performed in triplicate.

3.5. Statistical Analysis

The presence, relative frequency of *F. nucleatum*, and the relative expression of the *mlh1*, *msh2*, and *msh6* genes were analyzed in both the control group (n = 20)

and the CRC group (n = 40) based on biopsy samples. The relative expression of each MMR gene compared to *gapdh* RNA was determined using the formula $2^{(-\Delta Ct)}$, where ΔCt represents Ct (Target) - Ct (Reference). The fold change of target gene expression was calculated using the below formula:

$$\frac{2^{-(C_t \text{ Target} - C_t \text{ Reference})_{\text{Tumor}}}}{2^{-(C_t \text{ Target} - C_t \text{ Reference})_{\text{Normal}}}} \quad (1)$$

Data were analyzed using SPSS version 21 and GraphPad PRISM software version 8. Quantitative data were summarized as mean \pm standard deviation. The normality of the data was assessed, and, if appropriate, non-parametric analysis of variance (ANOVA) with a significance level (P-value < 0.05) was applied.

4. Results

4.1. Patients and Samples

The cancer group comprised 52% women and 48% men, with median ages of 55 and 65 years for women and men, respectively. The control group consisted of 45% women and 55% men, with the highest age ranges being 30 - 40 for women and 30 - 50 for men. Colonoscopy was performed based on signs and symptoms such as anemia (34%), abdominal pain (31%), blood in the stool (19%), and rectal bleeding (16%). The tissue samples analyzed included adenocarcinoma (87%) and adenoma (13%), taken from both proximal and distal sections of the intestine. A detailed description of the cancer samples is provided in [Table 1](#).

4.2. Expression Level of Target Genes

The relative expression levels of the *mlh1*, *msh2*, and *msh6* genes, assessed using real-time PCR with *gapdh* as the internal control, showed a significant increase (P < 0.05) in the cancer group compared to the control group. The fold change analysis revealed a 5-fold increase in *mlh1* expression, a 6.5-fold increase in *msh2* expression, and a 7-fold increase in *msh6* expression in the cancer group compared to the control group ([Figure 1](#)). To determine the relative abundance of *F. nucleatum*, real-time PCR with 16srRNA gene primers specific to *F. nucleatum* was employed. The results indicated a significantly higher frequency of *F. nucleatum* in the

cancer group compared to the control group (P < 0.05) ([Figure 2](#)).

4.3. Abundance of *Fusobacterium nucleatum* in Cancer and Control Groups

The relative frequency of *F. nucleatum* was significantly higher in cancer patients (70%) compared to healthy individuals (25%). Additionally, *F. nucleatum* was more prevalent among female cancer patients (58%) than male patients (42%). Females aged 70 - 80 and males aged 50 - 80 showed a higher presence of *F. nucleatum*. A correlation was found between the tumor location and the frequency of *F. nucleatum*; it was detected more frequently in tumors located in the distal part of the colon (63%) than in the proximal colon and rectum (27%). The prevalence of *F. nucleatum* was also higher in adenocarcinomas (71%) compared to adenomas (13%). The abundance of *F. nucleatum* varied across different sections of the large intestine, with the highest levels found in the sigmoid, followed by the rectum, cecum, ascending colon, hepatic flexure, descending colon, and transverse colon, respectively.

4.4. Relative Expression Levels of Selected Genes in the Presence and Absence of *Fusobacterium nucleatum*

In cancer groups, the expression levels of *mlh1*, *msh2*, and *msh6* were higher in the presence of *F. nucleatum* than in its absence. Fold change analysis showed a 7.5-fold increase in the expression levels of *msh2* and *msh6* and an 8-fold increase in *mlh1* expression in the presence of *F. nucleatum*.

5. Discussion

Global incidence estimates for CRC in 2020 were 19.8 per 100 000 people, with a higher rate in men (23.4) compared to women (16.2) (1). In Vietnam, from 1996 to 2015, 12 938 individuals were diagnosed with CRC, 53.9% of whom were men, and the mean age at diagnosis was consistently 60.0 years (14). In Iran, Zare-Bandamiri et al. reported that 57.4% of CRC patients were men, with a mean age of 55.8, and 45.5% of patients fell within the 50 - 70 age range (15). Another study in Iran showed that of 562 CRC patients, 39% had early-onset CRC (under 50 years old) and 60% had late-onset CRC (over 50 years old), with participant ages ranging from 20 to 90 years and an average age of 55.63 years (16). The higher occurrence of CRC in men, as observed in our study,

Table 1. Pathological Information of Patients with Colorectal Cancer

Patient ID	Tumor				
	Age, y	Sex	Location	Size	Morphology
C01	42	F	Ascending colon	0.3X0.2X0.1	Adenocarcinoma
C02	59	M	Hepatic flexure	0.5X0.4X0.2	Adenocarcinoma
C03	72	M	Rectum	1.5X1X0.3	Adenocarcinoma
C04	82	F	Sigmoid colon	1.5X1X0.7	Adenocarcinoma
C05	69	F	Sigmoid colon	0.5X0.4X0.2	Adenocarcinoma
C06	51	M	Descending colon	0.3X0.2X0.1	Adenocarcinoma
C07	49	M	Sigmoid colon	0.3X0.2X0.1	Adenocarcinoma
C08	78	M	Cecum	0.3X0.2X0.1	Adenocarcinoma
C09	68	M	Cecum	0.5X0.3X0.2	Adenocarcinoma
C10	48	F	Rectum	0.8X0.6X0.2	Adenoma
C11	76	F	Cecum	1X1X0.3	Adenocarcinoma
C12	27	M	Ascending colon	0.6X0.4X0.2	Adenocarcinoma
C13	51	F	Rectum	1X0.7X0.3	Adenocarcinoma
C14	84	M	Rectum	0.3X0.2X0.1	Adenoma
C15	70	F	Rectum	0.3X0.2X0.1	Adenocarcinoma
C16	76	F	Hepatic flexure	0.3X0.2X0.1	Adenocarcinoma
C17	56	F	Sigmoid colon	0.5X0.3X0.2	Adenocarcinoma
C18	65	M	Ascending colon	0.5X0.3X0.2	Adenocarcinoma
C19	51	M	Sigmoid colon	1X0.7X0.3	Adenocarcinoma
C20	49	M	Sigmoid colon	0.3X0.2X0.1	Adenocarcinoma
C21	63	F	Sigmoid colon	1X0.8X0.2	Adenoma
C22	58	M	Sigmoid colon	0.9X0.7X0.3	Adenocarcinoma
C23	64	M	Descending colon	0.3X0.2X0.1	Adenocarcinoma
C24	52	M	Rectum	1X0.9X0.2	Adenocarcinoma
C25	58	F	Ascending colon	0.6X0.2X0.2	Adenoma
C26	45	M	Descending colon	0.7X0.5X0.2	Adenocarcinoma
C27	56	F	Rectum	0.3X0.2X0.1	Adenocarcinoma
C28	86	M	Rectum	0.3X0.2X0.1	High grade glandular dysplasia
C29	73	M	Rectum	0.3X0.2X0.1	Adenocarcinoma
C30	59	F	Rectum	0.3X0.2X0.1	Adenocarcinoma
C31	63	F	Rectum	0.3X0.2X0.1	Adenocarcinoma
C32	73	M	Cecum	1X0.5X0.5	Adenocarcinoma
C33	57	M	Sigmoid colon	0.7X0.6X0.1	Adenocarcinoma
C34	58	F	Sigmoid colon	0.6X0.5X0.2	Adenocarcinoma
C35	71	F	Rectum	1.5X1X0.2	Adenoma
C36	62	M	Transverse colon	0.8X0.5X0.2	Adenocarcinoma
C37	78	M	Rectum	0.3X0.2X0.1	Adenocarcinoma
C38	78	F	Sigmoid colon	0.3X0.2X0.1	Adenocarcinoma
C39	53	F	Sigmoid colon	0.3X0.2X0.1	Adenocarcinoma
C40	66	F	Rectum	0.3X0.2X0.1	Adenocarcinoma

Abbreviations: F, female; M, male.

could be influenced by factors such as sample size, study duration, and regional differences. Interestingly, CRC was less common in the proximal area of the colon, aligning with findings from several Middle Eastern studies (17-19).

Differences in gene expression and tumor phenotype between proximal and distal lesions may explain the varying mechanisms of tumor progression. Proximal lesions, often smaller in size, might be more frequently overlooked during colonoscopy than distal lesions (20,

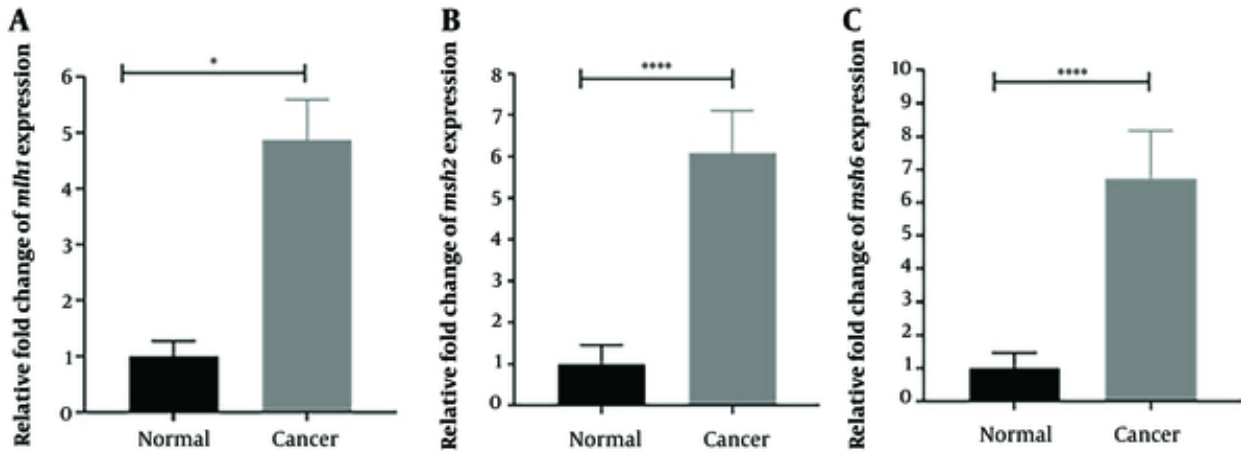


Figure 1. Fold change analysis of *mlh1*, *msh2*, and *msh6* gene expression in the cancer group relative to the control group (*P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001)

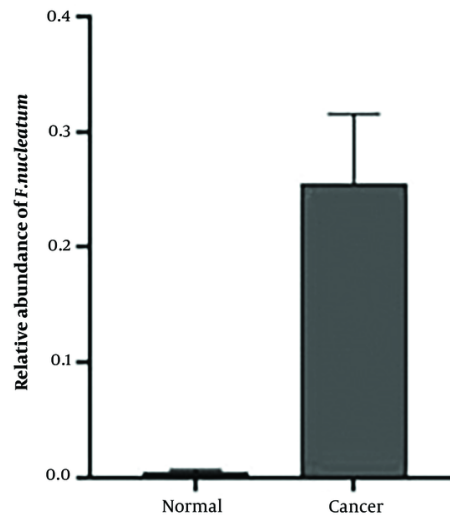


Figure 2. Comparison of the presence of *F. nucleatum*-16srRNA gene in cancer and control groups (P < 0.001)

21). Our study did not reveal any significant differences in age or tumor location across age groups, suggesting a similar distribution of distal tumors in both CRC groups (22-24). However, it's important to note that other studies have reported conflicting results on tumor localization, continuing the debate on this issue (25, 26). Similar findings were reported by Ramsoekh et al., who noted an older age at CRC onset among male carriers of

msh6 and *mlh1* mutations, as well as considerable variation in the age of CRC onset between carriers of *msh6* and *msh2* mutations (48 vs. 43 years) (27).

Ulreich et al. studied 165 individuals with CRC, finding that 86.6% had *mlh1*-proficient CRCs, and 13.3% had *mlh1*-deficient CRCs (28). Engel et al. reported that individuals with pathogenic *msh2* mutations had a 10% chance of developing advanced adenoma, compared to

a 7.7% risk among those with *mlh1* mutations. Moreover, a higher percentage of patients with pathogenic mutations in *mlh1* or *msh2* developed CRC within 10 years (11.3% and 11.4%, respectively) compared to those with *msh6* mutations (29). However, other studies have associated the *mlh1/msh2* phenotype with CRC (30, 31), suggesting that genetic variations may indirectly increase the risk of MSI-H CRC.

Our study found that 70% of cancer patient samples and 25% of control group samples were infected with *F. nucleatum*. The bacterium was predominantly found in the distal part of the colon, especially in the sigmoid and rectum, which are commonly associated with adenocarcinoma morphology. This aligns with a study that found a higher association of tumors in the distal part of the colon with *F. nucleatum* (32), suggesting that tumor development in these locations could be related to *F. nucleatum* colonization. However, other studies have reported varying results, with some indicating a preference for *F. nucleatum* colonization in the proximal colon over the distal colon (33, 34). Furthermore, while some studies report higher *F. nucleatum* distribution in the rectum compared to the distal sigmoid, others have indicated the opposite (35-37). A systematic review and meta-analysis by Idrissi Janati et al. supported *F. nucleatum* infection in the colon as a risk factor for CRC (38). Additionally, Tahara et al. discovered *F. nucleatum* in 74% of tumor tissues from 149 CRC patients (39).

The results of this study align with a review and meta-analysis that identified a strong correlation between increased *F. nucleatum* expression in CRCs and *mlh1* hypermethylation (40). Furthermore, research data has linked the quantity of *F. nucleatum* DNA in fresh-frozen CRC tissue with proximal tumor sites, greater depth of invasion, poorly differentiated tumors, and decreased expression of mismatch-repair proteins *mlh1*, *msh2*, and *pms2* (40). Studies also revealed that CRCs adjacent to normal colorectal tissues enriched with Fusobacterium were 15 times more likely to be Fusobacterium-enriched than CRCs close to normal Fusobacterium-free colorectal tissues (41).

The primary cause of MSI-H is deficits in MMR genes, including *msh2*, *mlh1*, and *msh6* (42). According to our results, several studies have shown that *F. nucleatum* promotes CRC carcinogenesis in animal models, stimulating CRC cell development through E-cadherin/ β -catenin signaling via the FadA adhesin, among other

virulence components linked to CRC (43, 44). Immune evasion and/or chemoresistance due to *F. nucleatum* may explain the poor prognosis of *F. nucleatum*-associated CRC, potentially involving a complex relationship between CIMP/MSI and *F. nucleatum* infection mediated by ROS and nucleotide excision repair processes (45).

5.1. Conclusions

In total, our study provides compelling evidence supporting the association between *F. nucleatum* and CRC development and its potential role in poor prognosis and chemoresistance. The findings highlight the importance of *F. nucleatum* as a potential molecular marker for predicting CRC development. The dysregulation of critical genes involved in CRC pathogenesis due to *F. nucleatum* infection further supports the bacterium's direct impact on cancer development. These findings contribute valuable insights into the role of *F. nucleatum* in CRC and pave the way for potential targeted therapies and predictive markers for this disease. However, additional research is necessary to fully elucidate the underlying mechanisms and validate these findings in larger and more diverse cohorts.

There are several limitations to our study that need to be acknowledged and taken into account. Firstly, the sample size, particularly within the CRC group, was relatively small, potentially affecting the generalizability of our findings. Secondly, the absence of access to CRC grades posed a significant challenge, impairing our ability to assess the relationship between gene expression and cancer grade. Thirdly, our study could have benefited from conducting additional molecular evaluations and gene expression analyses to delve deeper into the mechanisms underlying *F. nucleatum* contribution to gastrointestinal damage. Addressing these limitations in future research endeavors would undoubtedly enhance the comprehensiveness and robustness of the investigations.

Footnotes

Authors' Contribution: Study design: MD and BH; laboratory experiments: RT, MD, and BH; data analysis: MD, BH and HD; writing of the manuscript: RT, MD, BH,

and RHD; overall responsibility for the accuracy and integrity of the manuscript: MD, BH, RHD and HD.

Conflict of Interests: The authors declared that they have no conflicts of interests.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

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Informed Consent: Written consent was obtained from all the people included in the study to conduct the experiments of this research.

References

- Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi J, John A, et al. Colorectal cancer: A review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. *Cancers (Basel)*. 2022;**14**(7). [PubMed ID: 35406504]. [PubMed Central ID: PMC8996939]. <https://doi.org/10.3390/cancers14071732>.
- Hua H, Jiang Q, Sun P, Xu X. Risk factors for early-onset colorectal cancer: systematic review and meta-analysis. *Front Oncol*. 2023;**13**:1132306. [PubMed ID: 37213277]. [PubMed Central ID: PMC10196487]. <https://doi.org/10.3389/fonc.2023.1132306>.
- Zheng Z, Hou X, Bian X, Jia W, Zhao L. Gut microbiota and colorectal cancer metastasis. *Cancer Lett*. 2023;**555**:216039. [PubMed ID: 36528182]. <https://doi.org/10.1016/j.canlet.2022.216039>.
- Li Y, Rao G, Zhu G, Cheng C, Yuan L, Li C, et al. Dysbiosis of lower respiratory tract microbiome are associated with proinflammatory states in non-small cell lung cancer patients. *Thorac Cancer*. 2024;**15**(2):111-21. [PubMed ID: 38041547]. [PubMed Central ID: PMC10788479]. <https://doi.org/10.1111/1759-7714.15166>.
- Datorre JG, de Carvalho AC, Dos Reis MB, Dos Reis M, Matsushita M, Santos F, et al. Accuracy and clinical relevance of intra-tumoral Fusobacterium nucleatum detection in formalin-fixed paraffin-embedded (FFPE) tissue by droplet digital PCR (ddPCR) in colorectal cancer. *Diagnostics (Basel)*. 2022;**12**(1). [PubMed ID: 35054281]. [PubMed Central ID: PMC8775036]. <https://doi.org/10.3390/diagnostics12010114>.
- Wu Z, Ma Q, Guo Y, You F. The role of Fusobacterium nucleatum in colorectal cancer cell proliferation and migration. *Cancers (Basel)*. 2022;**14**(21). [PubMed ID: 36358769]. [PubMed Central ID: PMC9656216]. <https://doi.org/10.3390/cancers14215350>.
- Zhao T, Wang X, Fu L, Yang K. Fusobacterium nucleatum: a new player in regulation of cancer development and therapeutic response. *Cancer Drug Resist*. 2022;**5**(2):436-50. [PubMed ID: 35800370]. [PubMed Central ID: PMC9255244]. <https://doi.org/10.20517/cdr.2021.144>.
- Scheible M, Nguyen CT, Luong TT, Lee JH, Chen YW, Chang C, et al. The fused methionine sulfoxide reductase MsrAB promotes oxidative stress defense and bacterial virulence in Fusobacterium nucleatum. *mBio*. 2022;**13**(3). e0302221. [PubMed ID: 35420473]. [PubMed Central ID: PMC9239216]. <https://doi.org/10.1128/mbio.03022-21>.
- Fan S, Xing J, Jiang Z, Zhang Z, Zhang H, Wang D, et al. Effects of long non-coding RNAs induced by the gut microbiome on regulating the development of colorectal cancer. *Cancers (Basel)*. 2022;**14**(23). [PubMed ID: 36497293]. [PubMed Central ID: PMC9735521]. <https://doi.org/10.3390/cancers14235813>.
- Wang N, Fang JY. Fusobacterium nucleatum, a key pathogenic factor and microbial biomarker for colorectal cancer. *Trends Microbiol*. 2023;**31**(2):159-72. [PubMed ID: 36058786]. <https://doi.org/10.1016/j.tim.2022.08.010>.
- Somuncu B, Ekmekcioglu A, Antmen FM, Ertuzun T, Deniz E, Keskin N, et al. Targeting mitochondrial DNA polymerase gamma for selective inhibition of MLH1 deficient colon cancer growth. *PLoS One*. 2022;**17**(6). e0268391. [PubMed ID: 35657956]. [PubMed Central ID: PMC9165880]. <https://doi.org/10.1371/journal.pone.0268391>.
- Graham LS, Schweizer MT. Mismatch repair deficiency and clinical implications in prostate cancer. *Prostate*. 2022;**82** Suppl 1:S37-44. [PubMed ID: 35358351]. <https://doi.org/10.1002/pros.24343>.
- Rashid MU, Naeemi H, Muhammad N, Loya A, Lubinski J, Jakubowska A, et al. Prevalence and spectrum of MLH1, MSH2, and MSH6 pathogenic germline variants in Pakistani colorectal cancer patients. *Hered Cancer Clin Pract*. 2019;**17**:29. [PubMed ID: 31660093]. [PubMed Central ID: PMC6806584]. <https://doi.org/10.1186/s13053-019-0128-2>.
- Pham DX, Phung AHT, Nguyen HD, Bui TD, Mai LD, Tran BNH, et al. Trends in colorectal cancer incidence in Ho Chi Minh City, Vietnam (1996-2015): Joinpoint regression and age-period-cohort analyses. *Cancer Epidemiol*. 2022;**77**:102113. [PubMed ID: 35078008]. <https://doi.org/10.1016/j.canep.2022.102113>.
- Zare-Bandamiri M, Khanjani N, Jahani Y, Mohammadianpanah M. Factors affecting survival in patients with colorectal cancer in Shiraz, Iran. *Asian Pac J Cancer Prev*. 2016;**17**(1):159-63. [PubMed ID: 26838203]. <https://doi.org/10.7314/apjcp.2016.17.1.159>.
- Hoseini B, Rahmatinejad Z, Goshayeshi L, Bergquist R, Golabpour A, Ghaffarzagdegan K, et al. Colorectal Cancer in North-Eastern Iran: a retrospective, comparative study of early-onset and late-onset cases based on data from the Iranian hereditary colorectal cancer registry. *BMC Cancer*. 2022;**22**(1):48. [PubMed ID: 34998373]. [PubMed Central ID: PMC8742430]. <https://doi.org/10.1186/s12885-021-09132-5>.
- Maraqqa B, Al-Shbool G, Abu-Shawar O, Souleiman M, Alshakhatreh O, Al-Omari A, et al. Frequency of mismatch repair protein (MMRP) deficiency among young Jordanians diagnosed with colorectal carcinoma (CRC). *Gastroenterol Res Pract*. 2020;**2020**:5632984. [PubMed ID: 32382267]. [PubMed Central ID: PMC7195647]. <https://doi.org/10.1155/2020/5632984>.
- Zeinalian M, Hashemzadeh-Chaleshtori M, Akbarpour MJ, Emami MH. Epidemioclinical feature of early-onset colorectal cancer at-risk for lynch syndrome in central Iran. *Asian Pac J Cancer Prev*. 2015;**16**(11):4647-52. [PubMed ID: 26107218]. <https://doi.org/10.7314/apjcp.2015.16.11.4647>.
- Siraj AK, Prabhakaran S, Bavi P, Bu R, Beg S, Hazmi MA, et al. Prevalence of Lynch syndrome in a Middle Eastern population with colorectal cancer. *Cancer*. 2015;**121**(11):1762-71. [PubMed ID: 25712738]. <https://doi.org/10.1002/cncr.29288>.
- Lakoff J, Paszat LF, Saskin R, Rabeneck L. Risk of developing proximal versus distal colorectal cancer after a negative colonoscopy: a population-based study. *Clin Gastroenterol Hepatol*. 2008;**6**(10):1117-

21. quiz 1064. [PubMed ID: 18691942]. <https://doi.org/10.1016/j.cgh.2008.05.016>.
22. Wong R. Proximal tumors are associated with greater mortality in colon cancer. *J Gen Intern Med*. 2010;**25**(11):157-63. [PubMed ID: 20652758]. [PubMed Central ID: PMC2947632]. <https://doi.org/10.1007/s11606-010-1460-4>.
23. De Silva MV, Fernando MS, Fernando D. Comparison of some clinical and histological features of colorectal carcinoma occurring in patients below and above 40 years. *Ceylon Med J*. 2000;**45**(4):166-8. [PubMed ID: 11293963]. <https://doi.org/10.4038/cmj.v45i4.6722>.
24. Minardi AJ, Sittig KM, Zibari GB, McDonald JC. Colorectal cancer in the young patient. *Am Surg*. 1998;**64**(9):849-53. [PubMed ID: 9731812].
25. Chiang JM, Chen MC, Changchien CR, Chen JS, Tang R, Wang JY, et al. Favorable influence of age on tumor characteristics of sporadic colorectal adenocarcinoma: patients 30 years of age or younger may be a distinct patient group. *Dis Colon Rectum*. 2003;**46**(7):904-10. [PubMed ID: 12847364]. <https://doi.org/10.1007/s10350-004-6683-1>.
26. Myers EA, Feingold DL, Forde KA, Arnell T, Jang JH, Whelan RL. Colorectal cancer in patients under 50 years of age: a retrospective analysis of two institutions' experience. *World J Gastroenterol*. 2013;**19**(34):5651-7. [PubMed ID: 24039357]. [PubMed Central ID: PMC3769901]. <https://doi.org/10.3748/wjg.v19.i34.5651>.
27. Sudarshan V, Hussain N, Gahine R, Mourya J. Colorectal cancer in young adults in a tertiary care hospital in Chhattisgarh, Raipur. *Indian J Cancer*. 2013;**50**(4):337-40. [PubMed ID: 24369213]. <https://doi.org/10.4103/0019-509X.123621>.
28. Ramsoekh D, Wagner A, van Leerdam ME, Dooijes D, Tops CM, Steyerberg EW, et al. Cancer risk in MLH1, MSH2 and MSH6 mutation carriers; different risk profiles may influence clinical management. *Hered Cancer Clin Pract*. 2009;**7**(1):17. [PubMed ID: 20028567]. [PubMed Central ID: PMC2804564]. <https://doi.org/10.1186/1897-4287-7-17>.
29. Ulreich K, Firnau MB, Tagscherer N, Beyer S, Ackermann A, Plotz G, et al. High expression of casein kinase 2 alpha is responsible for enhanced phosphorylation of DNA mismatch repair protein MLH1 and increased tumor mutation rates in colorectal cancer. *Cancers (Basel)*. 2022;**14**(6). [PubMed ID: 35326704]. [PubMed Central ID: PMC8946085]. <https://doi.org/10.3390/cancers14061553>.
30. Engel C, Ahadova A, Seppala TT, Aretz S, Bigirwamungu-Bargeman M, Blaker H, et al. Associations of pathogenic variants in MLH1, MSH2, and MSH6 With risk of colorectal adenomas and tumors and with somatic mutations in patients with lynch syndrome. *Gastroenterol*. 2020;**158**(5):1326-33. [PubMed ID: 31926173]. <https://doi.org/10.1053/j.gastro.2019.12.032>.
31. Mrkonjic M, Roslin NM, Greenwood CM, Raptis S, Pollett A, Laird PW, et al. Specific variants in the MLH1 gene region may drive DNA methylation, loss of protein expression, and MSI-H colorectal cancer. *PLoS One*. 2010;**5**(10). e13314. [PubMed ID: 20967208]. [PubMed Central ID: PMC2954166]. <https://doi.org/10.1371/journal.pone.0013314>.
32. Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang J, et al. Fecal bacteria act as novel biomarkers for noninvasive diagnosis of colorectal cancer. *Clin Cancer Res*. 2017;**23**(8):2061-70. [PubMed ID: 27697996]. <https://doi.org/10.1158/1078-0432.CCR-16-1599>.
33. Bostanshirin N, Hajikhani B, Vaezi AA, Keranian F, Sameni F, Yaslianifard S, et al. Association between colorectal cancer and expression levels of miR-21, miR-17-5P, miR-155 genes and the presence of Fusobacterium nucleatum in biopsy samples obtained from Iranian patients. *Infect Agent Cancer*. 2023;**18**(1):14. [PubMed ID: 36859379]. [PubMed Central ID: PMC9979415]. <https://doi.org/10.1186/s13027-023-00494-y>.
34. Kwong TNY, Wang X, Nakatsu G, Chow TC, Tipoe T, Dai RZW, et al. Association between bacteremia from specific microbes and subsequent diagnosis of colorectal cancer. *Gastroenterol*. 2018;**155**(2):383-90.e8. [PubMed ID: 29729257]. <https://doi.org/10.1053/j.gastro.2018.04.028>.
35. Dahmus JD, Kotler DL, Kastenber DM, Kistler CA. The gut microbiome and colorectal cancer: a review of bacterial pathogenesis. *J Gastrointest Oncol*. 2018;**9**(4):769-77. [PubMed ID: 30151274]. [PubMed Central ID: PMC6087872]. <https://doi.org/10.21037/jgo.2018.04.07>.
36. Noshu K, Sukawa Y, Adachi Y, Ito M, Mitsuhashi K, Kurihara H, et al. Association of Fusobacterium nucleatum with immunity and molecular alterations in colorectal cancer. *World J Gastroenterol*. 2016;**22**(2):557-66. [PubMed ID: 26811607]. [PubMed Central ID: PMC4716059]. <https://doi.org/10.3748/wjg.v22.i2.557>.
37. Mima K, Cao Y, Chan AT, Qian ZR, Nowak JA, Masugi Y, et al. Fusobacterium nucleatum in colorectal carcinoma tissue according to tumor location. *Clin Transl Gastroenterol*. 2016;**7**(11). e200. [PubMed ID: 27811909]. [PubMed Central ID: PMC5543402]. <https://doi.org/10.1038/ctg.2016.53>.
38. Ito M, Kanno S, Noshu K, Sukawa Y, Mitsuhashi K, Kurihara H, et al. Association of Fusobacterium nucleatum with clinical and molecular features in colorectal serrated pathway. *Int J Cancer*. 2015;**137**(6):1258-68. [PubMed ID: 25703934]. <https://doi.org/10.1002/ijc.29488>.
39. Idrissi Janati A, Karp I, Sabri H, Emami E. Is a fusobacterium nucleatum infection in the colon a risk factor for colorectal cancer?: a systematic review and meta-analysis protocol. *Syst Rev*. 2019;**8**(1):114. [PubMed ID: 31077259]. [PubMed Central ID: PMC6511124]. <https://doi.org/10.1186/s13643-019-1031-7>.
40. Tahara T, Yamamoto E, Suzuki H, Maruyama R, Chung W, Garriga J, et al. Fusobacterium in colonic flora and molecular features of colorectal carcinoma. *Cancer Res*. 2014;**74**(5):1311-8. [PubMed ID: 24385213]. [PubMed Central ID: PMC4396185]. <https://doi.org/10.1158/0008-5472.CAN-13-1865>.
41. Wang Y, Wen Y, Wang J, Lai X, Xu Y, Zhang X, et al. Clinicopathological differences of high Fusobacterium nucleatum levels in colorectal cancer: A review and meta-analysis. *Front Microbiol*. 2022;**13**:945463. [PubMed ID: 36406461]. [PubMed Central ID: PMC9672069]. <https://doi.org/10.3389/fmicb.2022.945463>.
42. Mukherji R, Weinberg BA. The gut microbiome and potential implications for early-onset colorectal cancer. *Colorectal Cancer*. 2020;**9**(3). <https://doi.org/10.2217/crc-2020-0007>.
43. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998;**58**(22):5248-57. [PubMed ID: 9823339].
44. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*. 2013;**14**(2):207-15. [PubMed ID: 23954159]. [PubMed Central ID: PMC3772512]. <https://doi.org/10.1016/j.chom.2013.07.007>.

44. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe*. 2013;**14**(2):195-206. [PubMed ID: [23954158](#)]. [PubMed Central ID: [PMC3770529](#)]. <https://doi.org/10.1016/j.chom.2013.07.012>.
45. Koi M, Okita Y, Carethers JM. Fusobacterium nucleatum infection in colorectal cancer: Linking inflammation, DNA mismatch repair and genetic and epigenetic alterations. *J Anus Rectum Colon*. 2018;**2**(2):37-46. [PubMed ID: [30116794](#)]. [PubMed Central ID: [PMC6090547](#)]. <https://doi.org/10.23922/jarc.2017-055>.