Serum MicroRNA-21 as a Biomarker for Colorectal Cancer Diagnosis

Hatem Mahmoud Elyamany Yousef[1], Ali Abdel Rahman Sayed Ghweil[2], Shamardan Ezz-Eldin Sayed Bazeed[2], Tarek Khaled Abdella Elmaghraby[3], Abdallah Elaiw Mohamed Ali[4], Nourelhoda Elsaied Mohammad Hasan[5], Marwa Moustafa Saady Mahmoud[6]

1 Tropical medicine and gastroenterology department, Luxor faculty of medicine, Luxor university
2 Tropical medicine and gastroenterology department, South Valley University, Qena, Egypt
3 Egyptian Atomic Energy Authority
4 Clinical Pathology department, faculty of medicine, South Valley University , Qena, Egypt
5 Luxor GIT and Fever Hospital
6 Shefa Al-Orman Oncology Hospital

Corresponding author: Hatem Mahmoud Elyamany Yousef[1]
hatem.yamany@medicine.luxor.edu.eg

ABSTRACT

Colorectal cancer (CRC), a leading malignancy globally, is increasingly prevalent in low- and middle-income countries due to urbanization and lifestyle changes. In Egypt, CRC ranks as the 7th most common cancer, with most cases progressing over decades through adenoma–carcinoma sequences or serrated pathways. Early detection is crucial, as CRC is preventable and treatable in its early stages, but current diagnostic methods like colonoscopy are invasive, costly, and operator-dependent. Non-invasive biomarker tests, including carcinoembryonic antigen (CEA) and CA 19-9, have shown utility in monitoring CRC but lack optimal sensitivity and specificity. MicroRNA-21, a small non-coding RNA involved in gene regulation, is frequently overexpressed in CRC and has been linked to tumor proliferation, invasion, and metastasis. This study aimed to evaluate serum microRNA-21 levels as a diagnostic biomarker for CRC. Conducted at Qena University Hospital, Egypt, the cross-sectional study included 50 CRC patients and 50 healthy controls. Serum microRNA-21 levels were analyzed using RT-PCR and the 2-ΔΔCt method, excluding individuals with confounding factors such as prior cancer treatments or elevated CEA and CA 19-9 levels. The findings are expected to underscore microRNA-21's potential as a non-invasive diagnostic tool for CRC, offering high sensitivity and specificity. This could aid in reducing the reliance on invasive diagnostic techniques and improving early detection, ultimately lowering CRC-associated mortality.

Introduction:

CRC is considered a major health problem as it is the 3rd most common cancer worldwide and the 4th most common cancer cause of death globally. CRC is the seventh most frequent cancer in Egypt, representing 3.47% of male cancers and 3% of female cancers. Early detection is crucial for prevention and successful treatment, yet symptoms often appear only in advanced stages, emphasizing the need for widespread screening programs **(Ahmed et al., 2022).**

Although colonoscopy is considered the most reliable method for CRC diagnosis, it is invasive, expensive, and associated with risks, with its accuracy influenced by operator expertise. Non-invasive tests based on biomarkers have the potential to reduce the number of unnecessary colonoscopies but often fall short in achieving the required balance of accuracy, simplicity, and affordability **(Almughamsi et al., 2024).** Commonly used markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19.9 (CA 19.9) are helpful for CRC detection and monitoring when used together, as they improve diagnostic sensitivity and provide insights into disease progression and prognosis **(El-Korany et al., 2024).**

Advances in research on microRNAs, which are small non-coding RNA molecules that regulate gene expression, have highlighted their significant role in CRC development **(Elbadr et al., 2024).** MicroRNA-21, frequently overexpressed in CRC, has shown promise as a diagnostic marker for early detection due to its strong association with the disease. Studies suggest that analyzing microRNA-21 levels in blood or stool samples could enhance CRC screening and diagnosis, offering a non-invasive alternative to traditional methods while improving detection accuracy and treatment outcomes **(Qian et al., 2024).**

Colorectal Cancer (CRC)

Colorectal cancer (CRC) is the third most prevalent cancer globally, affecting both men and women, and is the second leading cause of cancer deaths. Its incidence is influenced by lifestyle, obesity, and diet. A protective effect is associated with physical activity, while a higher risk is linked to frequent consumption of red and processed meats, and alcohol (Morgan, et al. 2023). Westernized diets, characterized by high animal fat intake, low fiber, and reduced physical activity, contribute to obesity and CRC risk. Early diagnosis and balanced diet-based prevention are crucial for reducing CRC incidence (Silva, et al. 2020).

Epidemiology

In 2020, over 1.9 million new CRC cases were reported, causing nearly 935,000 deaths, and representing 11% of global cancer diagnoses. The highest increases in CRC incidence and mortality are seen in developed countries with a Western lifestyle. Key risk factors include obesity, sedentary lifestyle, red meat, alcohol, and tobacco consumption. CRC is the 7th most common cancer in Egypt, representing 3.47% of male and 3% of female cancers, with over 3,000 colon cancer patients estimated in 2015 (Hefni). Developed countries with a high Human Development Index (HDI) are most affected (Ferlay, et al. 2020; Sawicki, et al. 2021).

Classification

1. **Anatomical Site**: CRC is classified into three subtypes based on the tumor's location: proximal colon, distal colon, and rectum (Murphy, et al. 2019).
2. **Genetics**: CRC includes three main genetic alterations: chromosomal instability (CIN), CpG island methylator phenotype (CIMP), and microsatellite instability (MSI). Around 70% of MSI-high CRCs are also CIMP-high (Vodicka, et al. 2020).
3. **Polyps**: CRC is grouped into adenomatous (85-90%) and serrated polyps (10-15%). The adenoma-carcinoma pathway accounts for 70-90% of CRCs, while the serrated neoplasia pathway accounts for 10-20% (Dekker, et al. 2019).
4. **TNM Staging**: CRC is staged from stage 0 (carcinoma in situ) to stage IV (metastasis to distant organs). Stages range from early tumor growth (stage 0) to advanced spread to distant lymph nodes and organs (stage IV) (Kancha, 2024).

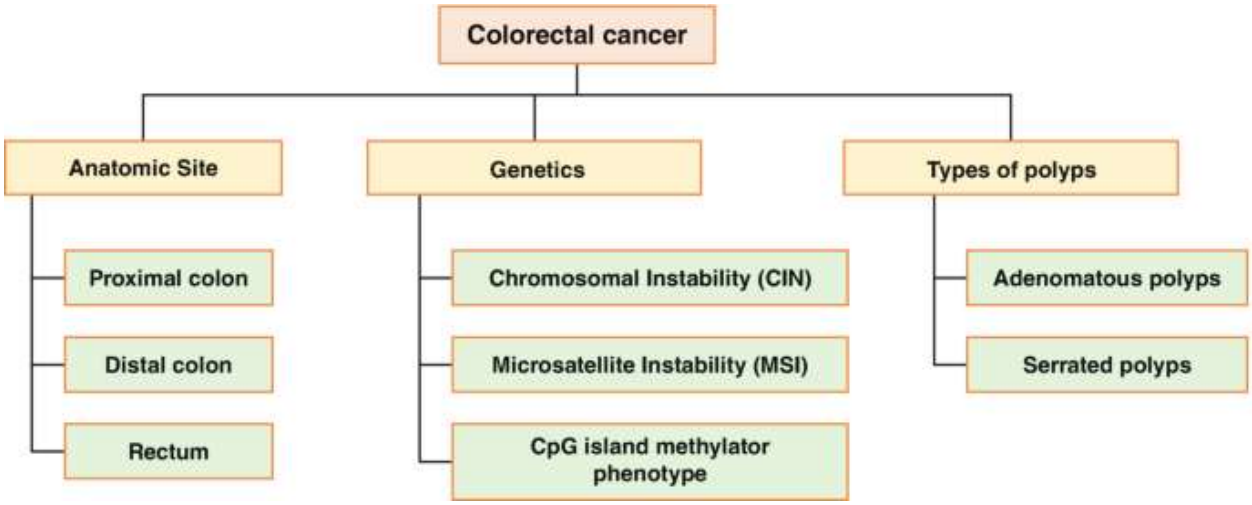

Fig (1): Classification of colorectal cancer (Kancha 2024)

Pathogenesis of CRC

Colorectal cancer (CRC) is genetically diverse, with high mutational loads, often categorized into hypermutated (>12 mutations per 10^6 bases) or non-hypermutated (<8.24 mutations per 10^6 bases) types. Genetic changes in epithelial cells lead to hyperproliferation, forming benign adenomas that can progress to cancer through pathways like microsatellite instability (MSI), chromosomal instability (CIN), and serrated neoplasia. The adenoma-carcinoma sequence describes cancer progression, with CIN-positive subtypes accounting for 10–15% of sporadic CRC (Hossain et al. 2022). Another pathway involves inflammation, leading to dysplasia and cancer, particularly in inflammatory bowel disease (Keum et al. 2019). CRC stages range from stage 0 (carcinoma in situ) to stage IV, with invasive adenocarcinomas accounting for 96% of CRC cases (Hossain et al. 2022).

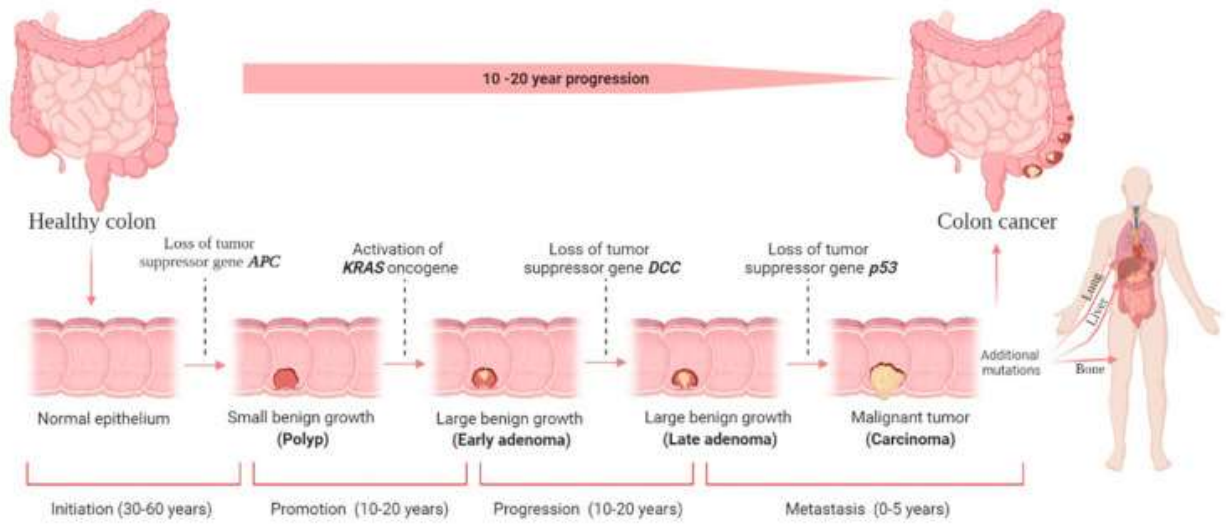

Fig (2): Colorectal cancer (CRC) stages and development. There are four stages in the development of CRC carcinogenesis: initiation, promotion, progression, and metastasis. The liver is the most common metastatic site, followed by the lung and bone. Although it is difficult to determine the duration required for each stage, decades will likely be required to form CRC. The figure was created using BioRender.com (accessed on 30 December 2021) (Hossain, et al. 2022).

Risk Factors of CRC

Non-modifiable Factors

Age, gender, and family history are major risk factors. Most CRC cases occur in individuals over 50, with men diagnosed at 68 years and women at 72 years on average (Steele et al. 2014). Family history doubles the risk of CRC (RR 1.80, 95% CI: 1.61–2.02) (Johnson et al. 2013). Hereditary conditions like familial adenomatous polyposis (FAP) and Lynch syndrome increase CRC risk (Bhai et al. 2020; Yılmaz et al. 2020).

Modifiable Factors

Obesity raises CRC risk by 10% per 8 kg/m² BMI increase (Johnson et al. 2013). Physical inactivity also increases CRC risk, while regular exercise improves survival (Wolin et al. 2009). Alcohol consumption (≥4 drinks/day) significantly raises CRC risk, with a 21% increased risk for moderate and 52% for heavy drinkers (Lin et al. 2020). Smoking increases CRC risk, with relative risks of 1.06 for 5 pack-years and 1.26 for 30 pack-years (Demb et al. 2019). Red and processed meat consumption raises CRC risk by 20–30%, while high fiber intake reduces it (Hossain et al. 2022; Bradbury et al. 2020).

Dietary Factors

A diet rich in vegetables, fruits, and whole grains is protective, while vitamin D deficiency and low calcium intake increase CRC risk (Vallès et al. 2018). Reducing carcinogens in meat through cooking methods can decrease CRC risk (Shinde et al. 2021).

Medication and Hormones

Aspirin and NSAIDs lower CRC risk, with a relative risk of 0.60 for five years of use (Johnson et al. 2013). However, long-term use carries risks, and low-dose aspirin is recommended for high-risk individuals (Hoskin et al. 2023). Hormone replacement therapy in postmenopausal women reduces CRC risk by 39% in current users (meta-analysis).

Colorectal Cancer Diagnosis

Colorectal cancer (CRC) diagnosis involves symptom assessment, physical exams, and diagnostic tests. Common symptoms include rectal bleeding, abdominal pain, changes in bowel habits, weight loss, and anemia. Colonoscopy is preferred for high-risk individuals, providing high sensitivity and specificity for tumor detection and biopsy (Sachdeo et al., 2020). Screening with fecal immunochemical tests (FIT) and guaiac fecal occult blood tests (gFOBT) is vital for early detection in individuals aged 50-75. Imaging techniques like CT colonography (CTC) and MRI offer non-invasive alternatives but may miss smaller lesions. Biomarkers such as CEA, CA 19-9, and microRNAs aid in prognosis and treatment monitoring. Genetic testing for KRAS, BRAF mutations, and microsatellite instability (MSI) refines diagnosis and therapy. Liquid biopsies and microbiota biomarkers are emerging tools for early detection and personalized treatment (García-Figueiras et al., 2021).

Symptoms

CRC suspicion arises from lower GI symptoms, with guidelines indicating referral based on rectal bleeding, abdominal mass, pain, weight loss, and anemia. Non-site-specific symptoms, such as appetite loss and deep vein thrombosis, also warrant attention (Monahan et al., 2022). Research shows single symptoms have low diagnostic utility, though symptom combinations (e.g., palpable abdominal mass and rectal bleeding) improve diagnostic sensitivity (Sawicki et al., 2021). Early diagnosis before symptom onset improves prognosis, highlighting the importance of urgent referrals for suspected CRC cases (Monahan et al., 2022).

Diagnostic Pathways for CRC Detection

Physical Examination & Family History

Primary care physicians should perform abdominal exams and assess health history for CRC suspicion. If CRC is suspected, referral to a gastroenterology clinic is recommended. Family history and risk factors should be evaluated, guiding the choice of diagnostic methods. Screening programs globally, targeting individuals aged 50–75, show varying participation rates from 16.1% to 68.2% (Sawicki, et al. 2021).

Screening Programs & Methods

Screening includes fecal immunochemical test (FIT), guaiac fecal occult blood test (gFOBT), colonoscopy, flexible sigmoidoscopy (FS), and digital rectal exam (DRE). Screening is common in Western countries with higher CRC rates (Navarro, et al. 2017). The presence of CRC in first-degree relatives (FDRs) or advanced adenomas affects screening frequency, starting from age 40–50 (Kastrinos, et al. 2020). Genetic syndromes like Lynch syndrome or familial adenomatous polyposis necessitate high-risk surveillance (Wilkinson, et al. 2019; Kirbiyik, et al. 2021).

Fecal Occult Blood Test (FOBT)

The fecal occult blood test is recommended for low-risk symptomatic patients. FIT, with higher sensitivity, is preferred over gFOBT for detecting CRC (Sawicki, et al. 2021). Recent studies suggest FIT's high sensitivity, especially at a 10 μg Hb/g cutoff, reducing colonoscopy rates by 75–80% (Vieito, et al. 2019; Bailey, et al. 2021).

Endoscopy

Colonoscopy remains the gold standard for CRC diagnosis, with high sensitivity and specificity. Colonoscopy detects tumors, enables biopsies, and assesses the entire large intestine. AI technologies, like deep learning, improve polyp detection (Lai, et al. 2021). Colon capsule endoscopy (CCE) is an alternative for moderate-risk patients, but it is not recommended as a first-line diagnostic tool (Fiorillo, et al. 2020; Spada, et al. 2020).

Imaging-Based Diagnosis

CT colonography (CTC) is a non-invasive, effective alternative to colonoscopy, though less sensitive for polyps <8 mm (Issa and Noureddine, 2017). New techniques like DW-MRI and FAPI-PET show promise in detecting metastases with high sensitivity (Kranenburg, et al. 2021).

Tumor Markers

CEA, while not useful for diagnosing CRC, serves as a prognostic marker for therapy (Halilovic, et al. 2020). CA 19-9, used for gastrointestinal cancers, is less sensitive than CEA but can enhance diagnostic accuracy when combined (Lee, et al. 2020).

MicroRNAs as Biomarkers

MicroRNAs (miRNAs), particularly miR-21 and miR-92a, show potential as CRC biomarkers, aiding early diagnosis and prognosis (Rapado-González, et al. 2019). Further research is needed for clinical application.

Additional Diagnostics

Once CRC is confirmed, imaging studies (CT, MRI) and genetic testing for KRAS and BRAF mutations guide treatment decisions. PET-CT is not recommended for routine CRC diagnosis, though it can be used for metastatic cases (Sawicki, et al. 2021). Testing for microsatellite instability (MSI) and mismatch repair (MMR) defects aids in treatment selection (Chung, 2022).

Emerging Biomarkers

Research continues to explore new biomarkers for CRC, including methylated genes and protein biomarkers, to improve early detection and predict treatment outcomes (Ogunwobi, et al. 2020).

Serum MicroRNA-21 in Cancer

MicroRNAs (miRNAs) are small non-coding RNA molecules (~20 nucleotides) that regulate gene expression. They are involved in various physiological processes such as differentiation, proliferation, and apoptosis. MiR-21 is an oncogenic miRNA that targets genes like PTEN, TPM1, and PDCD4 and is overexpressed in various cancers, including colorectal cancer (CRC). Its

elevated expression in CRC tissues has been linked to prognosis and prediction, highlighting its potential as a diagnostic biomarker for CRC (Fernandes et al., 2019).

Biogenesis of miRNAs

MiRNA biogenesis is a complex process starting in the nucleus and ending in the cytoplasm. Primary miRNAs are transcribed by RNA polymerase II, then processed by the Drosha-DGCR8 complex into pre-miRNAs (~65-70 nucleotides). These are exported to the cytoplasm by Exportin 5 and further cleaved by Dicer into a ~22 nt miRNA duplex. The mature miRNA is incorporated into the RNA-Induced Silencing Complex (RISC) (O'Brien et al., 2018).

Regulation of miR-21

MiR-21 is located on chromosome 17q23.2, overlapping with the TMEM49 gene. It is regulated by transcription factors such as AP-1 and STAT3, which activate miR-21 expression. A feedback loop between miR-21 and NFIB maintains its expression. Epigenetic modifications, such as hypomethylation, can also regulate miR-21 levels (Fujita et al., 2008; Singh et al., 2021).

Targets of miR-21

MiR-21 targets multiple genes, including PDCD4, TPM1, maspin, and RECK, involved in cell apoptosis, migration, and invasiveness. PDCD4, a negative regulator of AP-1, is downregulated by miR-21, leading to increased AP-1 activity. This contributes to the activation of the RAS/MAPK pathway. In breast cancer, miR-21 levels are inversely correlated with PDCD4 and maspin expression (Hatley et al., 2010; Thakur et al., 2022).

Carcinogenesis and miR-21

MiR-21 is the most upregulated miRNA in solid and hematological malignancies. It plays a significant role in tumor pathogenesis and carcinogenesis, as evidenced by several studies:

1. Knockdown of miR-21 in glioblastoma cells triggers caspase activation and increases apoptotic cell death, indicating its anti-apoptotic role.
2. MiR-21 is located at the FRA17B fragile site on chromosome 17q23.2, a site of HPV integration, potentially linking its overexpression in cervical cancer (Aloizou et al., 2020).
3. In hepatoma cells, miR-21 knockdown increases PTEN expression, reducing cell proliferation, migration, and invasion.
4. MiR-21 promotes hepatic lipid accumulation and cancer progression via the HMG-box-p53-SREBP1 pathway, with antisense oligonucleotides reducing lipid accumulation and xenograft tumor growth (Yang et al., 2023).

miR-21 in Colorectal Cancer Pathogenesis

MiR-21 is implicated in CRC, affecting chemoresistance and cancer stem cell enrichment. Downregulation of miR-21 may improve treatment response, while upregulation activates the Wnt signaling pathway, targeting cyclin D1 (CCND1) and c-Myc. MiR-21 also targets TGF-BR2,

inhibiting PDCD4 (Far et al., 2023). In a study of 765 CRC specimens, miR-21 expression was linked to cell invasion, proliferation, and tumor growth, while suppressing PTEN (Singh et al., 2021).

MiR-21 and PTEN in CRC

MiR-21 negatively correlates with PTEN expression and is associated with tumor stage and size. Elevated miR-21 expression in CRC patients contributes to PTEN downregulation, enhancing tumor progression. Suppression of miR-21 increases PTEN expression, reduces PI3K/AKT pathway activation, and decreases cell invasion and proliferation. Interestingly, downregulation of miR-21 increases PTEN protein expression without altering mRNA levels. Overexpression of miR-21 also reduces RhoB expression, promoting CRC proliferation and invasion.

Sec23A and miR-21 in CRC

MiR-21 affects Sec23A, a tumor suppressor involved in protein transport. In a study using SW-480 (high miR-21) and DLD-1 (low miR-21) cell lines, miR-21 inhibition in SW-480 cells reduced cancer cell proliferation and invasion, while overexpression in DLD-1 cells increased these activities. In vivo studies showed that overexpression of miR-21 promoted tumor growth and suppressed Sec23A expression. Additionally, miR-21 regulates Sprouty2 (SPR2), and downregulation of miR-21 promotes apoptosis in CRC cells, enhancing the effects of metformin and 5-fluorouracil (Far et al., 2023; Li et al., 2016).

Diagnostic Potency of miR-21 in Colorectal Cancer

MiR-21 expression correlates with CRC clinical stage, distant metastasis, and lymph node positivity. A systematic review identified miR-21 as the most reported miRNA biomarker for CRC diagnosis. A meta-analysis of 18 studies (1,129 CRC samples, 951 controls) showed miR-21's diagnostic potential with 83% specificity and 77% sensitivity. The positive likelihood ratio (PLR) was 4.20, and the negative likelihood ratio (NLR) was 0.30 (Liu et al., 2021). A more recent meta-analysis with 9 studies reported similar results, with 77% sensitivity and 82% specificity.

Prognostic Potency of miR-21 in Colorectal Cancer

High miR-21 expression in CRC tissues is linked to poor disease-free survival (DFS) and overall survival (OS). Ferraro et al. (2014) explored the ITGβ4/miR-21/PDCD4 pathway and its association with metastasis. Downregulation of ITGβ4 in tumor tissues differentiated metastatic from non-metastatic patients. ITGβ4, alongside pro-metastatic factors like PTEN and miR-21, regulates metastasis. Liver metastasis occurs in 25% of CRC patients at diagnosis and 50-70% after 3 years (Nordlinger & Rougier, 2002). A study on tumor budding cells in stage III CRC showed higher miR-21 expression in lymph node-positive cases, suggesting miR-21 as a stage indicator (Knudsen et al., 2018). Studies also showed that miR-21 expression in stage II CRC correlates with recurrence-free survival, indicating its prognostic value (Hansen et al., 2014; Kjaer-Frifeldt et al., 2012).

References

- Ahmed, S. F., Bernaba, C. N., & Muhammad, E. (2022). Immunohistochemical expression of Caspase-3 in colorectal carcinoma. SVU-International Journal of Medical Sciences, 5(1), 240-251.
- Almughamsi, A. M., Ban, A. M., Mohammed, M. A., Seraj, A. S., Domlo, H. A., Alayoubi, H. M., ... & Taha, I. (2024). Assessing Knowledge Gaps in Colorectal Cancer Screening Among Physicians in Al-Madinah, Saudi Arabia: Implications for Targeted Education Programs. SVU-International Journal of Medical Sciences, 7(2), 842-857.
- Aloizou, A.-M., Pateraki, G., Siokas, V., Mentis, A.-F. A., Liampas, I., Lazopoulos, G., . . . Paterakis, K. J. T. r. (2020). The role of MiRNA-21 in gliomas: Hope for a novel therapeutic intervention? , 7, 1514-1530.
- Amitay, E. L., Krilaviciute, A., & Brenner, H. J. G. m. (2018). Systematic review: Gut microbiota in fecal samples and detection of colorectal neoplasms. 9(4), 293-307.
- Bailey, S. E., Abel, G. A., Atkins, A., Byford, R., Davies, S.-J., Mays, J., . . . Renninson, J. J. B. J. o. C. (2021). Diagnostic performance of a faecal immunochemical test for patients with low-risk symptoms of colorectal cancer in primary care: an evaluation in the South West of England. 124(7), 1231-1236.
- Bhai, P., Kulshrestha, S., Puri, R. D., Bijarnia Mahay, S., Saxena, R., & Verma, I. C. J. I. J. o. G. (2020). Spectrum of mutations in genes associated with familial colorectal cancer syndrome (MLH1, MSH2, PMS2, MSH6, and APC): A not so common hereditary cancer syndrome in Indian population. 39, 599-607.
- Bradbury, K. E., Murphy, N., & Key, T. J. J. I. j. o. e. (2020). Diet and colorectal cancer in UK Biobank: a prospective study. 49(1), 246-258.
- Chung, C. J. J. o. O. P. P. (2022). Predictive and prognostic biomarkers with therapeutic targets in colorectal cancer: A 2021 update on current development, evidence, and recommendation. 28(4), 850-869.
- Cui, W.-Q., Wang, S.-T., Pan, D., Chang, B., & Sang, L.-X. J. W. j. o. g. o. (2020). Caffeine and its main targets of colorectal cancer. 12(2), 149.
- Dekker, E., Tanis, P. J., Vleugels, J., Kasi, P. M., & Wallace, M. J. L. (2019). Pure-amc. 394, 1467-1480.
- Demb, J., Earles, A., Martínez, M. E., Bustamante, R., Bryant, A. K., Murphy, J. D., . . . Gupta, S. J. B. o. g. (2019). Risk factors for colorectal cancer significantly vary by anatomic site. 6(1), e000313.
- Elbadr, M. M., Mahrous, M. M., Alorabi, M., Elbadre, H. M., Mohamed, R., & Ellisy, R. A. (2024). Role of dihydropyrimidine dehydrogenase genetic polymorphism and vitamin D assay for 5-Fluorouracil therapy in Upper Egypt colorectal cancer patients. SVU-International Journal of Medical Sciences, 7(2), 917-935.
- El-Korany, W. A., Zahran, W. E., Alm El-Din, M. A., Al-Shenawy, H. A., & Soliman, A. F. (2024). Clinical utility of hsa-miR-181a-5p expression level in Egyptian colorectal cancer patients. Egyptian Journal of Pure and Applied Science, 62(1), 76-84.
- Far, B. F., Vakili, K., Fathi, M., Yaghoobpoor, S., Bhia, M., & Naimi-Jamal, M. R. J. L. s. (2023). The role of microRNA-21 (miR-21) in pathogenesis, diagnosis, and prognosis of gastrointestinal cancers: A review. 316, 121340.
- Feng, Y.-H., & Tsao, C.-J. J. B. r. (2016). Emerging role of microRNA-21 in cancer. 5(4), 395-402.
- Ferlay, J., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M., . . . Bray, F. J. L. I. a. f. r. o. c. (2020). Global cancer observatory: cancer today. 20182020.
- Fernandes, J. C., Acuña, S. M., Aoki, J. I., Floeter-Winter, L. M., & Muxel, S. M. J. N.-c. R. (2019). Long non-coding RNAs in the regulation of gene expression: physiology and disease. 5(1), 17.

- Ferraro, A., Kontos, C. K., Boni, T., Bantounas, I., Siakouli, D., Kosmidou, V., . . . Zografos, G. J. E. (2014). Epigenetic regulation of miR-21 in colorectal cancer: ITGB4 as a novel miR-21 target and a three-gene network (miR-21-ITGB4-PDCD4) as predictor of metastatic tumor potential. 9(1), 129-141.
- Fiorillo, C., Quero, G., Longo, F., Mascagni, P., Delvaux, M., & Mutter, D. J. G. R. (2020). Capsule Endoscopy Versus Colonoscopy in Patients with Previous Colorectal Surgery: A Prospective Comparative Study. 13(5), 217.
- Fujita, S., Ito, T., Mizutani, T., Minoguchi, S., Yamamichi, N., Sakurai, K., & Iba, H. J. J. o. m. b. (2008). miR-21 Gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. 378(3), 492-504.
- García-Figueiras, R., Baleato-González, S., Canedo-Antelo, M., Alcalá, L., & Marhuenda, A. J. C. C. C. R. (2021). Imaging advances on ct and MRI in colorectal cancer. 17, 113-130.
- Gausman, V., Dornblaser, D., Anand, S., Hayes, R. B., O'Connell, K., Du, M., . . . Hepatology. (2020). Risk factors associated with early-onset colorectal cancer. 18(12), 2752-2759. e2752.
- Gopalakrishnan, V., Helmink, B. A., Spencer, C. N., Reuben, A., & Wargo, J. A. J. C. c. (2018). The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. 33(4), 570-580.
- Halilovic, E., Rasic, I., Sofic, A., Mujic, A., Rovcanin, A., Hodzic, E., & Kulovic, E. J. M. A. (2020). The importance of determining preoperative serum concentration of carbohydrate antigen 19-9 and carcinoembryonic antigen in assessing the progression of colorectal cancer. 74(5), 346.
- Hansen, T., Kjaer-Frifeldt, S., Christensen, R., Morgenthaler, S., Blondal, T., Lindebjerg, J., . . . Jakobsen, A. J. B. j. o. c. (2014). Redefining high-risk patients with stage II colon cancer by risk index and microRNA-21: results from a population-based cohort. 111(7), 1285-1292.
- Hatley, M. E., Patrick, D. M., Garcia, M. R., Richardson, J. A., Bassel-Duby, R., Van Rooij, E., & Olson, E. N. J. C. c. (2010). Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. 18(3), 282-293.
- Hefni, A. M. Clinicopathological and Survival Outcome of Young Onset Colorectal Cancer: 10 Years Retrospective Study in Upper Egypt.
- Hoskin, A. J., Holt, A. K., Legge, D. N., Collard, T. J., Williams, A. C., & Vincent, E. E. J. E. o. T. A.-t. T. (2023). Aspirin and the metabolic hallmark of cancer: Novel therapeutic opportunities for colorectal cancer. 4(4), 600.
- Hossain, M. S., Karuniawati, H., Jairoun, A. A., Urbi, Z., Ooi, D. J., John, A., . . . Ming, L. C. J. C. (2022). Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. 14(7), 1732.
- Huang, Y., Yang, Y. B., Zhang, X. H., Yu, X. L., Wang, Z. B., & Cheng, X. C. J. M. o. (2013). MicroRNA-21 gene and cancer. 30(1), 376.
- Issa, I. A., & Noureddine, M. J. W. j. o. g. (2017). Colorectal cancer screening: An updated review of the available options. 23(28), 5086.
- Ivancic, M. M., Megna, B. W., Sverchkov, Y., Craven, M., Reichelderfer, M., Pickhardt, P. J., . . . Kennedy, G. D. J. J. o. S. R. (2020). Noninvasive detection of colorectal carcinomas using serum protein biomarkers. 246, 160-169.
- Johnson, C. M., Wei, C., Ensor, J. E., Smolenski, D. J., Amos, C. I., Levin, B., . . . control. (2013). Meta-analyses of colorectal cancer risk factors. 24, 1207-1222.
- Kancha, R. K. (2024). Biomedical Aspects of Solid Cancers: Springer.
- Kasprzak, A. J. I. j. o. m. s. (2021). Insulin-like growth factor 1 (IGF-1) signaling in glucose metabolism in colorectal cancer. 22(12), 6434.

- Kastrinos, F., Samadder, N. J., & Burt, R. W. J. G. (2020). Use of family history and genetic testing to determine risk of colorectal cancer. 158(2), 389-403.
- Kennedy, B. M., & Harris, R. E. J. C. (2023). Cyclooxygenase and lipoxygenase gene expression in the inflammogenesis of colorectal cancer: Correlated expression of EGFR, JAK STAT and Src genes, and a natural antisense transcript, RP11-C67. 2.2. 15(8), 2380.
- Keum, N., Giovannucci, E. J. N. r. G., & hepatology. (2019). Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. 16(12), 713-732.
- Kirbiyik, O., Özyilmaz, B. J. C. P., & Cancer, C. (2021). Genetic Knowledge of Colorectal Cancer. 481-514.
- Kjaer-Frifeldt, S., Hansen, T., Nielsen, B., Joergensen, S., Lindebjerg, J., Soerensen, F., . . . Jakobsen, A. J. B. j. o. c. (2012). The prognostic importance of miR-21 in stage II colon cancer: a population-based study. 107(7), 1169-1174.
- Knudsen, K. N., Lindebjerg, J., Kalmár, A., Molnár, B., Sørensen, F. B., Hansen, T. F., . . . Metastasis, E. (2018). miR-21 expression analysis in budding colon cancer cells by confocal slide scanning microscopy. 35, 819-830.
- Kranenburg, O., Speeten, K. v. d., & Hingh, I. d. J. F. i. o. (2021). Peritoneal metastases from colorectal cancer: defining and addressing the challenges. 11, 650098.
- Lai, L. L., Blakely, A., Invernizzi, M., Lin, J., Kidambi, T., Melstrom, K. A., . . . Lu, T. J. J. o. B. O. (2021). Separation of color channels from conventional colonoscopy images improves deep neural network detection of polyps. 26(1), 015001-015001.
- Larson, E. J. A. R. o. P. H. (2007). Community factors in the development of antibiotic resistance. 28(1), 435-447.
- Lee, T., Teng, T. Z. J., & Shelat, V. G. J. W. j. o. g. s. (2020). Carbohydrate antigen 19-9—Tumor marker: Past, present, and future. 12(12), 468.
- Li, C., Zhao, L., Chen, Y., He, T., Chen, X., Mao, J., . . . Meng, Q. H. J. B. c. (2016). MicroRNA-21 promotes proliferation, migration, and invasion of colorectal cancer, and tumor growth associated with down-regulation of sec23a expression. 16, 1-11.
- Lin, T.-C., Chien, W.-C., Hu, J.-M., Tzeng, N.-S., Chung, C.-H., Pu, T.-W., . . . Chen, C.-Y. J. P. O. (2020). Risk of colorectal cancer in patients with alcoholism: A nationwide, population-based nested case-control study. 15(5), e0232740.
- Liu, T., Liu, D., Guan, S., & Dong, M. J. A. o. m. (2021). Diagnostic role of circulating MiR-21 in colorectal cancer: a update meta-analysis. 53(1), 87-102.
- Mena-Osuna, R. (2021). Molecular subtypes of colorectal cancer: mechanisms involved in tumor progression and immune evasion.
- Monahan, K. J., Davies, M. M., Abulafi, M., Banerjea, A., Nicholson, B. D., Arasaradnam, R., . . . Burling, D. J. G. (2022). Faecal immunochemical testing (FIT) in patients with signs or symptoms of suspected colorectal cancer (CRC): a joint guideline from the Association of Coloproctology of Great Britain and Ireland (ACPGBI) and the British Society of Gastroenterology (BSG). 71(10), 1939-1962.
- Morgan, E., Arnold, M., Gini, A., Lorenzoni, V., Cabasag, C., Laversanne, M., . . . Bray, F. J. G. (2023). Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. 72(2), 338-344.
- Murphy, N., Ward, H. A., Jenab, M., Rothwell, J. A., Boutron-Ruault, M.-C., Carbonnel, F., . . . Hepatology. (2019). Heterogeneity of colorectal cancer risk factors by anatomical subsite in 10 European countries: a multinational cohort study. 17(7), 1323-1331. e1326.
- Navarro, M., Nicolas, A., Ferrandez, A., & Lanas, A. J. W. j. o. g. (2017). Colorectal cancer population screening programs worldwide in 2016: An update. 23(20), 3632.

- Nguyen, L. H., Goel, A., & Chung, D. C. J. G. (2020). Pathways of colorectal carcinogenesis. 158(2), 291-302.
- Nordlinger, B., & Rougier, P. J. J. o. c. o. (2002). Liver metastases from colorectal cancer: the turning point. In (Vol. 20, pp. 1442-1445): American Society of Clinical Oncology.
- O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. J. F. i. e. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation. 9, 402.
- Ogunwobi, O. O., Mahmood, F., & Akingboye, A. J. I. j. o. m. s. (2020). Biomarkers in colorectal cancer: current research and future prospects. 21(15), 5311.
- Petrick, J. L., Barber, L. E., Warren Andersen, S., Florio, A. A., Palmer, J. R., & Rosenberg, L. J. F. i. O. (2021). Racial disparities and sex differences in early-and late-onset colorectal cancer incidence, 2001–2018. 11, 734998.
- Ponnatapura, J., & Lalwani, N. (2021). Imaging of Colorectal Cancer: Screening, Staging, and Surveillance. Paper presented at the Seminars in Roentgenology.
- Poore, G. D., Kopylova, E., Zhu, Q., Carpenter, C., Fraraccio, S., Wandro, S., . . . Song, S. J. J. N. (2020). Microbiome analyses of blood and tissues suggest cancer diagnostic approach. 579(7800), 567-574.
- Qian, H., Maghsoudloo, M., Kaboli, P. J., Babaeizad, A., Cui, Y., Fu, J., ... & Imani, S. (2024). Decoding the Promise and Challenges of miRNA-Based Cancer Therapies: An Essential Update on miR-21, miR-34, and miR-155. International Journal of Medical Sciences, 21(14), 2781.
- Rapado-González, Ó., Álvarez-Castro, A., López-López, R., Iglesias-Canle, J., Suárez-Cunqueiro, M. M., & Muinelo-Romay, L. J. C. (2019). Circulating microRNAs as promising biomarkers in colorectal cancer. 11(7), 898.
- Rossi, M., Jahanzaib Anwar, M., Usman, A., Keshavarzian, A., & Bishehsari, F. J. C. (2018). Colorectal cancer and alcohol consumption—populations to molecules. 10(2), 38.
- Sachdeo, R. A., Charde, M. S., & Chakole, R. D. J. A. J. o. R. i. P. S. (2020). Colorectal cancer: An overview. 10(3), 211-223.
- Sand, M. MicroRNAs in malignant tumors of the skin.
- Sawicki, T., Ruszkowska, M., Danielewicz, A., Niedźwiedzka, E., Arłukowicz, T., & Przybyłowicz, K. E. J. C. (2021). A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms and diagnosis. 13(9), 2025.
- Shah, M. S., DeSantis, T. Z., Weinmaier, T., McMurdie, P. J., Cope, J. L., Altrichter, A., . . . Hollister, E. B. J. G. (2018). Leveraging sequence-based faecal microbial community survey data to identify a composite biomarker for colorectal cancer. 67(5), 882-891.
- Shinde, S., Sinha, V., Dixit, V., Dwivedi, M., Vishwakarma, N. K., Tiwari, A. K., . . . Shukla, D. (2021). Dietary habits and global incidence of colon cancer. In Colon Cancer Diagnosis and Therapy: Volume 2 (pp. 15-52): Springer.
- Silva, A., Faria, G., Araújo, A., & Monteiro, M. P. J. C. R. i. O. H. (2020). Impact of adiposity on staging and prognosis of colorectal cancer. 145, 102857.
- Singh, A., Singh, A. K., Giri, R., Kumar, D., Sharma, R., Valis, M., . . . Garg, N. J. F. m. c. (2021). The role of microRNA-21 in the onset and progression of cancer. 13(21), 1885-1906.
- Spada, C., Hassan, C., Bellini, D., Burling, D., Cappello, G., Carretero, C., . . . Kaminski, M. F. J. E. (2020). Imaging alternatives to colonoscopy: CT colonography and colon capsule. European Society of gastrointestinal endoscopy (ESGE) and European Society of gastrointestinal and abdominal radiology (ESGAR) guideline–update 2020. 52(12), 1127-1141.
- Steele, S. R., Park, G. E., Johnson, E. K., Martin, M. J., Stojadinovic, A., Maykel, J., . . . rectum. (2014). The impact of age on colorectal cancer incidence, treatment, and outcomes in an equal-access health care system. 57(3), 303-310.

- Sun, G., Meng, J., Duan, H., Zhang, D., Tang, Y. J. P., & Research, O. (2019). Diagnostic assessment of septin9 DNA methylation for colorectal cancer using blood detection: A meta-analysis. 25, 1525-1534.
- Sur, D., Coza, O., Havasi, A., Cainap, C., Burz, C., Vlad, C., . . . Lisencu, C. J. J. o. B. O. O. J. o. t. B. U. o. O. (2020). Exosomal miRNAs in colorectal cancer: the carriers of useful news. 25(1), 23-34.
- Thakur, P., Saini, R. V., Chhillar, A. K., Saini, N. K., Thakur, V. K., Siwal, S. S., & Saini, A. K. J. B. (2022). Alteration in the expression of microRNA-21 regulated target genes: Role in breast cancer. 46(2), 309-324.
- Vallès, X., Alonso, M. H., López-Caleya, J. F., Díez-Obrero, V., Dierssen-Sotos, T., Lope, V., . . . Tardón, G. F. J. E. i. (2018). Colorectal cancer, sun exposure and dietary vitamin D and calcium intake in the MCC-Spain study. 121, 428-434.
- Vieito, N. P., Zarraquiños, S., & Cubiella, J. J. W. j. o. g. (2019). High-risk symptoms and quantitative faecal immunochemical test accuracy: systematic review and meta-analysis. 25(19), 2383.
- Villéger, R., Lopès, A., Veziant, J., Gagnière, J., Barnich, N., Billard, E., . . . Bonnet, M. J. W. j. o. g. (2018). Microbial markers in colorectal cancer detection and/or prognosis. 24(22), 2327.
- Vodicka, P., Urbanova, M., Makovicky, P., Tomasova, K., Kroupa, M., Stetina, R., . . . Schneiderova, M. J. I. J. o. M. S. (2020). Oxidative damage in sporadic colorectal cancer: molecular mapping of base excision repair glycosylases in colorectal cancer patients. 21(7), 2473.
- Wilkinson, A. N., Lieberman, D., Leontiadis, G. I., Tse, F., Barkun, A. N., Abou-Setta, A., . . . Telford, J. J. J. C. F. P. (2019). Colorectal cancer screening for patients with a family history of colorectal cancer or adenomas. 65(11), 784-789.
- Wolin, K. Y., Yan, Y., Colditz, G. A., & Lee, I. J. B. j. o. c. (2009). Physical activity and colon cancer prevention: a meta-analysis. 100(4), 611-616.
- Xiong, Y., Zhang, Y.-Y., Wu, Y.-Y., Wang, X.-D., Wan, L.-H., & Zhou, L.-M. J. L. s. (2014). Correlation of over-expressions of miR-21 and Notch-1 in human colorectal cancer with clinical stages. 106(1-2), 19-24.
- Yang, W. H., George, A. P., Wang, C.-M., Yang, R. H., Duncan, A. M., Patel, D., . . . Yang, W.-H. J. C. O. (2023). Tumor suppressor p53 down-regulates programmed cell death protein 4 (PDCD4) expression. 30(2), 1614-1625.
- Yılmaz, A., Mirili, C., Bilici, M., & Tekin, S. B. J. I. j. o. c. d. (2020). Colorectal cancer in Lynch syndrome associated with PMS2 and MSH6 mutations. 35, 351-353.