Medical and Research Publications
International Open Access

Review Article **Journal of MAR Pathology (Volume 1 Issue 5)**

A Review of Colorectal Neoplasia in Terms of Molecular Pathogenesis, Diagnosis, and Treatment

Jigar Katwala [1], Jeevan Divakaran *[2], Sunil Kumar Rai [3], Ila Chauhan [4]

1. Associate Professor, Clinical Skills, Medical University of the Americas, St Kitts and Nevis

2. Professor Pathology, Medical University of the Americas, St Kitts and Nevis

3. Assistant Professor, Cell and Molecular Biology, Medical University of the Americas, St Kitts and Nevis.

4. Assistant Professor, Clinical Skills, Medical University of the Americas, St Kitts and Nevis.

Corresponding Author: Jeevan Divakaran, Professor Pathology, Medical University of the Americas, St Kitts and Nevis.

Received Date: January 22, 2023

Published Date: March 01, 2023

Abstract

Colorectal neoplasia is the most frequent and lethal gastrointestinal tract malignancy worldwide in both males and females. According to the IARCG (International Agency for Research on Cancer Group), colorectal cancer is considered the third most diagnosed malignancy after lung and breast cancers and the second most common cause of death from cancer. More than 90% of cases of colorectal cancer occur in individuals over 50 years of age, but the more aggressive forms are observed in patients of younger age. It occurs more frequently in males compared to females. Most cases of neoplasia are sporadic, but germline mutations cause a few. Several diagnostic tests, including stool tests (DNA and immunological), guaiac test, barium enema, colonoscopy, and sigmoidoscopy, are available to diagnose colorectal cancer. This overview focuses on the molecular pathogenesis, diagnosis, and treatment of colorectal neoplasia.

Keywords: *Cancer, Colorectal neoplasia (CN), Molecular pathogenesis, Molecular Diagnosis, and Treatment.*

Introduction

Colorectal neoplasia (CN) is the most prevalent and devastating neoplasm of the gastrointestinal tract, accounting for 13% of all cancers **[**1**]**. In clinical practice, colon and rectal cancer are collectively called colorectal cancer. In oncology and pathology, CN is considered the third most diagnosed malignancy after lung and breast cancer and the second most malignant tumor in terms of deaths **[**2, 3**]**, and it has significantly increased the burden of patients in the healthcare system. Colon and rectal cancer account for 72% and 28% of CN cases, respectively, and the incidence of CN represents the collective incidence of colon and rectal cancer **[**4**]**. Globally, approximately 1.9 million cases were reported in 2020 **[**3**]**, while approximately 151,030 new cases are diagnosed annually in the USA **[**5**]**. The incidence of CN is increasing yearly, comprising 11% of all malignant neoplasms occurring in patients **[**6**]**. CN most frequently occurs in men, in contrast to females **[**7**]**. Existing studies demonstrate that CN cases are high in developed countries, especially in western countries **[**6-9**]**. The driving factors for the developmental risk of CN are a sedentary lifestyle, nutrition (diet with high fat), obesity, consumption of alcohol and tobacco, and a progressive increase in the age of the population **[**10, 11**]**.

Recent reports have analyzed that new CN cases will increase to 2.2 million by 2030 due to health-related behavior, socioeconomic factors, and environmental changes [6, 7, 10]. The most characteristic symptom associated with CN is blood in the stools, while non-specific symptoms include fever, nausea, fatigue, reduced appetite, weight loss, bowel obstruction, and abdominal pain [11, 12]. Laboratory studies showed that CN is a multistep genetic disease [13, 14, 15] as it occurs owing to the cumulative effect of various sequential genetic mutations, and these genetic alterations may be inherited or acquired.

Molecular Pathogenesis of Colorectal Neoplasia:

CN can result from mutations in oncogenes, tumor suppressor genes, and genes involved in DNA repair pathways. CN can be divided into three groups based on the mutation: sporadic, hereditary, and familial.

Sporadic CN: this is more common among older adults, comprises nearly 70% of all colorectal cancers, and is typically caused by point mutations. Because multiple genes might be affected by mutations, the molecular etiology of sporadic cancer is varied. [16]

Familial CN: this is more frequently seen in a first-degree relative, and molecular pathogenesis has not been established yet.[17]

Inherited CN: only about 5% of CN cases are caused by inherited malignancies. Polyposis and non-polyposis types are the two main types. Familial adenomatous polyposis (FAP) is an autosomal dominant disease mainly due to the APC gene mutation and is characterized by the development of several potentially cancerous polyps in the colon [10]. In contrast, DNA repair pathway mutations are linked to hereditary non-polyposis colorectal cancer (HNPCC). HNPCC is an autosomal dominant disease, also known as Lynch syndrome. [18] MUTYH-associated polyposis (MAP), Peutz-Jeghers syndrome (PJS), and Serrated polyposis syndrome (SPS) are also subtypes of inherited CN.

Molecular Pathways of Colorectal Neoplasia

Several molecular pathways are involved in the development of CN. Accumulation of epigenetic and genetic alterations transforms the normal epithelium into adenocarcinoma [19], while the exact molecular mechanisms underlying the transformation remain elusive. However, the pathogenetic mechanism leading to CN can be categorized into three types: CIN (chromosome instability), MSI (microsatellite instability), and CIMP (CpG island methylator phenotype) [20].

CIN (chromosome instability):

CIN accounts for approximately 80%-85% of CNs [21]; reduced apoptotic activity and increased growth-promoting activity are the characteristic features [22]. The molecular mechanisms include alterations in chromosome segregation, telomere dysfunction, and DNA damage response that affect genes such as APC, KRAS, PI3K, and TP53, amongst others.[23] All tumors initiate as adenomatous polyps owing to the inactivation of the Adenomatous Polyposis Coli (APC) gene. The progression of adenomatous polyps to adenocarcinoma results from enhanced KRAS and reduced SMAD4 activity sometimes referred to as the APC pathway [24].

The Microsatellite instability (MSI) pathway:

The Microsatellite instability (MSI) pathway is a hypermutable phenotype resulting from defects in the DNA repair mechanisms. These mutations can affect non-coding regions as well as codifying microsatellites. Loss of expression of mismatch repair genes (MMR) can be caused by a spontaneous event (promoter hypermethylation) or a germinal mutation such as found in Lynch syndrome.[23] MSI results from an alteration in the MMR DNA, leading to mutations in the MLH1, MSH2, and MSH6. (HNPCC). The hereditary form of CN is nominated as Lynch syndrome and occurs due to mutation in the MMR genes, including MSH2 & 6, PMS2, and MLH1 genes. Inactivation of MLH1 owing to biallelic hypermethylation of MLH1 promoter and double somatic mutation of MMR genes is responsible for MMR deficiency [25]. The patients with dMMR–MSI-H (high MSI and deficient MMR) exhibit a good prognosis, but they do not achieve therapeutic advantages from 5-fluorouracil [26].

CIMP (CpG island methylator phenotype):

A subset of CN called CIMP is characterized by hypermethylation of the CpG islands around the promoter of tumor suppressor genes, leading to gene silencing and a loss of protein expression. Due to the lack of a specific definition of high CIMP, it is challenging to translate this pathophysiological characteristic into treatment [21]. CIMP testing is performed on CDKN2A, CACNA1G, MINT1, and MLH1, while there is still no standard value to distinguish between CIMP+ and high CIMP [27].

New Molecular signaling pathways in Colorectal Neoplasia

Numerous genetic abnormalities linked to colorectal neoplasia have been discovered thanks to new genomic approaches. They have identified the role of important pathways (WNT, MAPK/PI3K, TGF-β) and functions within the cell (TP53 and cell-cycle regulation) in CN.

The WNT signaling pathway

Genetic alteration plays a significant role in the initiation and progression of polyp cancer. An abnormal crypt focus is referred to as an initial lesion associated with the development of CN **[**28**]**.

APC was the first tumor suppressor gene identified in association with colorectal cancer. Genetic alteration in the APC gene activates the WNT signaling pathway resulting in the development of adenomatous polyps. Subsequent mutations in the KRAS and TP53 genes lead to the transformation of these benign polyps into invasive cancer **[**18**]**. APC gene, b-catenin, and c-MYC are associated with the WNT pathway. C-MYC is a metastatic marker and an excellent survival-related prognostic factor. **[**23**]**

The MAPK and PI3K pathways

More than 50% of cases of CN have mutations in the KRAS, BRAF, and PIK3CA (PI3K) genes that stimulate cell proliferation through MAPK signaling and inhibit the apoptotic pathway. KRAS and BRAF mutations are associated with a poor prognosis and decreased survival. Further, KRAS and TP53 may accelerate the development of CN by altering the TGF-β signaling pathway **[**29**]**. There is a link between TP53 deficiency and worse CN survival rates. The TGF-β1-SMAD, Ras-Raf-MAPK pathway, Wnt-APC-CTNNB1, and PI3K mediated signaling pathways are also involved in the development of CN **[**30**]**.

Molecular Diagnosis of Colorectal Neoplasia

A biomarker or molecular marker is a biological entity that can measure the presence or progression of a particular disease or the effects of treatment. There are three important variations in CN: MSI, CIN, and CIMP. These mutations lead to changes in DNA, RNA, proteins, or metabolites, which can be detected in tumor specimens, blood, or stools and utilized as biomarkers or molecular diagnostic markers.**[**23**]** Molecular markers for diagnosis of CN are better than conventional diagnostic modalities

because they can be utilized for making better decisions for treatment, evaluating the prognosis, and predicting the response to chemotherapeutic agents, and serving as a screening tool for the recognition of family members of patients for the developmental risk of CN [31]; they also serve as an indication for prophylactic surgery. For instance, APC mutation is an initial event in familial adenomatous polyposis (FAP) linked with CNs. This molecular marker screens family members for CN development risk and indicates prophylactic surgery. In the case of equivocal histological findings, molecular markers can aid in distinguishing between tumor cells and normal cells.

PCR-based and Sanger DNA sequencing method

Sanger DNA sequencing method can screen known mutations and novel alterations like deletion, insertion, and substitution of nucleotides. A potential benefit of detecting genetic mutations is to make better decisions regarding treatment and prediction of chemotherapeutic drug response. According to ASCO (American society for clinical oncology), stage IV tumors in patients with sporadic CN must undergo mutational screening of KRAS before administering any anti-EGFR treatments [32-35]. These genetic mutations can be screened through PCR-based and DNA sequencing (Sanger) modality to evaluate the prognosis and predict the response to chemotherapeutic agents. The BRAF and KRAS genes are mutually exclusive, and mutational assessment of these genes is critical in predicting response to anti-EGFR monoclonal antibodies. There is evidence that sporadic CN patients with mutations in these genes failed to respond to anti-EGFR treatment [36, 37, 38].

Fluorophore-based quantitative RT-PCR and pyrosequencing

These novel approaches have greater specificity, sensitivity, and shorter turnover compared to PCR-based and Sanger sequencing methods. This technique's basic principle is to compare the lengths of specific microsatellite markers in tumor cells to normal cells. [39]

These tests help evaluate the prognosis of CN. Several previous studies found that the MSI status is a valuable prognostic factor for patients with sporadic CN because high MSI correlates with better survival rates, low recurrence, and a low tendency to metastasize. [40-43]. BRAF mutation screening is indicated in such cases, as this alteration is associated with high-MSI in sporadic CNs but not HNPCC cases. Mutation in BRAF appears to abrogate the distinctly better prognosis observed in high-MSI CNs [44-45].

In addition, these are also useful for screening. The genotype pattern is scored as MSI-L, MSI-H, or MSS after comparing the tumor with normal samples. Hypermethylation-mediated deactivation of the tumor suppressor genes leads to carcinogenesis. In Lynch syndrome, the second allele of the MRR gene is inactivated by hypermethylation following the allelic germline mutation of the MRR gene. Around 35%-40% of cases of sporadic CN are CIMP-positive. Hypermethylated CN cases are more prevalent in females especially linked with BRAF mutation **[**46-51**]**. Silencing of genes by hypermethylation is detected through bisulfite-conversion and PCR specific for methylation modality. Pyrosequencing can be employed to screen the hypermethylated genetic blueprint **[**52**]**. However, no established panel of biomarkers for detecting hypermethylation is currently available.

Amplification of microsatellite regions is helpful as an indicator to distinguish the tumor and normal cells, especially in the case of MSI CN. According to Bethesda guidelines, five molecular markers (BAT26, BAT25, D17S250, D2S123, and D5S346) are used for PCR-based detection of the HNPCC cases **[**53**]**. Mononucleotide molecular markers (BAT26 and BAT25) are highly sensitive compared to di-nucleotide markers. Pentaplex repeats of mononucleotide (NR-24, BAT-25, MONO-27, BAT-26, and NR-21) are more accurate (>90%) in the detection of altered genes associated with MMR in CN. Fluorescence-based PCR systems are utilized to amplify biomarkers associated with microsatellites to distinguish between tumors and normal cells, and analysis of products is performed by capillary electrophoresis **[**54**]**.

Tumor markers

Carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, tumor-associated glycoprotein (TAG-72), tissue polypeptide specific antigen (TPS), and TAG-72 are used in the diagnosis and monitoring of CN usage. Unfortunately, the sensitivity and organic specificity of these tumor markers lately used in CN diagnosis are poor.

Immunohistochemical (IHC) analysis

Histological assay plays a vital role in the diagnosis of Lynch syndrome, which involves screening antibodies against MMR proteins such as PMS2, MSH2, MLH1, and MSH6. IHC analysis can determine the loss of expression of one or more MMR proteins. Adenocarcinomas and colonic adenomas exhibit indistinguishable IHC staining features, and all carcinomas associated with CN show positive IHC staining for CK20. However, some are positive for CK7 **[**50, 51**]**. Other IHC biomarkers for detecting CN include cadherin-17, CDX-2, villin, SATB2, and β-catenin **[**57, 58**]**. Inactivation of

the SMAD4 gene is linked to poor prognosis of CN cases [59]. Laboratory studies indicate that IHC staining for CD166 and CD44 can be potential biomarkers of stem cells associated with carcinoma, which are involved in invasion, metastases, treatment response, and CN recurrence [60].

Micro-RNA

Identification of serum biomarkers has attracted many researchers' attention as this approach can diagnose CN at an initial stage, but only little success has been achieved so far in this field. Much evidence demonstrates the connection between miRNA in the blood and CN [61, 62]. Micro-RNA, including miR-92a, miR-135a, miR-211, miR-135b, and miR17-3p, has been linked with cases of CN [63, 64, 65]. Profiling of serum micro-RNA can be performed through the use of microarray and PCR-based modality [66, 67, 68]. Lateral flow nucleic acid-based assay can also be used to screen micro-RNA and serve as a POCT (point of care testing) tool [69, 70, 71]. Kits for evaluating the CpG island methylator phenotype and miRNA and gene microarrays detectable in stool or blood are in clinical trials and have a bright future.

Multitarget stool DNA testing

Considerable work has been done to recognize the CN-specific DNA biomarkers in stools because they are derived from tumor cells and are regarded as specific. This is a non-invasive test for CN that detects methylation patterns and genetic alterations in stool samples, i.e., aberrantly methylated NDRG4 and BMP3; any of seven KRAS point mutations linked with the adenoma-carcinoma sequence.

Proteomics

Proteomic profiling of the serum of patients with CN through a mass spectroscopy approach can be employed to determine the serum proteomic signature to distinguish the different kinds of CN. This technique determines the tumor-specific protein in blood and stool sample. It is under clinical evaluation.

Treatment of Colorectal Neoplasia

Early diagnosis is critical to enhancing the survival rate in patients with CN [72]. Surgery, radiation, and chemotherapy are the standard approaches to manage CN [73], and for advanced stages of malignancy, a combination of these approaches may be employed [74]. A multi-model approach is employed depending on various features like the size and location of the tumor, the extent of tumor metastasis, and the patient's health status [75, 76]. Several approaches have emerged for the management of metastatic and primary CN, which include: (a) Laparoscopic mediated surgery, especially for early-stage primary CN and for more aggressive surgical resection of metastatic CN (b) radiotherapy for rectal cancers (c) neo-adjuvant chemotherapy and (d) palliative chemotherapy [77]. Surgical intervention is the preferred approach for managing localized early-stage CN [76, 77, 78]. Radiotherapy and neo-adjuvant chemotherapy can be employed for CN before or after surgery, depending on the tumor stage [79]. The survival rate of the patients can be improved by surgical removal of solid metastases from distant organs like the liver or lungs. Systemic chemotherapy is the approach to enhance patients' survival rate with metastatic CN [80]. It is employed in combination with monoclonal antibodies developed against VEGF and EGFR to mitigate the process of angiogenesis and growth of tumors [78, 81]. Palliative chemotherapy is employed explicitly for non-surgical cases to manage late-stage metastatic CN and enhance expectancy and quality of life [78]. Gold-based drugs, anti-inflammatory agents, probiotics, and agarose macro beads are alternative treatments still under investigation to reduce the adverse effects [76]. Despite advancements in the management of CN, the effectiveness of chemotherapy drugs, safety issues, and late detection of disease are the primary hurdles against treatment efficacy.

Conclusion

Colorectal neoplasia is the third most common and the second most lethal among all kinds of carcinomas. Sporadic and acquired CN are the two types that result from mutations in various signaling pathways. Clinical studies are required to establish the relationship between CN and behavioral, lifestyle, genetic, and environmental factors. DNA sequencing, a PCR-based approach, and IHC staining of tissue samples are the primary modalities employed for detecting CN. Synergistic action of treatment efficacy must be explored through clinical trials at multicenter hospitals worldwide. Research studies must focus on establishing non-invasive serum biomarkers safer for patients. Further, integrating the proteomics, genomics, and transcriptomics databases may improve the diagnosis, prognosis, and treatment of patients with CN.

References

1. Granados-Romero JJ, Valderrama-Treviño AI, Contreras-Flores EH, Barrera-Mera B, Herrera Enríquez M, Uriarte-Ruíz K, Ceballos-Villalba JC, Estrada-Mata AG, Alvarado Rodríguez C, Arauz-Peña G. Colorectal cancer: a review. Int J Res Med Sci. 2017 Oct;5(11):4667.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021 May;71(3):209-49.
3. Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms, and diagnosis. Cancers. 2021 Jan;13(9):2025.
4. Centelles JJ. General aspects of colorectal cancer. International Scholarly Research Notices. 2012;2012.
5. Xia C, Dong X, Li H, Cao M, Sun D, He S, Yang F, Yan X, Zhang S, Li N, Chen W. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chinese medical journal. 2022 Mar 5;135(05):584-90.
6. Wong MC, Huang J, Lok V, Wang J, Fung F, Ding H, Zheng ZJ. Differences in incidence and mortality trends of colorectal cancer worldwide based on sex, age, and anatomic location. Clinical Gastroenterology and Hepatology. 2021 May 1;19(5):955-66.
7. World Health Organization. World health statistics 2016: monitoring health for the SDGs sustainable development goals. World Health Organization; 2016 Jun 8.
8. Fidler MM, Soerjomataram I, Bray F. A global view on cancer incidence and national levels of the human development index. International journal of cancer. 2016 Dec 1;139(11):2436-46.
9. Chetty R, Stepner M, Abraham S, Lin S, Scuderi B, Turner N, Bergeron A, Cutler D. The association between income and life expectancy in the United States, 2001-2014. Jama. 2016 Apr 26;315(16):1750-66.
10. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal F. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin.
11. Alzahrani SM, Al Doghaither HA, Al-Ghafari AB. General insight into cancer: An overview of colorectal cancer. Molecular and Clinical Oncology. 2021 Dec 1;15(6):1-8.
12. Jensen LF, Hvidberg L, Pedersen AF, Vedsted P. Symptom attributions in patients with colorectal cancer. BMC Family Practice. 2015 Dec;16(1):1-0.

13. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. JNCI: Journal of the National Cancer Institute. 1981 Jun 1;66(6):1192-308.
14. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996 Oct 18;87(2):159-70.
15. Marra G, Boland CR. Hereditary non-polyposis colorectal cancer: the syndrome, the genes, and historical perspectives. JNCI: Journal of the National Cancer Institute. 1995 Aug 2;87(15):1114-25.
16. Arvelo F, Sojo F, Cotte C. Biology of colorectal cancer. E cancer medical science. 2015;9:520.
17. Lin OS. Colorectal cancer screening in patients at moderately increased risk due to family history. World J Gastrointest Oncol. 2012;4:125–30.
18. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet. 2009;76:1–18.
19. Müller MF, Ibrahim AE, Arends MJ. Molecular pathological classification of colorectal cancer. Virchows Archiv. 2016 Aug;469(2):125-34.
20. Fischer J, Walker LC, Robinson BA, Frizelle FA, Church JM, Eglinton TW. Clinical implications of the genetics of sporadic colorectal cancer. ANZ Journal of Surgery. 2019 Oct;89(10):1224-9.
21. Al-Joufi FA, Setia A, Salem-Bekhit MM, Sahu RK, Alqahtani FY, Widyowati R, Aleanizy FS. Molecular pathogenesis of colorectal cancer with an emphasis on recent advances in biomarkers, as well as nanotechnology-based diagnostic and therapeutic approaches. Nanomaterials. 2022 Jan 4;12(1):169.
22. Raskov H, Søby JH, Troelsen J, Bojesen RD, Gögenur I. Driver gene mutations and epigenetics in colorectal cancer. Annals of Surgery. 2020 Jan 1;271(1):75-85.
23. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. Int J Mol Sci. 2017 Jan 19;18(1):197. doi: 10.3390/ijms18010197. PMID: 28106826; PMCID: PMC5297828.
24. Koveitypour Z, Panahi F, Vakilian M, Peymani M, Seyed Forootan F, Nasr Esfahani MH, Ghaedi K. Signaling pathways involved in colorectal cancer progression. Cell & bioscience. 2019 Dec;9(1):1-4.
25. Boland PM, Yurgelun MB, Boland CR. Recent progress in Lynch syndrome and other familial colorectal cancer syndromes. CA: a cancer journal for clinicians. 2018 May;68(3):217-31.

26. Watson MM. Microsatellite instability at tetranucleotides (EMAST) in colorectal cancer: clinical relevance, mechanisms, and immune markers.
27. Mitsuhashi K, Yamamoto I, Kurihara H, Kanno S, Ito M, Igarashi H, Ishigami K, Sukawa Y, Tachibana M, Takahashi H, Tokino T. Analysis of the molecular features of rectal carcinoid tumors to identify new biomarkers that predict biological malignancy. Oncotarget. 2015 Sep 8;6(26):22114.
28. Szylberg Ł, Janiczek M, Popiel A, Marszałek A. Serrated polyps and their alternative pathway to colorectal cancer: a systematic review. Gastroenterology research and practice. 2015 Oct;2015.
29. Novellasdemunt L, Antas P, Li VS. Targeting Wnt signaling in colorectal cancer. A review in the theme: cell signaling: proteins, pathways, and mechanisms. American Journal of Physiology-Cell Physiology. 2015 Oct 15;309(8):C511-21.
30. Ahronian LG, Sennott EM, Van Allen EM, Wagle N, Kwak EL, Faris JE, Godfrey JT, Nishimura K, Lynch KD, Mermel CH, Lockerman EL. Clinical Acquired Resistance to RAF Inhibitor Combinations in BRAF-Mutant Colorectal Cancer through MAPK Pathway AlterationsClinical Acquired Resistance in BRAF-Mutant Colorectal Cancer. Cancer discovery. 2015 Apr 1;5(4):358-67.
31. Bedeir A, Krasinskas AM. Molecular diagnostics of colorectal cancer. Archives of pathology & laboratory medicine. 2011 May;135(5):578-87.
32. Di Nicolantonio F, Martini M, Molinari F, Sartore Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer.
33. Kislitsin D, Lerner A, Rennert G, Lev Z. K-ras mutations in sporadic colorectal tumors in Israel: unusual high frequency of codon 13 mutations and evidence for the nonhomogeneous representation of mutation subtypes. Digestive Diseases and Sciences. 2002 May;47(5):1073-9.
34. De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. The lancet oncology. 2011 Jun 1;12(6):594-603.
35. Fornaro L, Lonardi S, Masi G, Loupakis F, Bergamo F, Salvatore L, Cremolini C, Schirripa M, Vivaldi C, Aprile G, Zaniboni A. FOLFOXIRI in combination with panitumumab as first-line treatment in quadruple wild-type (KRAS, NRAS, HRAS, BRAF) metastatic colorectal cancer patients: a phase II trial by the Gruppo Oncologico Nord Ovest (GONO). Annals of oncology. 2013 Aug 1;24(8):2062-7.

36. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. The lancet oncology. 2010 Aug 1;11(8):753-62.
37. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. New England journal of medicine. 2004 Jul 22;351(4):337-45.
38. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F. Panitumumab–FOLFOX4 treatment and RAS mutations in colorectal cancer. New England Journal of Medicine. 2013 Sep 12;369(11):1023-34.
39. Setaffy L, Langner C. Microsatellite instability in colorectal cancer: clinicopathological significance. Pol J Pathol. 2015;66:203–218.
40. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, Redston M, Gallinger S. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. New England Journal of Medicine. 2000 Jan 13;342(2):69-77.
41. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. Journal of clinical oncology. 2005 Jan 20;23(3):609-18.
42. Sinicrope FA, Rego RL, Halling KC, Foster N, Sargent DJ, La Plant B, French AJ, Laurie JA, Goldberg RM, Thibodeau SN, Witzig TE. Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology. 2006 Sep 1;131(3):729-37.
43. Benatti P, Gafà R, Barana D, Marino M, Scarselli A, Pedroni M, Maestri I, Guerzoni L, Roncucci L, Menigatti M, Roncari B. Microsatellite instability and colorectal cancer prognosis. Clinical Cancer Research. 2005 Dec 1;11(23):8332-40.
44. French AJ, Sargent DJ, Burgart LJ, Foster NR, Kabat BF, Goldberg R, Shepherd L, Windschitl HE, Thibodeau SN. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. Clinical Cancer Research. 2008 Jun 1;14(11):3408-15.
45. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL, Fuchs CS. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009 Jan 1;58(1):90-6.
46. Shi C, Washington K. Molecular testing in colorectal cancer: diagnosis of Lynch syndrome and personalized cancer medicine. American journal of clinical pathology. 2012 Jun 1;137(6):847-59.

47. Aaltonen LA, Peltomäki P, Mecklin JP, Järvinen H, Jass JR, Green JS, Lynch HT, Watson P, Tallqvist G, Juhola M, Sistonen P. Replication errors in benign and malignant tumors from hereditary non-polyposis colorectal cancer patients. Cancer research. 1994 Apr 1;54(7):1645-8.
48. Lipkin SM, Wang V, Stoler DL, Anderson GR, Kirsch I, Hadley D, Lynch HT, Collins FS. Germline and somatic mutation analyses in the DNA mismatch repair gene MLH3: evidence for somatic mutation in colorectal cancers. Human mutation. 2001 May;17(5):389-96.
49. Lynch HT, De la Chapelle A. Hereditary colorectal cancer. New England Journal of Medicine. 2003 Mar 6;348(10):919-32.
50. Wheeler JM, Loukola A, Aaltonen LA, Mortensen NM, Bodmer WF. The role of hypermethylation of the MLH1 promoter region in HNPCC versus MSI+ sporadic colorectal cancers. Journal of medical genetics. 2000 Aug 1;37(8):588-92.
51. Issa JP. Colon cancer: it's CIN or CIMP. Clinical Cancer Research. 2008 Oct 1;14(19):5939-40.
52. Herbst A, Kolligs FT. Detection of DNA hypermethylation in remote media of patients with colorectal cancer: new biomarkers for colorectal carcinoma. Tumor Biology. 2012 Apr;33(2):297-305.
53. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. 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 research. 1998 Nov 15;58(22):5248-57.
54. Xicola RM, Llor X, Pons E, Castells A, Alenda C, Piñol V, Andreu M, Castellví-Bel S, Payá A, Jover R, Bessa X. Performance of different microsatellite marker panels for detection of mismatch repair–deficient colorectal tumors. Journal of the national cancer institute. 2007 Feb 7;99(3):244-52.
55. Bayrak R, Yenidünya S, Haltas H. Cytokeratin 7 and cytokeratin 20 expression in colorectal adenocarcinomas. Pathology-Research and Practice. 2011 Mar 15;207(3):156-60.
56. Nojadeh JN, Behrouz Sharif S, Sakhinia E. Microsatellite instability in colorectal cancer. EXCLI J. 2018;17:159-168. Published 2018 Jan 22. doi:10.17179/excli2017-948
57. Ordóñez NG. Cadherin 17 is a novel diagnostic marker for adenocarcinomas of the digestive system. Advances in anatomic pathology. 2014 Mar 1;21(2):131-7.
58. Magnusson K, de Wit M, Brennan DJ, Johnson LB, McGee SF, Lundberg E, Naicker K, Klinger R, Kampf C, Asplund A, Wester K. SATB2 in combination with cytokeratin 20

identifies over 95% of all colorectal carcinomas. The American journal of surgical pathology. 2011 Jul 1;35(7):937-48.

59. Isaksson-Mettävainio M, Palmqvist R, Forssell J, Stenling R, Öberg Å. SMAD4/DPC4 expression and prognosis in human colorectal cancer. Anticancer research. 2006 Jan 1;26(1B):507-10.
60. Manhas J, Bhattacharya A, Agrawal SK, Gupta B, Das P, Deo SV, Pal S, Sen S. Characterization of cancer stem cells from different grades of human colorectal cancer. Tumor Biology. 2016 Oct;37(10):14069-81.
61. Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, Poon TC, Ng SS, Sung JJ. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut. 2009 Oct 1;58(10):1375-81.
62. Timoneda O, Balcells I, Córdoba S, Castelló A, Sánchez A. Determination of reference microRNAs for relative quantification in porcine tissues.
63. Nagel R, le Sage C, Diosdado B, van der Waal M, Oude Vrielink JA, Bolijn A, Meijer GA, Agami R. Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. Cancer research. 2008 Jul 15;68(14):5795-802.
64. Dong Y, Wu WK, Wu CW, Sung JJ, Yu J, Ng SS. MicroRNA dysregulation in colorectal cancer: a clinical perspective. British journal of cancer. 2011 Mar;104(6):893-8.
65. Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Jiang L, Tian Y, Lin TY. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. Journal of gastroenterology and hepatology. 2010 Oct;25(10):1674-80.
66. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods. 2010 Apr 1;50(4):298-301.
67. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic acids research. 2005 Jan 1;33(20):e179-.
68. Li W, Ruan K. MicroRNA detection by microarray. Analytical and bioanalytical chemistry. 2009 Jun;394(4):1117-24.
69. Hou SY, Hsiao YL, Lin MS, Yen CC, Chang CS. MicroRNA detection using lateral flow nucleic acid strips with gold nanoparticles. Talanta. 2012 Sep 15;99:375-9.

70. Mao X, Xu H, Zeng Q, Zeng L, Liu G. Molecular beacon-functionalized gold nanoparticles as probes in dry-reagent strip biosensor for DNA analysis. Chemical Communications. 2009(21):3065-7.
71. He Y, Zhang S, Zhang X, Baloda M, Gurung AS, Xu H, Zhang X, Liu G. Ultrasensitive nucleic acid biosensor based on enzyme–gold nanoparticle dual label and lateral flow strip biosensor. Biosensors and Bioelectronics. 2011 Jan 15;26(5):2018-24.
72. Jensen LF, Hvidberg L, Pedersen AF, Vedsted P. Symptom attributions in patients with colorectal cancer. BMC Family Practice. 2015 Dec;16(1):1-0.
73. Blecher E, Chaney-Graves K, DeSantis C, Edwards B, Ferlay J, Forman D, Grey N, Harford J, Kramer J, McMikel A, McNeal B. Global cancer facts and figures. American Cancer Society, Atlanta, GA, USA. 2011.
74. Samee A, Selvasekar CR. Current trends in staging rectal cancer. World journal of gastroenterology: WJG. 2011 Feb 2;17(7):828.
75. Mohammadian M, Zeynali S, Azarbaijani AF, Ansari MH, Kheradmand F. Cytotoxic effects of the newly-developed chemotherapeutic agents 17-AAG in combination with oxaliplatin and capecitabine in colorectal cancer cell lines. Research in pharmaceutical sciences. 2017 Dec;12(6):517.
76. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. International journal of molecular sciences. 2017 Jan 19;18(1):197.
77. Schatoff EM, Leach BI, Dow LE. Wnt signaling and colorectal cancer. Current colorectal cancer reports. 2017 Apr;13(2):101-10.
78. Recio-Boiles A, Cagir B. Colon cancer. InStatPearls **[**Internet**]** 2021 Jan 25. StatPearls Publishing.
79. Redondo-Blanco S, Fernández J, Gutiérrez-del-Río I, Villar CJ, Lombó F. New insights toward colorectal cancer chemotherapy using natural bioactive compounds. Frontiers in Pharmacology. 2017:109.
80. Li M, Zhang N, Li M. Capecitabine treatment of HCT-15 colon cancer cells induces apoptosis via mitochondrial pathway. Tropical Journal of Pharmaceutical Research. 2017 Aug 2;16(7):1529-36.
81. Centelles JJ. General aspects of colorectal cancer. International Scholarly Research Notices. 2012;2012.