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

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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.

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