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MicroRNAs and Inflammation in the Pathogenesis and Progression of Colon Cancer

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MicroRNA · Colorectal cancer · Inflammation · Biomarker · miR-21

Abstract
There is a strong connection between inflammation, altered microRNA (miRNA) expression and colon cancer. Longstanding inflammatory bowel diseases-related colitis leads to increased risk for the development of colorectal cancer (CRC), while sporadic CRC is in part driven by the inflammatory microenvironment. This supports a causative role for inflammation in colon carcinogenesis. miRNAs are a class of small noncoding RNAs that have recently emerged as key players in both inflammation and cancer. Some miRNAs act as inflammatory mediators, others can act as either oncogenes or tumor suppressors depending on the cellular environment in which they are expressed. In particular, miR-21 is an oncogenic miRNA that has been implicated as an inflammatory mediator and may promote inflammation-associated colon carcinogenesis. miRNAs have potential as biomarkers and therapeutic targets in CRC. They are currently being evaluated as early detection biomarkers and prognostic classifiers. Polymorphisms in miRNAs and miRNA-binding sites may alter one's risk of CRC. This review will focus on the role of inflammation and miRNAs in colon carcinogenesis and discuss the potential for miRNAs and inflammatory genes to be used as biomarkers and therapeutic targets of CRC.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer-related death worldwide. More than 1.2 million new cases and more than 600,000 deaths are estimated to occur annually [1]. Discovering risk factors, biomarkers and therapeutic targets for CRC may help reduce the burden of this cancer.

The connection between inflammation and cancer has been well established [2, 3]. Several chronic inflammatory diseases increase the risk of cancer, while certain anti-inflammatory therapies can decrease the risk of cancer. For example, chronic inflammation caused by inflammatory bowel disease (IBD), i.e. ulcerative colitis (UC) and Crohn's disease, is associated with increased risks of CRC [4, 5]. Furthermore, nonsteroidal anti-inflammatory drugs reduce CRC risk, and are considered to be chemopreventive agents [4, 5]. Infiltrating inflam-

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matory cells and inflammatory mediators in the tumor microenvironment play crucial roles in colon carcinogenesis [3]. These inflammatory mediators include cytokines, chemokines, transcription factors, reactive oxygen and nitrogen species, prostaglandins, and microRNAs (miRNAs).

MiRNAs are small (19–25 nucleotides) noncoding RNAs, which regulate the translation of specific genes through sequence-specific binding to the 3′ untranslated region of target mRNAs. Expression of miRNAs has been shown to be altered in every type of human cancer that has been examined, including CRC. Specific miRNAs have been shown to have oncogenic or tumor suppressive properties, which implicates these miRNAs as key players in carcinogenesis [6]. miRNAs also have important roles in inflammatory pathways. Inflammatory stimuli lead to altered miRNA expression and certain miRNAs act as mediators of inflammation [2]. miRNAs are more stable than mRNAs; therefore, they are easily detectable in formalin-fixed paraffin-embedded tissues as well as in plasma/serum by using reliable methods, including quantitative reverse-transcription PCR and microarray analysis. In view of that, miRNAs are highlighted as potentially useful biomarkers that may be sensitive and specific for early detection, prognostic classification and therapeutic decision of CRC.

This review discusses the interactive role of inflammation and altered miRNA expression in CRC, and provides a potential usefulness of miRNAs as biomarkers on the management of patients with CRC. We emphasize that oncogenic miR-21 may have a key role in colon carcinogenesis and is a potential prognostic classifier for CRC.

Inflammation and miRNAs, Linking to Colon Carcinogenesis

Colon Carcinogenesis in Sporadic and Colitis-Associated Cancer

CRCs develop through the sequential accumulation of genetic and epigenetic alterations, driving tumor initiation and progression from adenoma to adenocarcinoma. Loss of adenomatous polyposis coli (APC) tumor suppressor gene function (and subsequent β-catenin activation involved in Wnt signaling) occurs as an initiating event of adenoma, followed by activating mutations of KRAS oncogene for growth of the adenoma. Subsequently, additional pathways are dysregulated for adenoma to carcinoma progression, which include inactivation of p53 tumor suppressor as well as increased expression of an inducible inflammatory mediator, cyclooxygenase 2 (COX2). Genomic instability, including chromosomal instability and microsatellite instability (MSI), is recognized as an essential feature of cancer cells that accompanies the acquisition of these gene alterations. Colitis-associated cancer (CAC) is colon cancer that arises in the IBD patients. The risk of developing colon cancer increases with longer duration and larger extent of colitis in IBD patients. Unlike sporadic CRCs, CACs develop primarily through a colitis-dysplasia-carcinoma sequence without the formation of adenoma. Inactivation of p53 is an important early event in CAC development [5]. COX2 expression also occurs early in CAC, followed by KRAS activation and APC inactivation [5, 7]. Therefore, there is considerable overlap in genetic mechanisms of the pathogenesis between CRC and CAC, despite the difference in the timing and the frequency. In addition, DNA hypermethylation and genetic instability (chromosomal instability and MSI) are observed in both types [5, 7]. Inducible nitric oxide synthese (NOS2, iNOS) is also involved in the pathogenesis of CAC and CRC. NOS2 activity is positively correlated with the TP53 mutations of G:C to A:C transition at 5-methylcytosine sites in sporadic CRC and with increased TP53 mutations in inflamed colonic mucosa of UC patients [8, 9]. In addition to producing nitric oxide, NOS2 can bind to COX2 and S-nitrosylate, enhancing its activity [10]. Recent studies have highlighted the role of cellular senescence as a physiological barrier against progression from premalignant to malignant tumor, and two main pathways, p53 and Rb, are critically involved in this process (fig. 1) [11, 12]. The specific expression of p53 isoforms may signal an escape from the senescence barrier during the progression from colon adenoma to carcinoma [13]. In UC, senescence may also act as an antitumorigenic to prevent the transition from low-grade to high-grade dysplasia [14].

Inflammation has a clear role in both sporadic CRC and CAC. Both CRC and CAC tumors contain infiltrating inflammatory cells within the tumor microenvironment and these cells produce a variety of inflammatory mediators that can influence tumor progression. Both sporadic CRC and CAC tumors display increased expression of proinflammatory cytokines and exhibit constitutive activation of multiple inflammatory pathways, including nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3), which are activated by tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 [5, 15]. Transcription factors NF-κB and STAT3 are key players in both CRC and CAC,

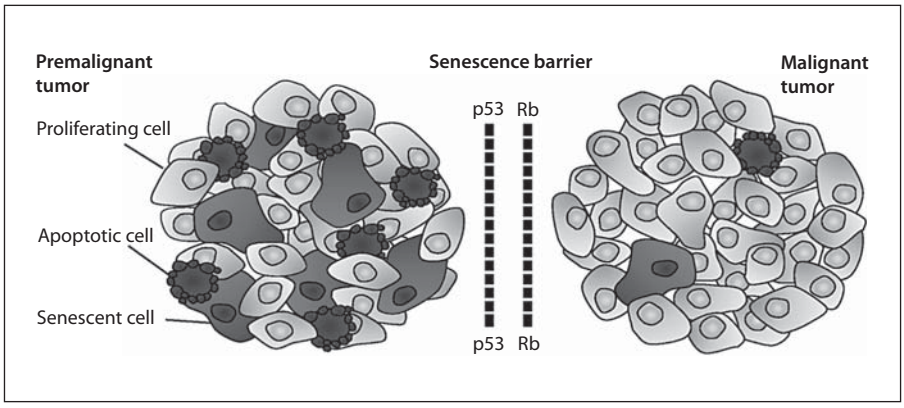

Fig. 1. p53 and Rb tumor suppressor networks are senescence barriers to progression from premalignant to malignant tumor. Premalignant tumors can be characterized by the cells undergoing apoptosis and/or senescence. Senescence barriers may be bypassed by inactivation of p53 and Rb tumor suppressor pathways.

which can induce positive signaling loops that increase cytokines, chemokines and recruitment of inflammatory cells in the tumor microenvironment. These components of cancer-associated inflammation can promote colon carcinogenesis by regulating angiogenesis, cell proliferation and apoptosis, leading to tumor progression and metastasis [3, 5, 15]. Consistent with the fact that inflammation contributes to colon carcinogenesis, we have reported that the expression signature of inflammatory genes can predict CRC prognosis [16].

miRNAs Contribute to Inflammation and Colon Carcinogenesis

miRNA expression patterns are altered in inflammatory diseases, including IBD. Wu et al. [17] reported that active inflammation in UC was associated with the differential expression of 11 miRNAs, particularly, increased miR-21 and decreased miR-192 expression. Increased miR-21 was further confirmed in inflamed UC tissues as well as intestinal inflammation in Crohn's disease [18]. Notably, elevated miR-21 expression is observed not only in the site of inflammation, including colitis, but also in many types of malignancies, including CRC, suggesting that elevated miR-21 may have a causative role in inflammation-associated carcinogenesis [2, 6, 19]. In fact, many in vitro studies indicate that miR-21 is an oncogenic miRNA (oncomiR), which can promote cell proliferation, inhibit apoptosis, and enhance invasion and metastasis by targeting putative tumor suppressive genes, including phosphatase and tensin homolog (PTEN), programmed cell death 4 (PDCD4), sprouty 2 (SPRY2) and others [20, 21]. Recently, Hatley et al. [22] revealed an in vivo miR-21 oncogenic pathway by using gain-of-function and loss-of-function mice of miR-21 in combination with a KRAS-induced lung cancer model. Medina et al. [23] demonstrated that inducible miR-21 overexpression was sufficient to induce pre-B cell lymphoma and was required to maintain malignancy, suggesting that tumors can be 'addicted' to high miR-21 expression. Consistent with its oncomiR functions, miR-21 expression can be enhanced by oncogenic signaling, including epidermal growth factor receptor (EGFR) and KRAS pathways [22, 24]. It is noteworthy that oncogenic KRAS induces not only miR-21, but also proinflammatory cytokines IL-6 and IL-8 [2]. Furthermore, various inflammatory stimuli can also induce miR-21 expression. Proinflammatory cytokines IL-6 and interferon can induce miR-21 expression in a STAT3-dependent manner [25, 26], in agreement with our report demonstrating a positive correlation between miR-21 and IL-6 expression in colon cancer tissues [16]. Iliopoulos et al. [27] suggested a positive-feedback loop involving inflammation and colon cancer, in which IL-6-induced STAT3 activates miR-21 and miR-181b-1, leading to NF-κB activation required to maintain the transformed state by inhibiting PTEN and cylindromatosis (CYLD) tumor suppressors, respectively. In view of those findings, evidence is accumulating that miR-21 plays an interactive role in both inflammation and carcinogenesis.

Several studies implicate altered expression of miRNAs as a causal factor in colon carcinogenesis. miR-143 and miR-145 are downregulated in CRC and can act as tumor suppressors in colon cancer cells in vitro. These miRNAs can affect cell growth by directly targeting genes involved in multiple oncogenic pathways, such as KRAS and MYC [20, 28, 29]. Also, miR-143 can inhibit DNA methyltransferase 3A [30], and miR-145 can repress pluripotency genes in embryonic stem cells [31]. The let-7 miRNA family is another regulator of KRAS by binding to the 3′ untranslated region of KRAS, thereby blocking subsequent RAS pathway activation and inhibiting cell

growth in colon cancer cell lines [6, 20]. Increased expression of COX2, a frequent event in CRC, can be negatively regulated by miR-101, suggesting that an impairment of miR-101 expression might contribute to colon carcinogenesis [32]. The oncogenic miR-17–92 cluster is transactivated by MYC and inhibits E2F1 to contribute to CRC development [33]. MiR-135a/b can directly suppress the expression of APC and induce Wnt signaling activity, suggesting their role in CRC pathogenesis [34]. p53 induces miR-34a, leading to apoptosis, cell cycle arrest and senescence, while its expression is repressed by promoter hypermethylation, allelic loss or TP53 mutations [6]. Overexpression of miR-155 can downregulate mismatch repair enzymes, including MSH2, MSH6 and MLH1, resulting in a mutator phenotype and MSI [35]. In addition, MSH2 expression is negatively regulated by miR-21 [36]. Collectively, there is growing evidence that miRNAs can affect crucial pathways responsible for initiation and progression of CRC.

miRNAs as Biomarkers for CRC

Circulating miRNAs and Early Detection Biomarkers

Early diagnosis can provide the increased opportunity for successful curative resection. Colonoscopy is the most reliable tool to detect CRC and has been shown to be effective at reducing deaths caused by CRC. However, the invasiveness and high cost of colonoscopies reduce screening rates. Fecal occult blood test have lower cost and are less invasiveness, but fecal occult blood test has limitations of low sensitivity which make it a less than ideal screening biomarker. Noninvasive biomarkers with high sensitivity and specificity are required to increase CRC screening rates.

Given that miRNAs are stable and detectable in serum and plasma, it raises the possibility of circulating miRNAs as noninvasive biomarkers for CRC detection [2]. Ng et al. [37] discovered that miR-17-3p and miR-92a, both members of the oncogenic miR-17–92 cluster, were elevated in plasma in patients with CRC. These miRNAs could discriminate CRC from control subjects with 89% sensitivity and 70% specificity, demonstrating some potential of circulating miRNAs as a noninvasive test to detect CRC. Plasma levels of these two miRNAs were reduced after surgical resection, suggesting that circulating miRNAs may be potential biomarkers to detect relapse following surgery. The diagnostic value of miR-92a, along with a second candidate miR-29a, in plasma was further validated by Huang et al. [38]. Circulating miR-221 in plasma was also reported as a potential CRC biomarker [39]. Recently, a circulating miRNA study which employed two independent CRC cohorts suggested that the expression of miR-141 in plasma could be a prognostic biomarker for advanced CRC patients [40]. Future studies will have to develop standardized, sensitive and specific detection methods with relatively low cost for CRC screening.

Prognostic Utility

Although surgical resection is the only curative treatment for patients with localized CRC, many of these patients will recur and die from this disease. Adjuvant chemotherapy after surgery improves survival for TNM stage III patients, while its clinical benefit for stage II patients remains controversial [41]. Therefore, developing prognostic biomarkers that can identify CRC patients at high risk for disease recurrence may identify patients that will benefit from additional chemotherapy.

We have previously published a large-scale study of miRNA expression analysis on paired tumor and nontumor tissues from two independent CRC cohorts: an American cohort using microarray to identify miRNAs that were associated with survival and a Chinese cohort using quantitative reverse-transcription PCR to validate those findings [42]. We identified five highly expressed miRNAs in tumor (miR-21, miR-20a, miR-181b, miR-203 and miR-106a) that were each associated with poor outcome in the American cohort. miR-21 expression was validated in the Chinese cohort as it was significantly associated with worse cancer-specific mortality in each cohort, independent of other clinical parameters. High miR-21 expression was also associated with worse prognosis in TNM stage II patients which demonstrates that miR-21 expression may be a promising prognostic biomarker for early-stage CRC patients. Our findings were further verified by other studies [20]. Also, miR-21 is one of the most dysregulated miRNAs and is associated with prognosis in many human cancers, thus, miR-21 might act as an oncomiR in broad malignancies, which can contribute to tumor aggressiveness and influence clinical outcome [21].

There have been a number of miRNAs identified as potential prognostic biomarkers. Decreased expression of miR-320 and miR-498 were each associated with poor survival in stage II CRC patients [43]. Other studies indicated that higher levels of miR-200c, miR-17, miR-125b, miR-185, miR-215 and lower levels of miR-106a, miR-133b were each associated with poor prognosis [20, 44–47]. However, these studies still remain to be validated.

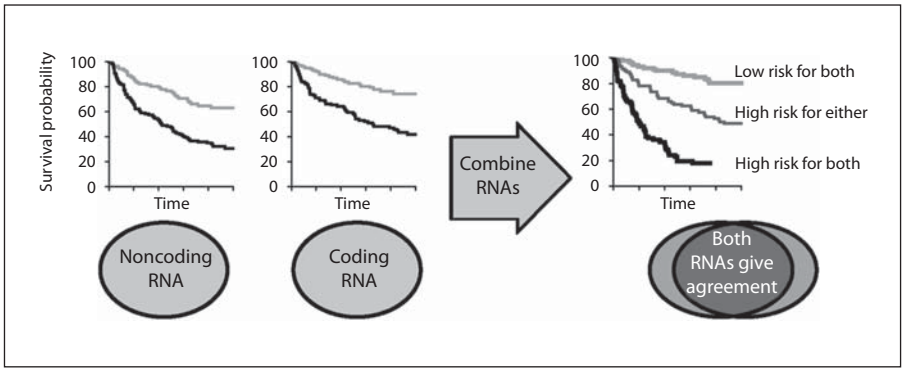

Fig. 2. The combination of multiple, validated biomarkers may improve predictions of clinical outcomes. In this example, protein noncoding RNA and protein coding RNA expression are statistically and mechanistically independent and can be combined to improve associations with prognosis. Each biomarker misclassifies a different subset of patients and combining them provides a more robust prediction.

Although the association between miRNAs and CRC outcome has been largely explored, prospective studies based on standardized methods for miRNA analysis are required to determine their usefulness in clinic.

Combining multiple validated biomarkers may provide a more accurate prognostic risk stratification of cancer patients than using single biomarkers. We have reported that the combination of an inflammatory gene signature with miR-21 expression improved predictions of cancer-specific mortality in CRC patients, including stage II patients [16]. This indicates a potential utility of protein coding and noncoding gene biomarkers, alone or in combination, to identify high-risk patients with early-stage CRC (fig. 2).

Therapeutic Response

Each patient may have a different susceptibility to chemotherapeutic drugs, depending on their genetic background and acquired drug resistance during tumor progression. Chemotherapy in general is costly and has significant side effects, thus highlighting the need for predictive biomarkers that can individualize treatment for each patient. For instance, KRAS mutation testing has become routine in the clinic to determine eligibility for EGFR targeting therapy, as KRAS-mutant tumors do not respond to the anti-EGFR treatment [48].

We previously reported that high miR-21 expression was associated with poor therapeutic outcome in CRC patients who received 5-fluorouracil (5-FU)-based adjuvant chemotherapy [42]. CRC with MSI phenotype, caused by the impairment of mismatch repair genes, including MSH2, are resistant to 5-FU [49]. Thus, it may be possible that miR-21 can repress MSH2, leading to chemoresistance to 5-FU [36]. The expression of let-7g and miR-181b are associated with chemoresponse to S-1, which contains a 5-FU prodrug [50].

Single-nucleotide polymorphisms in miRNAs or miRNA-binding sites may also influence prognosis and response to therapy [2, 20]. A KRAS 3′ untranslated region polymorphism in a let-7 miRNA complementary site was found to be positively associated with anti-EGFR agent cetuximab responsiveness in metastatic CRC patients with KRAS-wild-type tumor [51]. Single-nucleotide polymorphisms in pre-miR-423 and pre-miR-608 were associated with prognosis, especially in patients receiving 5-FU and platinum-based chemotherapy [52]. Single-nucleotide polymorphisms in pri-miR-26a-1 and pri-miR-100 are associated with treatment outcome in metastatic CRC patients treated with 5-FU and irinotecan [53]. These studies should be validated in independent populations and the underlying mechanisms responsible for the response to specific chemotherapies need to be examined before these discoveries can be translated into the clinic.

Conclusions and Future Perspectives

Although there is little doubt that miRNAs can link inflammation and colon cancer, further mechanistic studies are required to elucidate the mechanisms by which miRNAs contribute to colon carcinogenesis along with modifying the inflammatory tumor microenvironment. Also, more in vivo evidence for a causative role of miRNAs in CRC is needed.

Although recent studies have highlighted the potential usefulness of miRNAs in CRC management, further validation in prospective independent clinical panels would be the next desirable step. Future efforts will also be required to develop clinical tools based on the expression and/or genetic polymorphisms of miRNAs that can serve as sensitive, specific and noninvasive biomarkers for CRC

detection as well as personalized therapeutic strategies. Among a large number of candidate miRNAs, miR-21 in particular not only represents one of the most important oncomiRs involved in CRC development and progression, but also has the potential utility as a CRC biomarker that predicts prognosis and therapeutic outcome. miR-21 is also a potential target supported by preclinical studies.

Disclosure Statement

The authors have no conflict of interest to declare.

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