

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2020 November 14; 26(42): 6514-6705



**EDITORIAL**

- 6514 Nonalcoholic fatty liver disease in lean subjects: Prognosis, outcomes and management  
*Chrysavgis L, Ztriva E, Protopapas A, Tziomalos K, Cholongitas E*

**REVIEW**

- 6529 Simultaneous colorectal and parenchymal-sparing liver resection for advanced colorectal carcinoma with synchronous liver metastases: Between conventional and mini-invasive approaches  
*De Raffele E, Mirarchi M, Cuicchi D, Lecce F, Casadei R, Ricci C, Selva S, Minni F*
- 6556 What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis?  
*Peruhova M, Peshevska-Sekulovska M, Krastev B, Panayotova G, Georgieva V, Konakchieva R, Nikolaev G, Velikova TV*

**MINIREVIEWS**

- 6572 Modern surgical strategies for perianal Crohn's disease  
*Zabot GP, Cassol O, Saad-Hossne R, Bemelman W*
- 6582 Vascular anomalies associated with hepatic shunting  
*Schmalz MJ, Radhakrishnan K*

**ORIGINAL ARTICLE****Basic Study**

- 6599 Reactive oxygen species-induced activation of Yes-associated protein-1 through the c-Myc pathway is a therapeutic target in hepatocellular carcinoma  
*Cho Y, Park MJ, Kim K, Kim SW, Kim W, Oh S, Lee JH*
- 6614 Fedora-type magnetic compression anastomosis device for intestinal anastomosis  
*Chen H, Ma T, Wang Y, Zhu HY, Feng Z, Wu RQ, Lv Y, Dong DH*

**Retrospective Cohort Study**

- 6626 Attention deficit hyperactivity disorder and gastrointestinal morbidity in a large cohort of young adults  
*Kedem S, Yust-Katz S, Carter D, Levi Z, Kedem R, Dickstein A, Daher S, Katz LH*
- 6638 Nomograms and risk score models for predicting survival in rectal cancer patients with neoadjuvant therapy  
*Wei FZ, Mei SW, Chen JN, Wang ZJ, Shen HY, Li J, Zhao FQ, Liu Z, Liu Q*

**Observational Study**

- 6658** Estimation of visceral fat is useful for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease

*Hernández-Conde M, Llop E, Fernández Carrillo C, Tormo B, Abad J, Rodríguez L, Perelló C, López Gomez M, Martínez-Porras JL, Fernández Puga N, Trapero-Marugan M, Fraga E, Ferre Aracil C, Calleja Panero JL*

**Prospective Study**

- 6669** Accuracy of carbon dioxide insufflation for endoscopic retrograde cholangiopancreatography using double-balloon endoscopy

*Niwa Y, Nakamura M, Kawashima H, Yamamura T, Maeda K, Sawada T, Mizutani Y, Ishikawa E, Ishikawa T, Kakushima N, Furukawa K, Ohno E, Honda T, Ishigami M, Fujishiro M*

**SYSTEMATIC REVIEWS**

- 6679** Prognostic role of artificial intelligence among patients with hepatocellular cancer: A systematic review

*Lai Q, Spoletini G, Mennini G, Larghi Laureiro Z, Tsilimigras DI, Pawlik TM, Rossi M*

**CASE REPORT**

- 6689** Case series of three patients with hereditary diffuse gastric cancer in a single family: Three case reports and review of literature

*Hirakawa M, Takada K, Sato M, Fujita C, Hayasaka N, Nobuoka T, Sugita S, Ishikawa A, Mizukami M, Ohnuma H, Murase K, Miyanishi K, Kobune M, Takemasa I, Hasegawa T, Sakurai A, Kato J*

- 6698** Intussusception due to hematogenous metastasis of hepatocellular carcinoma to the small intestine: A case report

*Mashiko T, Masuoka Y, Nakano A, Tsuruya K, Hirose S, Hirabayashi K, Kagawa T, Nakagohri T*

**ABOUT COVER**

Editorial Board Member of *World Journal of Gastroenterology*, Dr. Misha Luyer is a Senior Consultant for upper gastrointestinal and pancreas surgery and Chair of the Department of Surgery. After finishing his surgical training in 2010, he started as a Fellow at the Catharina Hospital in Eindhoven (Netherlands), where he received specialized training in upper gastrointestinal, bariatric and pancreatic surgery. In 2012, he was appointed consultant at the Catharina Hospital. Since then, he has been involved in national training courses for minimally invasive upper gastrointestinal surgery and pancreatic surgery. He has authored more than 150 articles published in peer-reviewed scientific journals and book chapters in the fields of upper gastroenterology and pancreas. He currently serves on the Editorial Board of several peer-reviewed scientific journals. (L-Editor: Filipodia)

**AIMS AND SCOPE**

The primary aim of *World Journal of Gastroenterology* (*WJG, World J Gastroenterol*) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJG* mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

**INDEXING/ABSTRACTING**

The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2020 edition of Journal Citation Report® cites the 2019 impact factor (IF) for *WJG* as 3.665; IF without journal self cites: 3.534; 5-year IF: 4.048; Ranking: 35 among 88 journals in gastroenterology and hepatology; and Quartile category: Q2.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Production Editor: Ji-Hong Liu; Production Department Director: Yun-Xiaoqian Wu; Editorial Office Director: Ze-Mao Gong.

**NAME OF JOURNAL**

*World Journal of Gastroenterology*

**ISSN**

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

**LAUNCH DATE**

October 1, 1995

**FREQUENCY**

Weekly

**EDITORS-IN-CHIEF**

Andrzej S Tarnawski, Subrata Ghosh

**EDITORIAL BOARD MEMBERS**

<http://www.wjgnet.com/1007-9327/editorialboard.htm>

**PUBLICATION DATE**

November 14, 2020

**COPYRIGHT**

© 2020 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjgnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjgnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjgnet.com/bpg/gerinfo/240>

**PUBLICATION ETHICS**

<https://www.wjgnet.com/bpg/GerInfo/288>

**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis?

Milena Peruhova, Monika Peshevska-Sekulovska, Boris Krastev, Gabriela Panayotova, Viktoriya Georgieva, Rossitza Konakchieva, Georgi Nikolaev, Tsvetelina Veselinova Velikova

**ORCID number:** Milena Peruhova 0000-0002-6618-2324; Monika Peshevska-Sekulovska 0000-0002-8468-0132; Boris Krastev 0000-0003-4196-0828; Gabriela Panayotova 0000-0002-2029-104X; Viktoriya Georgieva 0000-0003-1245-8900; Rossitza Konakchieva 0000-0001-7981-4506; Georgi Nikolaev 0000-0003-0584-415X; Tsvetelina Veselinova Velikova 0000-0002-0593-1272.

**Author contributions:** Peruhova M wrote the draft; all the authors wrote additional sections in the paper; and all authors revised and approved the final version of the manuscript.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Milena Peruhova, Monika Peshevska-Sekulovska, Gabriela Panayotova, Viktoriya Georgieva,** Department of Gastroenterology, University Hospital Lozenetz, Sofia 1407, Bulgaria

**Milena Peruhova, Gabriela Panayotova, Tsvetelina Veselinova Velikova,** Medical Faculty, Sofia University St. Kliment Ohridski, Sofia 1407, Bulgaria

**Boris Krastev,** Department of Clinical Oncology, MHAT Hospital for Women Health Nadezhda, Sofia 1330, Bulgaria

**Rossitza Konakchieva, Georgi Nikolaev,** Faculty of Biology, Sofia University St. Kliment Ohridski, Sofia 1407, Bulgaria

**Tsvetelina Veselinova Velikova,** Department of Clinical Immunology, University Hospital Lozenetz, Sofia 1407, Bulgaria

**Corresponding author:** Tsvetelina Veselinova Velikova, MD, PhD, Assistant Professor, Research Scientist, Department of Clinical Immunology, University Hospital Lozenetz, Kozyak 1 Street, Sofia 1407, Bulgaria. [tsvelikova@medfac.mu-sofia.bg](mailto:tsvelikova@medfac.mu-sofia.bg)

### Abstract

In the last two decades, the vision of a unique carcinogenesis model for colorectal carcinoma (CRC) has completely changed. In addition to the adenoma to carcinoma transition, colorectal carcinogenesis can also occur *via* the serrated pathway. Small non-coding RNA, known as microRNAs (miRNAs), were also shown to be involved in progression towards malignancy. Furthermore, increased expression of certain miRNAs in premalignant sessile serrated lesions (SSLs) was found, emphasizing their role in the serrated pathway progression towards colon cancer. Since miRNAs function as post-transcriptional gene regulators, they have enormous potential to be used as useful biomarkers for CRC and screening in patients with SSLs particularly. In this review, we have summarized the most relevant information about the specific role of miRNAs and their relevant signaling pathways among different serrated lesions and polyps as well as in serrated adenocarcinoma. Additional focus is put on the correlation between gut immunity and miRNA expression in the serrated pathway, which remains unstudied.

**Key Words:** MicroRNA; Serrated pathway; Carcinogenesis; Colorectal carcinoma; Sessile

[p://creativecommons.org/licenses/by-nc/4.0/](https://creativecommons.org/licenses/by-nc/4.0/)

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Bulgaria

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0  
Grade B (Very good): B, B  
Grade C (Good): 0  
Grade D (Fair): 0  
Grade E (Poor): 0

**Received:** August 29, 2020

**Peer-review started:** August 29, 2020

**First decision:** September 12, 2020

**Revised:** September 24, 2020

**Accepted:** October 20, 2020

**Article in press:** October 20, 2020

**Published online:** November 14, 2020

**P-Reviewer:** De Palma FDE

**S-Editor:** Huang P

**L-Editor:** A

**P-Editor:** Liu JH



serrated lesions; Adenocarcinoma

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** In addition to the adenoma to carcinoma transition, colorectal carcinogenesis can also occur *via* the serrated pathway. In most serrated polyps, the pathway is believed to include the acquisition of a mutation in a gene that regulates mitogen-activated protein kinase (MAPK) pathway, disruptions to the Wnt signaling pathway and widespread methylation of CpG islands. Moreover, there are less data about different microRNAs (miRNAs) expression profiling in serrated adenomas with different grades of dysplasia. In contrast to the conventional colorectal carcinogenesis, the pivotal role of miRNAs and their relevant signaling pathways in the serrated pathway of carcinogenesis is still to be elucidated because of an insufficient number of studies conducted to clarify separate steps in the process.

**Citation:** Peruhova M, Peshevska-Sekulovska M, Krastev B, Panayotova G, Georgieva V, Konakchieva R, Nikolaev G, Velikova TV. What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis? *World J Gastroenterol* 2020; 26(42): 6556-6571

**URL:** <https://www.wjgnet.com/1007-9327/full/v26/i42/6556.htm>

**DOI:** <https://dx.doi.org/10.3748/wjg.v26.i42.6556>

## INTRODUCTION

Colorectal cancer (CRC) is the most prevalent cancer in Western countries and the second cause of cancer-related death<sup>[1]</sup>. Obesity, sedentary lifestyle, tobacco and alcohol consumption are considered the driving factor behind the growth of CRC<sup>[2]</sup>. In the last two decades the vision of a unique carcinogenesis model for CRC has completely changed. The most prevalent genetic events accompanying CRC development are mutations that de-regulate the Wnt signaling cascade. In particular, inactivating mutations in the tumor suppressor adenomatous polyposis coli (APC) are considered the earliest genetic lesions sufficient to initiate tumorigenesis<sup>[3]</sup>.

In addition to the adenoma to carcinoma sequence, colorectal carcinogenesis can also occur *via* the serrated pathway. After the identification of serrated carcinomas by Jass *et al*<sup>[4]</sup> in 1992, the underlying genetic and epigenetic alterations have been described. In most serrated polyps, the pathway is believed to be the acquisition of a mutation in a gene that regulates mitogen-activated protein kinase (MAPK) pathway, disruptions to the Wnt signaling pathway and widespread methylation of CpG islands<sup>[5,6]</sup>.

A class of small non-coding RNAs, designated as microRNAs (miRNAs), are involved in progression towards malignancy. miRNAs act as tumor suppressors or oncogenes depending on the characteristics of their downstream targets<sup>[7]</sup>. They function as post-transcriptional gene regulators and have been increasingly recognized as useful biomarkers for CRC<sup>[8]</sup>.

A plethora of studies have documented aberrant miRNA levels in CRC, but only a few of them relate to serrated pathway carcinogenesis<sup>[9]</sup>. There is even less data about different miRNA expression profiling in serrated adenomas with different grades of dysplasia<sup>[10]</sup>. In contrast to the conventional colorectal carcinogenesis, the pivotal role of miRNAs in the serrated pathway is still to be elucidated because of the insufficient number of studies conducted to clarify separate steps in serrated carcinogenesis<sup>[11]</sup>.

Many of the published reviews in the English literature about the serrated pathway have been focused on histological, endoscopic, and molecular features<sup>[12,13]</sup>. However, there are a few data about post-transcriptional gene regulation, in particular, the expression of miRNAs in the serrated pathway in CRC. We aimed to interrogate the role of miRNAs in relevant signaling pathways in serrated carcinogenesis.

Emerging new approaches revealed increased expression of certain miRNAs in premalignant sessile serrated lesions (SSLs), emphasizing their role in the serrated pathway progression towards colon cancer<sup>[14]</sup>. This could make miRNAs potential biomarkers for screening in patients with SSLs<sup>[15,16]</sup>.

In this review, we summarized the most relevant information about the specific role of miRNAs among different serrated lesions and polyps as well as in serrated adenocarcinoma (SAC). Additionally, the review is the first that looks at the correlation between gut immunity and miRNA expression in the serrated pathway.

## MORPHOLOGICAL ASPECTS OF SERRATED POLYPS AND SAC

Based on the literature, the percentage prevalence of serrated pathway is highly variable, ranging from 15% up to 30% of all CRCs<sup>[17-20]</sup>.

According to the 5th edition of WHO classification of colorectal serrated lesions and polyps, they are classified into three histopathological subtypes: Hyperplastic polyps (HPs), SSLs, and traditional serrated adenomas (TSAs)<sup>[21]</sup> (Figure 1). TSAs are extremely rare < 1% of all colorectal polyps, while HPs are the most common, comprising approximately 75% of all serrated polyps. SSLs (previously known as sessile serrated adenomas or sessile serrated polyps) cause nearly 25% of serrated polyps<sup>[22]</sup>.

HPs are usually small, rarely cause symptoms, and have minimal malignant potential. However, it was established that HPs could progress to SSLs or TSAs for a period of 7.5 years<sup>[23]</sup>. In this context, HPs may predispose to cancer because of their ability to transform into serrated lesions<sup>[24]</sup>. These lesions could be found anywhere in the colon, but they are mostly placed in the distal colon (70%-80%)<sup>[25]</sup>. It was established that HPs, with right-side localization, are more likely to have malignant potential<sup>[26-28]</sup>.

Clinical characteristics, such as size, location, and endoscopic appearance, can support the identification of SSLs but are not sufficient for their identification. Approximately 10% of SSLs could lead to sporadic CRCs *via* the serrated polyp-carcinoma sequence<sup>[29]</sup>.

In most series, TSAs account for < 1% of all colorectal polyps, represent about 1%-2% of the serrated lesions and are located predominantly in the left colon<sup>[30-32]</sup>.

SAC is characterized by mainly right-sided location of the colon, specific molecular features and female predominance. Percentage prevalence of SAC is about 7.5%-8.7% of all CRCs and according to the literature it has worse prognosis than conventional CRC<sup>[6,33]</sup>.

## EPIGENETIC AND GENETIC ASPECTS IN SERRATED PATHWAY

### **CpG methylator phenotype**

Toyota *et al*<sup>[34]</sup> introduced the CpG island methylator phenotype (CIMP) in 1999. Methylation is an epigenetic process where a methyl group (CH<sub>3</sub>) is added to the cytosine nucleotide at a CpG dinucleotide group. The process of methylation of gene promoters is a physiological mechanism by which gene expression is regulated without altering the DNA sequence<sup>[35,36]</sup>.

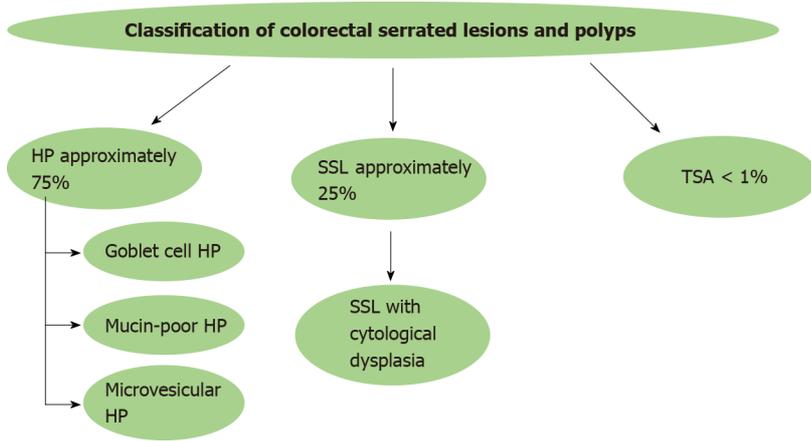
Transcriptional silencing of essential tumor suppressor genes, caused by aberrant DNA methylation, could promote neoplastic growth. This aberrant methylator has been called the CIMP and is thought to be important in the serrated pathway in CRC<sup>[37]</sup>.

Using eight markers, Ogino *et al*<sup>[38]</sup> classified CIMP in CRC into three subgroups, CIMP-low (CIMP-L), CIMP-high (CIMP-H), and CIMP-negative, according to the numbers of methylated promoters.

With the growing impact of translational research and molecular pathology, the CRC pathogenesis became more elucidated based on the association of CIMP and key mutations in *KRAS*, *BRAF*, *PIK3CA*, *TP53*, and *APC*. Furthermore, microsatellite instability (MSI), caused by dysfunction of DNA mismatch repair (MMR) genes, is considered another critical pathway in carcinogenesis<sup>[39]</sup>.

### **MSI mechanism in CRC**

The MSI mechanism in CRC was first described in relation to Lynch syndrome, where germline mutations take place in specific MMR genes such as *MLH1*, *MSH2*, *MSH6*, and *PMS2*<sup>[40]</sup>. Germline deletions at 3' end of the *EPCAM* gene which lead to decreased *MSH2* expression were also demonstrated as a recurrent cause of Lynch syndrome<sup>[41]</sup>. Furthermore, functional relevance of *MSH3* mutations for the development and inheritance of CRC were reported, but their role in the serrated pathway needs further



**Figure 1** Schematic presentation of classification of colorectal serrated lesions and polyps. HP: Hyperplastic polyp; SSL: Sessile serrated lesion; TSA: Traditional serrated adenoma.

analysis and more cohort studies<sup>[42,43]</sup>. Evidence has shown that mutations in MSI are vital points in the developing malignancy in 3%-15% of all CRC<sup>[42,43]</sup>. About 80% of MSI CRCs are characterized by the hypermethylation of *MLH1*, while 20% of MSI CRCs by mutations in MMR genes<sup>[44]</sup>. MSI status could be subclassified into MSI-high (MSI-H), MSI-low (MSI-L) and microsatellite stable (MSS) according to the number of mutations in microsatellite sequences<sup>[45]</sup>.

Alteration of MMR genes due to epigenetic silencing by sporadic, acquired hypermethylation of the *MLH1* gene promoter leads to the serrated pathway in CRC<sup>[44]</sup>.

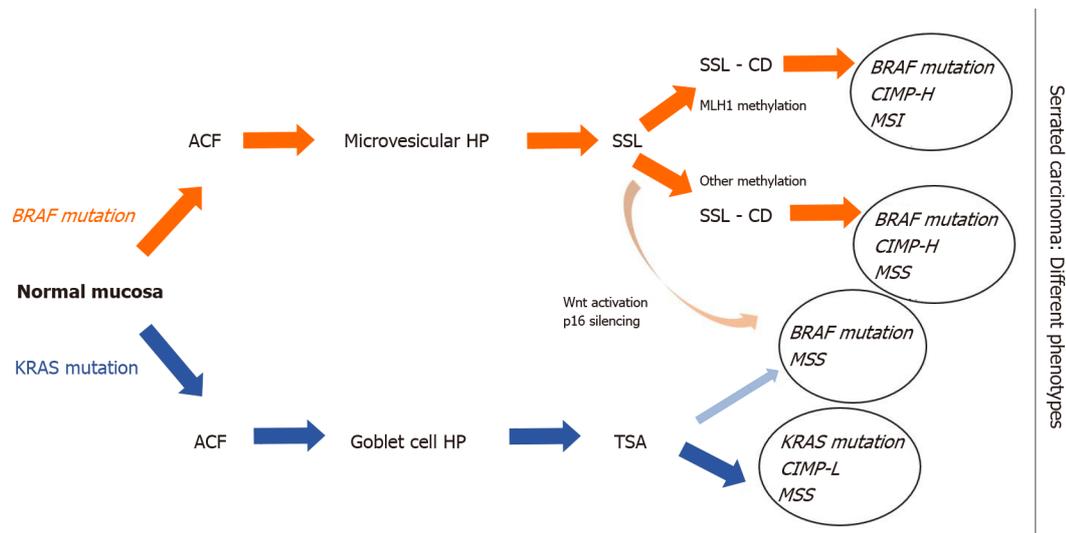
Serrated colorectal malignancies are characterized by CIMP-H, *MLH1* promoter hypermethylation, and MSI and *BRAF* mutations<sup>[46]</sup>.

### ***BRAF / KRAS gene mutations in serrated CRC***

Serrated colorectal lesions rarely bear truncating *APC* mutations, but the most frequent genetic alterations involve *BRAF* mutations, whereas *KRAS* mutations are less common<sup>[47]</sup>. Both *KRAS* and *BRAF* belong to the MAPK signaling pathway, mediating cell proliferation, apoptosis and differentiation<sup>[48]</sup>.

*BRAF* gene encodes a protein called B-Raf, which plays a pivotal role in regulating the MAPK/ERKs signaling pathway<sup>[49]</sup>. Recent findings in molecular biology demonstrated that mutations in *BRAF* are found in about 10% of CRC patients<sup>[50]</sup>. *BRAF*-mutated CRCs are associated with the female gender, often right-sided, mucinous histology, and advanced stage<sup>[51]</sup>. *BRAF* mutations are considered as early events in CIMP cancers by inhibition of normal apoptosis in colonic mucosa<sup>[52]</sup>. Many recent studies classified two different molecular phenotypes of CRC based on *BRAF* mutation status: *BRAF* V600E- and non-V600-mutated CRC<sup>[53]</sup>. A correlation between serrated carcinogenesis and *BRAF* V600E mutation was established, which induce CIMP-H status and methylation of *MLH1* promoter<sup>[54]</sup>. In contrast to the conventional adenomas, the earliest event in serrated precursor lesions are *BRAF* mutations and hypermethylation, which leads to transformation of aberrant crypt foci (ACF) to microvesicular HP and then to SSLs. Methylation and loss of key tumor suppressor genes such as *p16* and *MLH1* are the key points in SSLs' progression to SAC<sup>[55]</sup>. Interesting information about the *BRAF* mutated/MSS SACs was reported by Bond *et al*<sup>[55]</sup>. They found out that hypermethylation events occurred in *BRAF* mutated SACs more often than in conventional pathway (respectively 60% and 3%)<sup>[55]</sup>. *BRAF* V600E-mutated CRCs are with dismal prognosis and resistance to standard systemic chemotherapy<sup>[56,57]</sup>.

Another significant driver in the serrated pathway is *KRAS* mutations<sup>[58]</sup>. Opposite to the traditional model of Vogelstein, where aberrant activation of Wnt pathway has been observed, high frequency of *KRAS* mutations was established in TSAs. In contrast to SSLs, TSA lesions showed *MGMT* hypermethylation, but not *MLH1* promoter hypermethylation. Based on this evidence, a non-*MLH1* mutating SSL could progress to a TSA and ultimately develop into a *BRAF*-mutated MSS tumor (Figure 2)<sup>[59,60]</sup>.



**Figure 2 Outline of the schematic serrated pathway progression.** In red color we indicate the steps of transformation of *BRAF*-mutated serrated lesions. *BRAF* mutations and hypermethylation lead to transformation of aberrant crypt foci to microvesicular hyperplastic polyp then to sessile serrated lesions (SSLs). Methylation and loss of key tumor suppressor genes such as *p16* and *MLH1* are the key points in SSLs' progression to serrated adenocarcinoma. In blue color we indicate *KRAS* mutations in traditional serrated adenomas (TSAs), which showed *MGMT* hypermethylation, but not *MLH1* promoter hypermethylation. In light red shading we indicate a non-*MLH1* mutating SSL, which could progress to a TSA and ultimately develop into a *BRAF*-mutated microsatellite stability tumor. ACF: Aberrant crypt foci; HP: Hyperplastic polyp; SSL: Sessile serrated lesion; SSL-CD: Sessile serrated lesion with cytological dysplasia; TSA: Traditional serrated adenoma; CIMP: CpG island hypermethylator phenotype; CIMP-H: CIMP-high; CIMP-L: CIMP-low; MSI: Microsatellite instability; MSS: Microsatellite stability.

## MIRNA PROFILE FROM PREMALIGNANT SERRATED LESIONS TO CRC

miRNAs were discovered in *Caenorhabditis elegans* by Lee *et al*<sup>[61]</sup> in 1993 while studying the gene *lin-14*. However, the scientific community became aware of the importance of miRNAs seven years later when they were recognized as a specific class of biological regulators. miRNAs are small, single-stranded, non-coding RNAs (18-24 nucleotides) that can post-transcriptionally regulate the expression of various oncogenes and tumor suppressor genes<sup>[62]</sup>. Also, they play an essential role in cancer development, proliferation, regression, and metastasis. Even though their role in cancer progression is yet to be elucidated, several studies reported the influence of specific miRNA alterations in premalignant and malignant lesions<sup>[63-66]</sup>. miRNA expression profiling gives us the opportunity to understand and identify differences between benign and malignant lesions of the colon mucosa, as well as to stratify benign lesions according to their malignant potential<sup>[67]</sup>.

### The role of miRNA-125b, miRNA-222, miRNA-214, miRNA-335 in CRC carcinogenesis

In this scenario, several studies showed a unique miRNA signature in different types of colonic polyps, as well as in the progression of serrated lesions.

Tsikitis *et al*<sup>[68]</sup> profiled miRNA patterns in screen-detected polyps in relation to histologic features and cancer-related risk. miRNA expression analysis was carried out on biopsy specimens from 109 patients. The specimens were obtained from normal mucosa (NM), HPs, tubular adenomas (TAs), tubulovillous adenomas, or high-grade dysplasia (TVHGs), SSLs, and TSAs. They have not found a significant difference in the expression of miRNA between TSAs and SSLs. miRNAs expression pattern was similar in TSAs and HGTVs, whilst there were several differentially expressed miRNAs between HPNMs and TSAs. Additionally, they performed pairwise comparisons of non-serrated tissues and serrated lesions. miRNAs-222 and miRNA-214 were significantly downregulated by 2.35- and 1.51-fold respectively in serrated polyps, whereas miRNA-335 was significantly overexpressed by two-fold in non-serrated tissues. Tsikitis *et al*<sup>[68]</sup> drew the conclusion that the downregulation of miRNA-125b and miRNA-320a in the serrated pathway may be used as independent predictors of progression with a concordance index of 84.7%.

Opposite to the serrated pathway, in the conventional adenoma-carcinoma sequence, many studies showed a high expression of miRNA-125b in advanced tumor size. Another correlation was found between the overexpression of miRNA-125b, which leads to repression of the endogenous level of p53 protein in human CRC cells. Cancer progression and poor outcomes were associated with overexpression of miRNA-125b in the conventional colorectal pathway<sup>[69]</sup>.

### **The role of miRNA-31 in carcinogenesis of serrated pathway of the colorectum**

However, many studies showed that miRNA-31 plays a pivotal role in serrated carcinogenesis. In this scenario, miRNA-31 is located at 9p21.3 and is frequently overexpressed in sessile serrated adenomas. Aoki *et al*<sup>[70]</sup> analyzed in their case report miRNA-31 expression using quantitative reverse transcription-PCR in patients with early invasive CRC with HP component. Their results showed higher miRNA-31 expression in the carcinoma component compared to HP component. They revealed that progression of HP (or SSLs) to SAC is likely to be associated with overexpression of miRNA-31.

To shed light on the role of miRNA31 on the serrated pathway, Kanth *et al*<sup>[11]</sup> conducted a study of 108 colon biopsies with distinct histology types. Different expression was established in 23 miRNAs between NM and serrated lesions. Additionally, six miRNAs showed a different expression pattern between SSLs and HPs, as miRNA-31-5p has been the most significantly modulated.

Nosho *et al*<sup>[71]</sup> based on miRNA array analysis, identified that miRNA-31 was the most upregulated in *BRAF* (V600E) mutation, compared to *BRAF*-wild type CRCs. Moreover, they performed transfection of the miRNA-31 inhibitor and consequently showed that miRNA-31 might regulate *BRAF* activation in CRCs. Therefore, miRNA-31 could be used as a diagnostic biomarker as well as a feasible therapeutic target in the future. Finally, they proved that high miRNA-31 expression was associated with shorter prognosis in patients with CRC.

Higher miRNA-31 expression was associated with cell proliferation and survival in development in CRC, as well as tumor invasion and poor prognosis<sup>[72-73]</sup>. Kubota *et al*<sup>[76]</sup> pointed out that miRNA-31 could be a potential prognostic biomarker in their study of patients with stage IV of CRC. They also found out a correlation between miRNA-31 overexpression and poor tumor differentiation, as well as advanced disease stages.

Recent studies showed the presence of miRNA-31 in the serum of patients with metastatic CRC, who were treated with anti-EGFR therapy. Igarashi *et al*<sup>[77]</sup> found out a correlation between high miRNA-31-5p expression and shorter PFS in CRC patients treated with anti-EGFR therapeutics. Their theory suggested that miRNA-31-5p could be a useful prognostic biomarker for anti-EGFR therapy.

Even though the underlying mechanisms of the role of miRNA-31-5p in CRC remain unknown. It has been postulated that miRNA-31 can directly bind to the 3' untranslated region (3' UTR) of *SATB2*, which takes part in regulation of transcription and chromatin remodeling. Overexpression of miRNA-31-5p could induce epithelial-mesenchymal transition, tumorigenesis, and progression in CRC<sup>[78]</sup>.

Furthermore, another correlation between the expression of miRNA-31 and CRC-associated fibroblast (CAFs) was established, but not *in vivo* experimental models. Yang *et al*<sup>[79]</sup> elucidated that miRNA-31 inhibits autophagy in CAFs and alters colorectal proliferation and invasion of CRC cells. Thus, more studies must be conducted in this direction because of the lack of *in vivo* experimental models.

### **Relevance of miRNA-135-B in CRC**

In many studies, it has been reported that overexpression of miRNA-135-B has been associated with *APC* dysfunction in CRC, leading to the promotion of tumor-proliferation, progression, and invasion<sup>[63,80]</sup>. It was established that miRNA-135-B had been associated with the serrated pathway and colorectal carcinogenesis.

Only few studies indicate that specific miRNA profiles can be used to distinguish neoplastic from benign lesions in colon mucosa<sup>[6]</sup>. A study by Kanth *et al*<sup>[11]</sup> was the first that showed the overexpression of specific miRNAs in serrated polyps or serrated carcinoma. In summary, they provided a comprehensive analysis of miRNA gene expression in SSLs, by identifying miRNA-135B, miRNA-378A, miRNA-548, miRNA-9, and miRNA-196B. miRNA-378A-3p was significantly downregulated in SSLs compared to normal colon mucosa. They suggested that these miRNAs are good predictors in SSLs to carcinoma transformation. Additionally, they discovered that miRNA-9 and miRNA-196b were also de-regulated in SSL compared to HP. These miRNAs showed different expression patterns in *BRAF* mutated-MSI tumors. Interestingly, reduced expression of miRNA-196B has been detected in the plasma of patients with CIMP-positive SSLs or MSI colon cancers<sup>[11]</sup>.

### **The involvement of miRNA-21 in CRC**

MiRNA-21 is one of the most eminent miRNAs involved in the genesis and progression of CRC. Evidence implied that miRNA-21 negatively regulates tumor suppressor phosphatase and tensin homolog (*PTEN*) gene, which played an essential role in cell proliferation and invasion in CRC<sup>[81-84]</sup>. An interesting study by Ghareib

*et al*<sup>[85]</sup> established that miRNA-21 in serum could be feasible, non-invasive biomarker with high sensitivity and specificity (95.8% and 91.7%) for early detection and prognosis in patients with CRC.

In addition, Chen *et al*<sup>[86]</sup> report a correlation between tissue and serum miRNA-21 overexpression and poor prognosis in patients with CRC. It is more significant in colon cancers, compared to rectal.

Another interesting study by Yau *et al*<sup>[87]</sup> presents the potential role of fecal-based miRNA-21 and miRNA-92a as non-invasive biomarkers for CRC screening. They reported higher expression of miRNA-21 and miRNA-92a in patients with advanced distal CRC compared to the proximal localization, without significant value in the detection of early CRC.

miRNA-21 down-regulates tumor suppressor PDCD4, thus stimulating cancer cell invasion and intravasation. Moreover, the high level of miRNA-21 was associated with metastasis and resistance to chemotherapy of 5-FU in CRC. Thus, it makes miRNA-21 a potential non-invasive biomarker for diagnostic and prognosis for CRC<sup>[88]</sup>.

Recently, several studies have reported the correlation between expression of miRNA-21 and serrated pathway in CRC. A study by Schmitz *et al*<sup>[89]</sup> demonstrated different expression of miRNA-21 among NM, HPs, and SSLs. They found overexpression of miRNA-21 in SSLs, whereas normal colon mucosa and HPs exhibited no differences. Opposite to them, Kanth *et al*<sup>[11]</sup> proved that there was no statistically significant expression of miRNA-21 in SSLs.

Future investigations are necessary to find out the correlation between expression levels of miRNA-21 and genetic and epigenetic alterations of SSLs.

### **The role of miRNA-181a-2 in the development of serrated pathway in CRC**

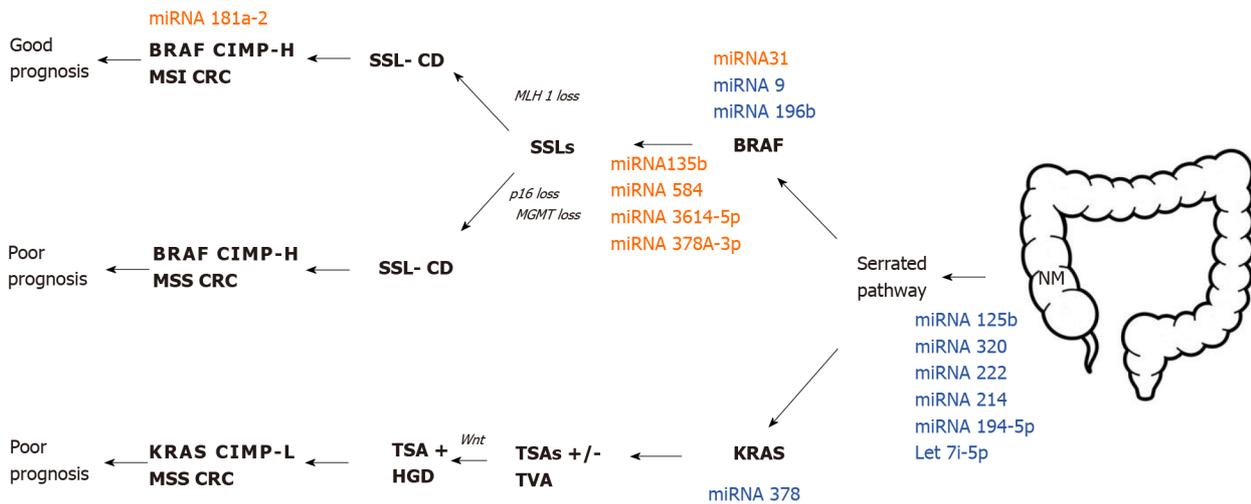
miRNA-181 plays a pivotal role in regulation at the post-transcriptional level in many different types of cancer. More specifically, the expression of miRNA-181a and miRNA-181b are strongly associated with the mutation status of the tumor suppressor gene *p53* in colorectal carcinogenesis<sup>[90]</sup>. The underlying mechanism of how miRNA-181a influences conventional colorectal carcinogenesis could be based on up-regulation miRNA-181a through the activation of the Wnt/ $\beta$ -catenin pathway<sup>[91]</sup>.

Little is known about the expression of miRNA-181a in the serrated pathway. A comprehensive analysis of miRNA profile in SACs and MSI-H CRC has been carried out by Kondelova *et al*<sup>[10]</sup> Interesting information about the molecular features of miRNA expression in SACs and MSI-H CRC has been elucidated. Microarray assay showed that 223 miRNAs were differently expressed, as 75 of them were downregulated in SACs compared to MSI-H CRC. On the other hand, 148 miRNAs were upregulated in the same comparison group. Notably, only miRNA-181a-2 showed significant overexpression in MSI-H CRC compared to SACs. It has been established that miRNA-181a-2 has an inverse correlation with nicotinamide phosphoribosyl transferase, which is a transcription factor playing a significant role in organogenesis and stem cell development<sup>[92]</sup>.

In conclusion, their analysis showed that miRNA-181a-2 plays a role in development in different subtypes of CRC from the serrated pathological pathway. Additionally, the up-regulation of miRNA-181a-2 was associated with MSI-H status. This study may be a foundation for further researches aiming to elucidate the function of miRNA-181a-2 in CRC<sup>[10]</sup>.

### **Other significant miRNAs in serrated pathway**

Slattery *et al*<sup>[15]</sup> have carried out promising research about different miRNA expression between NM and different types of polyps. They made a comprehensive analysis of miRNA expression among adenomatous polyp (AD), SSLs, and HPs. This study identified 19 differently expressed miRNAs between AD and HP such as let-7i-5p, miRNA-1229-5p, miRNA-1234-5p, miRNA-1249, miRNA-1268B, miRNA-1275, miRNA-194-5p, miRNA-215, miRNA-2392, miRNA-30b-5p, miRNA-331-3p, miRNA-3653, miRNA-3960, miRNA-4281, miRNA-4689, miRNA-4739, miRNA-518a-5p, miRNA-6510-5p and miRNA-939-5p. They concluded that the expression of the above-mentioned miRNAs in HP and SSLs are down-regulated and are related to MSI and CIMP. On the other hand, ADs have upregulated miRNA expression and are associated with TP53 and KRAS-mutations. Additionally, their study aimed to identify different miRNA expression and molecular pathways in colorectal carcinogenesis through genomic landscaping of colon polyps<sup>[15]</sup>. An overview of putative miRNA profile expression in the serrated colorectal pathway is presented in **Figure 3**.



**Figure 3 Putative microRNA profile expression in the serrated colorectal pathway.** microRNAs in red color showed up-regulation, while the ones in blue color showed down-regulation. miRNA: microRNA; SSL: Sessile serrated lesion; SSL-CD: Sessile serrated lesion with cytological dysplasia; TSA: Traditional serrated adenoma; HGD-H: High-grade dysplasia; TVA: Tubulovillous adenoma; CIMP: CpG island methylator phenotype; CIMP-H: CIMP-high; CIMP-L: CIMP-low; MSI: Microsatellite instability; MSS: Microsatellite stability; NM: Normal mucosa.

### HUMAN GUT MICROBIOTA, MUCOSAL IMMUNITY, AND MIRNA IN SERRATED PATHWAY

Human gut microbiota comprises approximately 39 trillion microorganisms that colonize the adult gut system<sup>[93]</sup>. It plays a significant role in maintaining homeostasis of the intestinal immune system, which represents a natural barrier to pathogen infection<sup>[94]</sup> but also maintain oral tolerance in the gut. Gut homeostasis can be disturbed by environmental factors such as lifestyle, diets, infections, and antibiotics, leading to dysbiosis. Many recent studies have demonstrated the association between gut dysbiosis and colorectal carcinogenesis<sup>[95]</sup>. Evidence suggest that *Fusobacterium nucleatum* (*F. nucleatum*) has overabundance in gut microbiota in dysbiosis<sup>[96]</sup>. This finding is in agreement with the fact that *F. nucleatum* is involved in mucosal inflammation and contributes to the progression of CRC<sup>[97,98]</sup>. There are plenty of studies that investigate interactions between *F. nucleatum* and conventional adenoma to carcinoma sequences<sup>[99-101]</sup>. Ito *et al*<sup>[102]</sup> focused on *F. nucleatum* and serrated carcinoma pathway. In particular, they investigated the putative correlation between *F. nucleatum* and miRNA-31 expression. However, the results of the study did not indicate a significant association between miRNA-31 and *F. nucleatum*. Nevertheless, Yu *et al*<sup>[103]</sup> showed that invasive *F. nucleatum* might play a role in developing proximal colon carcinogenesis through the serrated neoplasia process, which may play a less significant role in the traditional adenomas-carcinoma sequence. Bacterial biofilms may not support *F. nucleatum* infiltrate tumor tissues.

Longitudinal studies of immune infiltrate in resected CRC tumors have shown the role of the immune response in the pathophysiology of CRC. miRNAs, as non-coding RNAs, are capable of controlling several post-transcription target genes and performing essential roles in cell proliferation, differentiation, and apoptosis, including the immune cells<sup>[104]</sup>. In other words, miRNAs are necessary for maintaining the functioning of the immune system. However, abnormal expression of miRNAs is often found in various forms of tumors that contributes to immune deficiencies or immune evasion. Li *et al*<sup>[104]</sup> focused on the possible functions of miRNAs in CRC immune response control and the use of specific miRNA targets for CRC therapy. It is assumed that miRNAs possess an immunomodulatory role and can potentially be a part of the anti-cancer target pipeline. However, there may be some drawbacks and threats of using miRNAs as immunotherapeutics.

As discussed above, different miRNA profile variations from the transition of NM to adenoma and CRC identified some miRNA as contributors to those transformations. Moreover, serum miRNAs may be used as markers to track certain changes accompanying carcinogenesis<sup>[105]</sup>. miRNA profiles obtained in standard colorectal mucosa differ from those in adenomas and CRC. Oncogenes such as *c-Met* and *KRAS*, together with the miRNAs could also have pro- or anti-CRC effects, including influencing the immune system. More interestingly, some miRNAs increased their

expression in developing CRC, whereas others reduced their expression, such as miRNA-30b<sup>[106]</sup>. Furthermore, evidence indicates that miRNAs not only participate in colorectal carcinogenesis, but can be used as biomarkers for diagnosing, managing, and follow up the patients.

It is well-known that one of the mechanisms for cancer invasion is to establish complex pathways for disarming the immune system and evading immune surveillance. Nakanishi *et al*<sup>[106]</sup> demonstrated that in human serrated tumors, the expression of atypical protein kinases C (PKC) is decreased. Simultaneous inactivation of the encoding genes in the intestinal epithelium of the mouse culminated in random serrated tumorigenesis with a highly reactive and immunosuppressive stroma leading to advanced cancer development. Whereas epithelial PKC deficiency resulted in the death of immunogenic cells and the infiltration of CD8+ T cells that repressed tumor initiation, IFN, and CD8+ T cell responses were impaired by PKC loss, resulting in tumorigenesis<sup>[106]</sup>.

Some tumors may stimulate the immune cells in the tumor stroma to produce a variety of inhibiting cytokines such as transforming growth factor (TGF- $\beta$ ) and IL-10, which suppress the recruitment and activation of antitumor T lymphocytes<sup>[107]</sup>. Furthermore, IL-6 suppresses the ability of dendritic cells to present antigens by activating the signal transducer and transcription activator 3 (STAT3) and lessens CD4+ T cell-mediated immune response<sup>[108]</sup>. Thus, an immunotherapy that utilizes monoclonal antibodies that antagonize immunosuppressive cytokines or inactivate immunosuppressive cells may enhance tolerance to cancer and prevent tumor growth<sup>[106]</sup>. Our team also documented that IL-6 upregulation is crucial for developing both IBD and CRC well before the upregulation of other Th17/Treg associated genes (TGF $\beta$ 1, IL-10, IL-23, and FoxP3 transcription factor) that are critical primarily for the development of CRC<sup>[109]</sup>. An additional study revealed that intratumoral IL-17-mediated signaling might inhibit immunotherapy responses<sup>[110]</sup>.

In line with this, synergistic therapeutic efficacy was demonstrated by combined therapy with TGF- $\beta$  receptor inhibitor and anti-PD-L1 checkpoint blockade. A study of human samples confirmed the importance of atypical PKCs during the immunosurveillance defects in human serrated CRC. These results give insight into how this poor-prognosis subtype of CRC to be diagnosed and treated<sup>[106]</sup>.

Since miRNAs modify the differentiation, activation, and distribution of the various immune cells and the intricate cytokine network, miRNAs play an essential role in both innate and adaptive immune responses. miRNAs are closely involved in processes such as control of innate and adaptive immunity activation, regulation of inflammation and cytokine network, trafficking and cytokine crosstalk between the tumor and its microenvironment, miRNAs are promising targets for immunotherapy of different gastroenterological cancers<sup>[111]</sup>. Thus, miRNAs exert regulatory and protective functions in the digestive system and antitumor defense against gastroenterological cancers development.

In line with this, KRAS-IRF2 (interferon regulatory factor 2) axis also impacts the immune system towards immune suppression<sup>[112]</sup>. The clinical significance of this observation is the immunotherapy resistance in CRC. However, the biological functions and mechanisms of oncogenic KRAS in resistance to immune checkpoint blockade therapy are not fully understood.

Additionally, although various studies have examined the immune environment of CRCs with MSI, only one analysis assessed the immune microenvironment of serrated precursor lesions, including sessile serrated adenoma with dysplasia (SSA-D)<sup>[113]</sup>. Rau *et al*<sup>[113]</sup> studied the density of intraepithelial lymphocytes (IELs) in various serrated polyps and SSAs-D. The investigators observed that the amount of IELs was substantially higher in SSA-D than in SSAs, which displayed significantly higher numbers of IELs relative to HPs and typical adenomas. In their research, Acosta-Gonzalez *et al*<sup>[114]</sup> examined the immune properties of the serrated carcinogenesis system and its association with morphological stepwise dysplasia-carcinoma development and MSI status. They confirmed the higher density of IELs in lesions of MSI-H tumors. Additionally, other studies have shown that the total number of frameshift mutations in MSI CRCs correlates with lymphocyte infiltrating tumor density, specifically CD8+ lymphocyte density<sup>[115]</sup>.

Nevertheless, the serrated pathway has two outcomes that differ in their clinical and prognostic characteristics as well as in their methylome profile and histological and molecular characteristics: (1) SSLs; or (2) Sporadic CRC showing MSI-H<sup>[42]</sup>. The latter subtype of CRC is correlated with deep immune invasion and has a better prognosis than the former<sup>[116]</sup>.

The latest approaches in transcriptomics used to classify human CRC have shown that mesenchymal and/or desmoplastic involvement, together with an

immunosuppressive microenvironment, are essential determinants of the worst prognosis of CRC. Importantly, these aggressive CRCs harbor the traits of serrated tumors, suggesting that how aggressive the CRC becomes is determined by initiation by this alternate mechanism. Moreover, molecular markers and profiles of gene expression have indicated that at least two CRC subgroups exist within the serrated pathway: (1) An inflammatory subtype with features of stromal/mesenchymal high immune infiltration (referred to “mesenchymal serrated” CRCs); and (2) MSI (“classical serrated”). *BRAF* mutation characterized with immune suppression in the tumor environment<sup>[117]</sup>.

However, the tumor stroma's possible activation and the type of immune response associated with the CRC tumor stroma are not yet well understood. SAC may be infiltrated by CD45+ cells that express PD-L1 and decrease CD8+ T cells, which determines that there are multiple immune mechanisms to avoid the immune response<sup>[106]</sup>. Nevertheless, to create more efficient therapies, understanding the pathogenesis, including the tumor environment on the immunological settings, for both forms of serrated CRC is essential. Although emerging data show that immunotherapy is a promising choice for patients with multiple cancer forms still, there is a substantial clinical gap between the identification of serrated precursor lesions and the effective therapies for treating them.

## CONCLUSION

With the growing influence of translational research and molecular pathology, the serrated pathway carcinogenesis became more elucidated based on the association of CIMP and key mutations in *BRAF*, *KRAS*, *PIK3CA*, *TP53*, and *APC*. Furthermore, MSI caused by dysfunction of DNA MMR genes, is considered as another critical pathway in carcinogenesis.

In this review we summarized the most relevant information that have been published in the literature so far about miRNA expression in serrated pathway. Furthermore, we intended to answer the question could miRNA expression tell us more about colorectal serrated pathway carcinogenesis. The answer may come from several studies that have been published related to this issue. The data showed a unique miRNA signature in different types of colonic polyps, as well as in the progression of serrated lesions. Besides, those miRNAs play an important role in serrated carcinogenesis, proliferation, regression, and metastasis. Existing evidence support that miRNAs expression profiling, including miRNA-125b, miRNA-222, miRNA-214, miRNA-335 miRNA-31 miRNA-135-B miRNA-21 miRNA-181a-2, *etc.*, allows us to understand and identify differences between benign and malignant lesions of the colon mucosa, as well as to stratify benign lesions according to their malignant potential.

Moreover, serum miRNAs may be used as markers to track specific changes accompanying serrated carcinogenesis. This assertion is based on the fact that there is a significant difference of miRNA expression between serrated and conventional pathway in colorectal carcinogenesis.

The immunopathology of CRC attracted growing attention since an association between gut dysbiosis and colorectal carcinogenesis was suggested by recent authors. miRNAs are putative regulators of several post-transcription target genes and are thought to play essential role in differentiation and proliferation of immune cells. It is assumed that, different miRNA profile pattern may contribute to alterations in gut immunity and dysbiosis, leading to transition events of NM to adenoma.

The specific miRNA expression in serrated pathway, could be useful tool to find appropriate diagnostic, prognostic and treatment response markers in clinical practice. Thus, in order to understand the real significance of miRNAs in this clinical setting, further studies must be conducted.

## REFERENCES

- 1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- 2 **Rawla P**, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* 2019; **14**: 89-103 [PMID: 31616522 DOI: 10.5114/pg.2018.81072]
- 3 **Farooqi AA**, de la Roche M, Djamgoz MBA, Siddik ZH. Overview of the oncogenic signaling pathways in

- colorectal cancer: Mechanistic insights. *Semin Cancer Biol* 2019; **58**: 65-79 [PMID: 30633978 DOI: 10.1016/j.semcancer.2019.01.001]
- 4 **Jass JR**, Smith M. Sialic acid and epithelial differentiation in colorectal polyps and cancer—a morphological, mucin and lectin histochemical study. *Pathology* 1992; **24**: 233-242 [PMID: 1289763 DOI: 10.3109/00313029209068874]
  - 5 **O'Brien MJ**, Yang S, Clebanoff JL, Mulcahy E, Farraye FA, Amoroso M, Swan N. Hyperplastic (serrated) polyps of the colorectum: relationship of CpG island methylator phenotype and K-ras mutation to location and histologic subtype. *Am J Surg Pathol* 2004; **28**: 423-434 [PMID: 15087661 DOI: 10.1097/00000478-200404000-00001]
  - 6 **Mäkinen MJ**. Colorectal serrated adenocarcinoma. *Histopathology* 2007; **50**: 131-150 [PMID: 17204027 DOI: 10.1111/j.1365-2559.2006.02548.x]
  - 7 **Bartley AN**, Yao H, Barkoh BA, Ivan C, Mishra BM, Rashid A, Calin GA, Luthra R, Hamilton SR. Complex patterns of altered MicroRNA expression during the adenoma-adenocarcinoma sequence for microsatellite-stable colorectal cancer. *Clin Cancer Res* 2011; **17**: 7283-7293 [PMID: 21948089 DOI: 10.1158/1078-0432.CCR-11-1452]
  - 8 **Schee K**, Fodstad Ø, Flatmark K. MicroRNAs as biomarkers in colorectal cancer. *Am J Pathol* 2010; **177**: 1592-1599 [PMID: 20829435 DOI: 10.2353/ajpath.2010.100024]
  - 9 **Wong K**, Xie G. Updates on the Molecular Genetics of Colorectal Cancer. *Colorec Cancer* 2017; **3**: 1 [DOI: 10.21767/2471-9943.100032]
  - 10 **Kondelova A**, Albuquerque-González B, Vychytilova-Faltejskova P, García-Solano J, Prochazka V, Kala Z, Pérez F, Slaby O, Conesa-Zamora P. miR-181a-2\* expression is different amongst carcinomas from the colorectal serrated route. *Mutagenesis* 2020; **35**: 233-241 [PMID: 31784758 DOI: 10.1093/mutage/gez039]
  - 11 **Kanth P**, Hazel MW, Boucher KM, Yang Z, Wang L, Bronner MP, Boylan KE, Burt RW, Westover M, Neklason DW, Delker DA. Small RNA sequencing of sessile serrated polyps identifies microRNA profile associated with colon cancer. *Genes Chromosomes Cancer* 2019; **58**: 23-33 [PMID: 30265426 DOI: 10.1002/gcc.22686]
  - 12 **Satorres C**, García-Campos M, Bustamante-Balén M. Molecular Features of the Serrated Pathway to Colorectal Cancer: Current Knowledge and Future Directions. *Gut Liver* 2020 [PMID: 32340435 DOI: 10.5009/gnl19402]
  - 13 **Zhang XT**, Zhang QW, Liu F, Lin XL, Chen JN, Li XB. Endoscopic features of sessile serrated adenoma/polyps under narrowband imaging: A retrospective study. *J Dig Dis* 2019; **20**: 135-142 [PMID: 30693669 DOI: 10.1111/1751-2980.12706]
  - 14 **Okugawa Y**, Grady WM, Goel A. Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. *Gastroenterology* 2015; **149**: 1204-1225. e12 [PMID: 26216839 DOI: 10.1053/j.gastro.2015.07.011]
  - 15 **Slaterry ML**, Herrick JS, Wolff RK, Mullany LE, Stevens JR, Samowitz W. The miRNA landscape of colorectal polyps. *Genes Chromosomes Cancer* 2017; **56**: 347-353 [PMID: 27925331 DOI: 10.1002/gcc.22436]
  - 16 **Ito M**, Mitsuhashi K, Igarashi H, Noshō K, Naito T, Yoshii S, Takahashi H, Fujita M, Sukawa Y, Yamamoto E, Takahashi T, Adachi Y, Nojima M, Sasaki Y, Tokino T, Baba Y, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. MicroRNA-31 expression in relation to BRAF mutation, CpG island methylation and colorectal continuum in serrated lesions. *Int J Cancer* 2014; **135**: 2507-2515 [PMID: 24752710 DOI: 10.1002/ijc.28920]
  - 17 **Leggett B**, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. *Gastroenterology* 2010; **138**: 2088-2100 [PMID: 20420948 DOI: 10.1053/j.gastro.2009.12.066]
  - 18 **Snover DC**. Update on the serrated pathway to colorectal carcinoma. *Hum Pathol* 2011; **42**: 1-10 [PMID: 20869746 DOI: 10.1016/j.humpath.2010.06.002]
  - 19 **O'Brien MJ**, Zhao Q, Yang S. Colorectal serrated pathway cancers and precursors. *Histopathology* 2015; **66**: 49-65 [PMID: 25263173 DOI: 10.1111/his.12564]
  - 20 **Bettington M**, Walker N, Clouston A, Brown I, Leggett B, Whitehall V. The serrated pathway to colorectal carcinoma: current concepts and challenges. *Histopathology* 2013; **62**: 367-386 [PMID: 23339363 DOI: 10.1111/his.12055]
  - 21 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]
  - 22 **Fan C**, Younis A, Bookhout CE, Crockett SD. Management of Serrated Polyps of the Colon. *Curr Treat Options Gastroenterol* 2018; **16**: 182-202 [PMID: 29445907 DOI: 10.1007/s11938-018-0176-0]
  - 23 **Yang S**, Farraye FA, Mack C, Posnik O, O'Brien MJ. BRAF and KRAS Mutations in hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology and CpG island methylation status. *Am J Surg Pathol* 2004; **28**: 1452-1459 [PMID: 15489648 DOI: 10.1097/01.pas.0000141404.56839.6a]
  - 24 **Hawkins NJ**, Bariol C, Ward RL. The serrated neoplasia pathway. *Pathology* 2002; **34**: 548-555 [PMID: 12555993]
  - 25 **Cao H**, He N, Song S, Xu M, Piao M, Yan F, Wang B. Is surveillance colonoscopy necessary for patients with sporadic gastric hyperplastic polyps? *PLoS One* 2015; **10**: e0122996 [PMID: 25874940 DOI: 10.1371/journal.pone.0122996]
  - 26 **Goldstein NS**, Bhanot P, Odish E, Hunter S. Hyperplastic-like colon polyps that preceded microsatellite-unstable adenocarcinomas. *Am J Clin Pathol* 2003; **119**: 778-796 [PMID: 12817424 DOI: 10.1309/DRFQ-0WFW-F1G1-3CTK]
  - 27 **Longacre TA**, Fenoglio-Preiser CM. Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. *Am J Surg Pathol* 1990; **14**: 524-537 [PMID: 2186644 DOI: 10.1097/00000478-199006000-00003]
  - 28 **Torlakovic E**, Skovlund E, Snover DC, Torlakovic G, Nesland JM. Morphologic reappraisal of serrated colorectal polyps. *Am J Surg Pathol* 2003; **27**: 65-81 [PMID: 12502929 DOI: 10.1097/00000478-200301000-00008]
  - 29 **Rosenberg DW**, Yang S, Pleau DC, Greenspan EJ, Stevens RG, Rajan TV, Heinen CD, Levine J, Zhou Y,

- O'Brien MJ. Mutations in BRAF and KRAS differentially distinguish serrated *versus* non-serrated hyperplastic aberrant crypt foci in humans. *Cancer Res* 2007; **67**: 3551-3554 [PMID: 17440063 DOI: 10.1158/0008-5472.CAN-07-0343]
- 30 Carr NJ, Mahajan H, Tan KL, Hawkins NJ, Ward RL. Serrated and non-serrated polyps of the colorectum: their prevalence in an unselected case series and correlation of BRAF mutation analysis with the diagnosis of sessile serrated adenoma. *J Clin Pathol* 2009; **62**: 516-518 [PMID: 19126563 DOI: 10.1136/jcp.2008.061960]
- 31 Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, Simms LA, Young J, James M, Montgomery GW, Appleyard M, Hewett D, Togashi K, Jass JR, Leggett BA. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. *Gastroenterology* 2006; **131**: 1400-1407 [PMID: 17101316 DOI: 10.1053/j.gastro.2006.08.038]
- 32 McCarthy AJ, Serra S, Chetty R. Traditional serrated adenoma: an overview of pathology and emphasis on molecular pathogenesis. *BMJ Open Gastroenterol* 2019; **6**: e000317 [PMID: 31413858 DOI: 10.1136/bmjgast-2019-000317]
- 33 García-Solano J, Pérez-Guillermo M, Conesa-Zamora P, Acosta-Ortega J, Trujillo-Santos J, Cerezuela-Fuentes P, Mäkinen MJ. Clinicopathologic study of 85 colorectal serrated adenocarcinomas: further insights into the full recognition of a new subset of colorectal carcinoma. *Hum Pathol* 2010; **41**: 1359-1368 [PMID: 20594582 DOI: 10.1016/j.humpath.2010.04.002]
- 34 Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999; **96**: 8681-8686 [PMID: 10411935 DOI: 10.1073/pnas.96.15.8681]
- 35 Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003; **349**: 2042-2054 [PMID: 14627790 DOI: 10.1056/NEJMra023075]
- 36 Nagasaka T, Koi M, Kloor M, Gebert J, Vilkin A, Nishida N, Shin SK, Sasamoto H, Tanaka N, Matsubara N, Boland CR, Goel A. Mutations in both KRAS and BRAF may contribute to the methylator phenotype in colon cancer. *Gastroenterology* 2008; **134**: 1950-1960, 1960. e1 [PMID: 18435933 DOI: 10.1053/j.gastro.2008.02.094]
- 37 Fernando WC, Miranda MS, Worthley DL, Togashi K, Watters DJ, Leggett BA, Spring KJ. The CIMP Phenotype in BRAF Mutant Serrated Polyps from a Prospective Colonoscopy Patient Cohort. *Gastroenterol Res Pract* 2014; **2014**: 374926 [PMID: 24812557 DOI: 10.1155/2014/374926]
- 38 Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. *J Mol Diagn* 2007; **9**: 305-314 [PMID: 17591929 DOI: 10.2353/jmoldx.2007.060170]
- 39 Advani SM, Advani P, DeSantis SM, Brown D, VonVille HM, Lam M, Loree JM, Mehrvarz Sarshekeh A, Bressler J, Lopez DS, Daniel CR, Swartz MD, Kopetz S. Clinical, Pathological, and Molecular Characteristics of CpG Island Methylator Phenotype in Colorectal Cancer: A Systematic Review and Meta-analysis. *Transl Oncol* 2018; **11**: 1188-1201 [PMID: 30071442 DOI: 10.1016/j.tranon.2018.07.008]
- 40 Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature* 1998; **396**: 643-649 [PMID: 9872311 DOI: 10.1038/25292]
- 41 Kuiper RP, Vissers LE, Venkatchalam R, Bodmer D, Hoenselaar E, Goossens M, Haufe A, Kamping E, Niessen RC, Hogervorst FB, Gille JJ, Redeker B, Tops CM, van Gijn ME, van den Ouweland AM, Rahner N, Steinke V, Kahl P, Holinski-Feder E, Morak M, Kloor M, Stemmler S, Betz B, Hutter P, Bunyan DJ, Syngal S, Culver JO, Graham T, Chan TL, Nagtegaal ID, van Krieken JH, Schackert HK, Hoogerbrugge N, van Kessel AG, Ligtenberg MJ. Recurrence and variability of germline EPCAM deletions in Lynch syndrome. *Hum Mutat* 2011; **32**: 407-414 [PMID: 21309036 DOI: 10.1002/humu.21446]
- 42 De Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The Molecular Hallmarks of the Serrated Pathway in Colorectal Cancer. *Cancers (Basel)* 2019; **11** [PMID: 31330830 DOI: 10.3390/cancers11071017]
- 43 Hashimoto T, Yamashita S, Yoshida H, Taniguchi H, Ushijima T, Yamada T, Saito Y, Ochiai A, Sekine S, Hiraoka N. WNT Pathway Gene Mutations Are Associated With the Presence of Dysplasia in Colorectal Sessile Serrated Adenoma/Polyps. *Am J Surg Pathol* 2017; **41**: 1188-1197 [PMID: 28614199 DOI: 10.1097/PAS.0000000000000877]
- 44 Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; **138**: 2073-2087. e3 [PMID: 20420947 DOI: 10.1053/j.gastro.2009.12.064]
- 45 Murphy KM, Zhang S, Geiger T, Hafez MJ, Bacher J, Berg KD, Eshleman JR. Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. *J Mol Diagn* 2006; **8**: 305-311 [PMID: 16825502 DOI: 10.2353/jmoldx.2006.050092]
- 46 Kim JH, Bae JM, Cho NY, Kang GH. Distinct features between MLH1-methylated and unmethylated colorectal carcinomas with the CpG island methylator phenotype: implications in the serrated neoplasia pathway. *Oncotarget* 2016; **7**: 14095-14111 [PMID: 26883113 DOI: 10.18632/oncotarget.7374]
- 47 Travaglino A, D'Armiento FP, Cassese G, Campanino MR, Borrelli G, Pignatiello S, Luglio G, Maione F, De Palma GD, D'Armiento M. Clinicopathological factors associated with BRAF-V600E mutation in colorectal serrated adenomas. *Histopathology* 2019; **75**: 160-173 [PMID: 30815911 DOI: 10.1111/his.13846]
- 48 Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nat Rev Cancer* 2004; **4**: 937-947 [PMID: 15573115 DOI: 10.1038/nrc1503]
- 49 Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodríguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. *Int J Mol Sci* 2017; **18** [PMID: 28106826 DOI: 10.3390/ijms18010197]
- 50 Tejpar S, Bertagnolli M, Bosman F, Lenz HJ, Garraway L, Waldman F, Warren R, Bild A, Collins-Brennan D, Hahn H, Harkin DP, Kennedy R, Ilyas M, Morreau H, Proutski V, Swanton C, Tomlinson I, Delorenzi M, Fiocca R, Van Cutsem E, Roth A. Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. *Oncologist*

- 2010; **15**: 390-404 [PMID: 20350999 DOI: 10.1634/theoncologist.2009-0233]
- 51 **Caputo F**, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, Andrikou K, Cascinu S, Gelsomino F. BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. *Int J Mol Sci* 2019; **20** [PMID: 31661924 DOI: 10.3390/ijms20215369]
- 52 **Hughes LA**, Khalid-de Bakker CA, Smits KM, van den Brandt PA, Jonkers D, Ahuja N, Herman JG, Weijnenberg MP, van Engeland M. The CpG island methylator phenotype in colorectal cancer: progress and problems. *Biochim Biophys Acta* 2012; **1825**: 77-85 [PMID: 22056543 DOI: 10.1016/j.bbcan.2011.10.005]
- 53 **Jones JC**, Renfro LA, Al-Shamsi HO, Schrock AB, Rankin A, Zhang BY, Kasi PM, Voss JS, Leal AD, Sun J, Ross J, Ali SM, Hubbard JM, Kipp BR, McWilliams RR, Kopetz S, Wolff RA, Grothey A. <sup>Non-V600</sup> BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *J Clin Oncol* 2017; **35**: 2624-2630 [PMID: 28486044 DOI: 10.1200/JCO.2016.71.4394]
- 54 **Kambara T**, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, Barker MA, Arnold S, McGivern A, Matsubara N, Tanaka N, Higuchi T, Young J, Jass JR, Leggett BA. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004; **53**: 1137-1144 [PMID: 15247181 DOI: 10.1136/gut.2003.037671]
- 55 **Bond CE**, Whitehall VLJ. How the BRAF V600E Mutation Defines a Distinct Subgroup of Colorectal Cancer: Molecular and Clinical Implications. *Gastroenterol Res Pract* 2018; **2018**: 9250757 [PMID: 30598662 DOI: 10.1155/2018/9250757]
- 56 **Sinicrope FA**, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J, Bot BM, Tejpar S, Delorenzi M, Goldberg RM, Mahoney M, Sargent DJ, Alberts SR. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology* 2015; **148**: 88-99 [PMID: 25305506 DOI: 10.1053/j.gastro.2014.09.041]
- 57 **O'Brien MJ**, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amoroso M, Farraye FA. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 2006; **30**: 1491-1501 [PMID: 17122504 DOI: 10.1097/01.pas.0000213313.36306.85]
- 58 **Yamane L**, Scapulatempo-Neto C, Reis RM, Guimarães DP. Serrated pathway in colorectal carcinogenesis. *World J Gastroenterol* 2014; **20**: 2634-2640 [PMID: 24627599 DOI: 10.3748/wjg.v20.i10.2634]
- 59 **Bettington ML**, Walker NI, Rosty C, Brown IS, Clouston AD, McKeone DM, Pearson SA, Klein K, Leggett BA, Whitehall VL. A clinicopathological and molecular analysis of 200 traditional serrated adenomas. *Mod Pathol* 2015; **28**: 414-427 [PMID: 25216220 DOI: 10.1038/modpathol.2014.122]
- 60 **Bettington M**, Walker N, Rosty C, Brown I, Clouston A, McKeone D, Pearson SA, Leggett B, Whitehall V. Clinicopathological and molecular features of sessile serrated adenomas with dysplasia or carcinoma. *Gut* 2017; **66**: 97-106 [PMID: 26475632 DOI: 10.1136/gutjnl-2015-310456]
- 61 **Lee RC**, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993; **75**: 843-854 [PMID: 8252621 DOI: 10.1016/0092-8674(93)90529-Y]
- 62 **To KK**, Tong CW, Wu M, Cho WC. MicroRNAs in the prognosis and therapy of colorectal cancer: From bench to bedside. *World J Gastroenterol* 2018; **24**: 2949-2973 [PMID: 30038463 DOI: 10.3748/wjg.v24.i27.2949]
- 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 Res* 2008; **68**: 5795-5802 [PMID: 18632633 DOI: 10.1158/0008-5472.CAN-08-0951]
- 64 **Schetter AJ**, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM, Harris CC. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* 2008; **299**: 425-436 [PMID: 18230780 DOI: 10.1001/jama.299.4.425]
- 65 **Balaguer F**, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR, Goel A. Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. *Cancer Res* 2010; **70**: 6609-6618 [PMID: 20682795 DOI: 10.1158/0008-5472.CAN-10-0622]
- 66 **Nagy ZB**, Wichmann B, Kalmár A, Galamb O, Barták BK, Spisák S, Tulassay Z, Molnár B. Colorectal adenoma and carcinoma specific miRNA profiles in biopsy and their expression in plasma specimens. *Clin Epigenetics* 2017; **9**: 22 [PMID: 28289479 DOI: 10.1186/s13148-016-0305-3]
- 67 **Oberg AL**, French AJ, Sarver AL, Subramanian S, Morlan BW, Riska SM, Borralho PM, Cunningham JM, Boardman LA, Wang L, Smyrk TC, Asmann Y, Steer CJ, Thibodeau SN. miRNA expression in colon polyps provides evidence for a multihit model of colon cancer. *PLoS One* 2011; **6**: e20465 [PMID: 21694772 DOI: 10.1371/journal.pone.0020465]
- 68 **Tsikitis VL**, Potter A, Mori M, Buckmeier JA, Preece CR, Harrington CA, Bartley AN, Bhattacharyya AK, Hamilton SR, Lance MP, Thompson PA. MicroRNA Signatures of Colonic Polyps on Screening and Histology. *Cancer Prev Res (Phila)* 2016; **9**: 942-949 [PMID: 27658891 DOI: 10.1158/1940-6207.CAPR-16-0086]
- 69 **Nishida N**, Yokobori T, Mimori K, Sudo T, Tanaka F, Shibata K, Ishii H, Doki Y, Kuwano H, Mori M. MicroRNA miR-125b is a prognostic marker in human colorectal cancer. *Int J Oncol* 2011; **38**: 1437-1443 [PMID: 21399871 DOI: 10.3892/ijo.2011.969]
- 70 **Aoki H**, Noshō K, Igarashi H, Ito M, Mitsuhashi K, Naito T, Yamamoto E, Tanuma T, Nomura M, Maguchi H, Shinohara T, Suzuki H, Yamamoto H, Shinomura Y. MicroRNA-31 expression in colorectal serrated pathway progression. *World J Gastroenterol* 2014; **20**: 12346-12349 [PMID: 25232271 DOI: 10.3748/wjg.v20.i34.12346]
- 71 **Noshō K**, Igarashi H, Nojima M, Ito M, Maruyama R, Yoshii S, Naito T, Sukawa Y, Mikami M, Sumioka W, Yamamoto E, Kurokawa S, Adachi Y, Takahashi H, Okuda H, Kusumi T, Hosokawa M, Fujita M, Hasegawa T, Okita K, Hirata K, Suzuki H, Yamamoto H, Shinomura Y. Association of microRNA-31 with BRAF mutation, colorectal cancer survival and serrated pathway. *Carcinogenesis* 2014; **35**: 776-783 [PMID: 24242331 DOI: 10.1093/carcin/bgt374]

- 72 **Yu T**, Ma P, Wu D, Shu Y, Gao W. Functions and mechanisms of microRNA-31 in human cancers. *Biomed Pharmacother* 2018; **108**: 1162-1169 [PMID: 30372817 DOI: 10.1016/j.biopha.2018.09.132]
- 73 **Strubberg AM**, Madison BB. MicroRNAs in the etiology of colorectal cancer: pathways and clinical implications. *Dis Model Mech* 2017; **10**: 197-214 [PMID: 28250048 DOI: 10.1242/dmm.027441]
- 74 **Cekaite L**, Rantala JK, Bruun J, Guriby M, Agesen TH, Danielsen SA, Lind GE, Nesbakken A, Kallioniemi O, Lothe RA, Skotheim RI. MiR-9, -31, and -182 deregulation promote proliferation and tumor cell survival in colon cancer. *Neoplasia* 2012; **14**: 868-879 [PMID: 23019418 DOI: 10.1593/neo.121094]
- 75 **Cottonham CL**, Kaneko S, Xu L. miR-21 and miR-31 converge on TIAM1 to regulate migration and invasion of colon carcinoma cells. *J Biol Chem* 2010; **285**: 35293-35302 [PMID: 20826792 DOI: 10.1074/jbc.M110.160069]
- 76 **Kubota N**, Taniguchi F, Nyuya A, Umeda Y, Mori Y, Fujiwara T, Tanioka H, Tsuruta A, Yamaguchi Y, Nagasaka T. Upregulation of microRNA-31 is associated with poor prognosis in patients with advanced colorectal cancer. *Oncol Lett* 2020; **19**: 2685-2694 [PMID: 32218819 DOI: 10.3892/ol.2020.11365]
- 77 **Igarashi H**, Kurihara H, Mitsuhashi K, Ito M, Okuda H, Kanno S, Naito T, Yoshii S, Takahashi H, Kusumi T, Hasegawa T, Sukawa Y, Adachi Y, Okita K, Hirata K, Imamura Y, Baba Y, Imai K, Suzuki H, Yamamoto H, Noshio K, Shinomura Y. Association of MicroRNA-31-5p with Clinical Efficacy of Anti-EGFR Therapy in Patients with Metastatic Colorectal Cancer. *Ann Surg Oncol* 2015; **22**: 2640-2648 [PMID: 25472647 DOI: 10.1245/s10434-014-4264-7]
- 78 **Yang MH**, Yu J, Chen N, Wang XY, Liu XY, Wang S, Ding YQ. Elevated microRNA-31 expression regulates colorectal cancer progression by repressing its target gene SATB2. *PLoS One* 2013; **8**: e85353 [PMID: 24386467 DOI: 10.1371/journal.pone.0085353]
- 79 **Yang X**, Xu X, Zhu J, Zhang S, Wu Y, Wu Y, Zhao K, Xing C, Cao J, Zhu H, Li M, Ye Z, Peng W. miR-31 affects colorectal cancer cells by inhibiting autophagy in cancer-associated fibroblasts. *Oncotarget* 2016; **7**: 79617-79628 [PMID: 27793031 DOI: 10.18632/oncotarget.12873]
- 80 **Valeri N**, Braconi C, Gasparini P, Murgia C, Lampis A, Paulus-Hock V, Hart JR, Ueno L, Grivennikov SI, Lovat F, Paone A, Cascione L, Sumani KM, Veronese A, Fabbri M, Carasi S, Alder H, Lanza G, Gafa' R, Moyer MP, Ridgway RA, Cordero J, Nuovo GJ, Frankel WL, Rugge M, Fassan M, Groden J, Vogt PK, Karin M, Sansom OJ, Croce CM. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. *Cancer Cell* 2014; **25**: 469-483 [PMID: 24735923 DOI: 10.1016/j.ccr.2014.03.006]
- 81 **Schee K**, Boye K, Abrahamsen TW, Fodstad Ø, Flatmark K. Clinical relevance of microRNA miR-21, miR-31, miR-92a, miR-101, miR-106a and miR-145 in colorectal cancer. *BMC Cancer* 2012; **12**: 505 [PMID: 23121918 DOI: 10.1186/1471-2407-12-505]
- 82 **Asangani IA**, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, Allgayer H. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdc4d and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene* 2008; **27**: 2128-2136 [PMID: 17968323 DOI: 10.1038/sj.onc.1210856]
- 83 **Xiong B**, Cheng Y, Ma L, Zhang C. MiR-21 regulates biological behavior through the PTEN/PI-3 K/Akt signaling pathway in human colorectal cancer cells. *Int J Oncol* 2013; **42**: 219-228 [PMID: 23174819 DOI: 10.3892/ijo.2012.1707]
- 84 **Wu Y**, Song Y, Xiong Y, Wang X, Xu K, Han B, Bai Y, Li L, Zhang Y, Zhou L. MicroRNA-21 (Mir-21) Promotes Cell Growth and Invasion by Repressing Tumor Suppressor PTEN in Colorectal Cancer. *Cell Physiol Biochem* 2017; **43**: 945-958 [PMID: 28957811 DOI: 10.1159/000481648]
- 85 **Ghareib AF**, Mohamed RH, Abd El-Fatah AR, Saadawy SF. Assessment of Serum MicroRNA-21 Gene Expression for Diagnosis and Prognosis of Colorectal Cancer. *J Gastrointest Cancer* 2020; **51**: 818-823 [PMID: 31482406 DOI: 10.1007/s12029-019-00306-w]
- 86 **Chen Z**, Liu H, Jin W, Ding Z, Zheng S, Yu Y. Tissue microRNA-21 expression predicted recurrence and poor survival in patients with colorectal cancer - a meta-analysis. *Onco Targets Ther* 2016; **9**: 2615-2624 [PMID: 27226723 DOI: 10.2147/OTT.S103893]
- 87 **Yau TO**, Tang CM, Harriss EK, Dickins B, Polytrachou C. Faecal microRNAs as a non-invasive tool in the diagnosis of colonic adenomas and colorectal cancer: A meta-analysis. *Sci Rep* 2019; **9**: 9491 [PMID: 31263200 DOI: 10.1038/s41598-019-45570-9]
- 88 **Ding L**, Lan Z, Xiong X, Ao H, Feng Y, Gu H, Yu M, Cui Q. The Dual Role of MicroRNAs in Colorectal Cancer Progression. *Int J Mol Sci* 2018; **19** [PMID: 30227605 DOI: 10.3390/ijms19092791]
- 89 **Schmitz KJ**, Hey S, Schinwald A, Wohlschlaeger J, Baba HA, Worm K, Schmid KW. Differential expression of microRNA 181b and microRNA 21 in hyperplastic polyps and sessile serrated adenomas of the colon. *Virchows Arch* 2009; **455**: 49-54 [PMID: 19547998 DOI: 10.1007/s00428-009-0804-0]
- 90 **Xi Y**, Formentini A, Chien M, Weir DB, Russo JJ, Ju J, Kornmann M, Ju J. Prognostic Values of microRNAs in Colorectal Cancer. *Biomark Insights* 2006; **2**: 113-121 [PMID: 18079988]
- 91 **Han P**, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, Liu YL, Cui BB. The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/ $\beta$ -catenin signaling. *Mol Cancer* 2017; **16**: 9 [PMID: 28086904 DOI: 10.1186/s12943-017-0583-1]
- 92 **Khan JA**, Forouhar F, Tao X, Tong L. Nicotinamide adenine dinucleotide metabolism as an attractive target for drug discovery. *Expert Opin Ther Targets* 2007; **11**: 695-705 [PMID: 17465726 DOI: 10.1517/14728222.11.5.695]
- 93 **Sender R**, Fuchs S, Milo R. Are We Really Vastly Outnumbered? *Cell* 2016; **164**: 337-340 [PMID: 26824647 DOI: 10.1016/j.cell.2016.01.013]
- 94 **Gagnière J**, Raisch J, Veziat J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501-518 [PMID: 26811603 DOI: 10.3748/wjg.v22.i2.501]
- 95 **Zitvogel L**, Ayyoub M, Routy B, Kroemer G. Microbiome and Anticancer Immunosurveillance. *Cell* 2016; **165**: 276-287 [PMID: 27058662 DOI: 10.1016/j.cell.2016.03.001]

- 96 **Holt RA**, Cochrane K. Tumor Potentiating Mechanisms of *Fusobacterium nucleatum*, A Multifaceted Microbe. *Gastroenterology* 2017; **152**: 694-696 [PMID: 28143770 DOI: 10.1053/j.gastro.2017.01.024]
- 97 **Shang FM**, Liu HL. *Fusobacterium nucleatum* and colorectal cancer: A review. *World J Gastrointest Oncol* 2018; **10**: 71-81 [PMID: 29564037 DOI: 10.4251/wjgo.v10.i3.71]
- 98 **Bullman S**, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, Neuberg D, Huang K, Guevara F, Nelson T, Chipashvili O, Hagan T, Walker M, Ramachandran A, Diosdado B, Serna G, Mulet N, Landolfi S, Ramon Y Cajal S, Fasani R, Aguirre AJ, Ng K, Élez E, Ogino S, Tabernero J, Fuchs CS, Hahn WC, Nuciforo P, Meyerson M. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science* 2017; **358**: 1443-1448 [PMID: 29170280 DOI: 10.1126/science.aal5240]
- 99 **Jahani-Sherafat S**, Alebouyeh M, Moghim S, Ahmadi Amoli H, Ghasemian-Safaei H. Role of gut microbiota in the pathogenesis of colorectal cancer; a review article. *Gastroenterol Hepatol Bed Bench* 2018; **11**: 101-109 [PMID: 29910850]
- 100 **Peters BA**, Dominianni C, Shapiro JA, Church TR, Wu J, Miller G, Yuen E, Freiman H, Lustbader I, Salik J, Friedlander C, Hayes RB, Ahn J. The gut microbiota in conventional and serrated precursors of colorectal cancer. *Microbiome* 2016; **4**: 69 [PMID: 28038683 DOI: 10.1186/s40168-016-0218-6]
- 101 **Yoon H**, Kim N, Park JH, Kim YS, Lee J, Kim HW, Choi YJ, Shin CM, Park YS, Lee DH, Jung HC. Comparisons of Gut Microbiota Among Healthy Control, Patients With Conventional Adenoma, Sessile Serrated Adenoma, and Colorectal Cancer. *J Cancer Prev* 2017; **22**: 108-114 [PMID: 28698865 DOI: 10.15430/JCP.2017.22.2.108]
- 102 **Ito M**, Kanno S, Noshio K, Sukawa Y, Mitsuhashi K, Kurihara H, Igarashi H, Takahashi T, Tachibana M, Takahashi H, Yoshii S, Takenouchi T, Hasegawa T, Okita K, Hirata K, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. Association of *Fusobacterium nucleatum* with clinical and molecular features in colorectal serrated pathway. *Int J Cancer* 2015; **137**: 1258-1268 [PMID: 25703934 DOI: 10.1002/ijc.29488]
- 103 **Yu J**, Chen Y, Fu X, Zhou X, Peng Y, Shi L, Chen T, Wu Y. Invasive *Fusobacterium nucleatum* may play a role in the carcinogenesis of proximal colon cancer through the serrated neoplasia pathway. *Int J Cancer* 2016; **139**: 1318-1326 [PMID: 27130618 DOI: 10.1002/ijc.30168]
- 104 **Li X**, Nie J, Mei Q, Han WD. MicroRNAs: Novel immunotherapeutic targets in colorectal carcinoma. *World J Gastroenterol* 2016; **22**: 5317-5331 [PMID: 27340348 DOI: 10.3748/wjg.v22.i23.5317]
- 105 **Liu G**, Li B. Role of miRNA in transformation from normal tissue to colorectal adenoma and cancer. *J Cancer Res Ther* 2019; **15**: 278-285 [PMID: 30964098 DOI: 10.4103/jcrt.JCRT\_135\_18]
- 106 **Nakanishi Y**, Duran A, L'Hermitte A, Shelton PM, Nakanishi N, Reina-Campos M, Huang J, Soldevila F, Baaten BJJ, Tauriello DVF, Castilla EA, Bhango MS, Bao F, Sigal D, Diaz-Meco MT, Moscat J. Simultaneous Loss of Both Atypical Protein Kinase C Genes in the Intestinal Epithelium Drives Serrated Intestinal Cancer by Impairing Immunosurveillance. *Immunity* 2018; **49**: 1132-1147. e7 [PMID: 30552022 DOI: 10.1016/j.immuni.2018.09.013]
- 107 **Takeuchi Y**, Nishikawa H. Roles of regulatory T cells in cancer immunity. *Int Immunol* 2016; **28**: 401-409 [PMID: 27160722 DOI: 10.1093/intimm/dxw025]
- 108 **Kitamura H**, Ohno Y, Toyoshima Y, Ohtake J, Homma S, Kawamura H, Takahashi N, Taketomi A. Interleukin-6/STAT3 signaling as a promising target to improve the efficacy of cancer immunotherapy. *Cancer Sci* 2017; **108**: 1947-1952 [PMID: 28749573 DOI: 10.1111/cas.13332]
- 109 **Velikova TV**, Miteva L, Stanilov N, Spassova Z, Stanilova SA. Interleukin-6 compared to the other Th17/Treg related cytokines in inflammatory bowel disease and colorectal cancer. *World J Gastroenterol* 2020; **26**: 1912-1925 [PMID: 32390702 DOI: 10.3748/wjg.v26.i16.1912]
- 110 **Llosa NJ**, Luber B, Tam AJ, Smith KN, Siegel N, Awan AH, Fan H, Oke T, Zhang J, Domingue J, Engle EL, Roberts CA, Bartlett BR, Aulakh LK, Thompson ED, Taube JM, Durham JN, Sears CL, Le DT, Diaz LA, Pardoll DM, Wang H, Anders RA, Housseau F. Intratumoral Adaptive Immunosuppression and Type 17 Immunity in Mismatch Repair Proficient Colorectal Tumors. *Clin Cancer Res* 2019; **25**: 5250-5259 [PMID: 31061070 DOI: 10.1158/1078-0432.CCR-19-0114]
- 111 **Yang Y**, Alderman C, Schlaoui A, Xiao Y, Wang W. MicroRNAs as Immunotherapy Targets for Treating Gastroenterological Cancers. *Can J Gastroenterol Hepatol* 2018; **2018**: 9740357 [PMID: 30046565 DOI: 10.1155/2018/9740357]
- 112 **Liao W**, Overman MJ, Boutin AT, Shang X, Zhao D, Dey P, Li J, Wang G, Lan Z, Li J, Tang M, Jiang S, Ma X, Chen P, Katkhuda R, Korphaisarn K, Chakravarti D, Chang A, Spring DJ, Chang Q, Zhang J, Maru DM, Maeda DY, Zebala JA, Kopetz S, Wang YA, DePinho RA. KRAS-IRF2 Axis Drives Immune Suppression and Immune Therapy Resistance in Colorectal Cancer. *Cancer Cell* 2019; **35**: 559-572. e7 [PMID: 30905761 DOI: 10.1016/j.ccell.2019.02.008]
- 113 **Rau TT**, Atreya R, Aust D, Baretton G, Eck M, Erlenbach-Wünsch K, Hartmann A, Lugli A, Stöhr R, Vieth M, Wirsing AM, Zlobec I, Katzenberger T. Inflammatory response in serrated precursor lesions of the colon classified according to WHO entities, clinical parameters and phenotype-genotype correlation. *J Pathol Clin Res* 2016; **2**: 113-124 [PMID: 27499921 DOI: 10.1002/cjp2.41]
- 114 **Acosta-Gonzalez G**, Ouseph M, Lombardo K, Lu S, Glickman J, Resnick MB. Immune environment in serrated lesions of the colon: intraepithelial lymphocyte density, PD-1, and PD-L1 expression correlate with serrated neoplasia pathway progression. *Hum Pathol* 2019; **83**: 115-123 [PMID: 30172913 DOI: 10.1016/j.humpath.2018.08.020]
- 115 **Maby P**, Tougeron D, Hamieh M, Mlecnik B, Kora H, Bindea G, Angell HK, Fredriksen T, Elie N, Fauquembergue E, Drouet A, Leprince J, Benichou J, Mauillon J, Le Pessot F, Sesboué R, Tuech JJ, Sabourin JC, Michel P, Frébourg T, Galon J, Latouche JB. Correlation between Density of CD8+ T-cell Infiltrate in Microsatellite Unstable Colorectal Cancers and Frameshift Mutations: A Rationale for Personalized Immunotherapy. *Cancer Res* 2015; **75**: 3446-3455 [PMID: 26060019 DOI: 10.1158/0008-5472.CAN-14-3051]
- 116 **García-Solano J**, Turpin MC, Torres-Moreno D, Huertas-López F, Tuomisto A, Mäkinen MJ, Conesa A, Conesa-Zamora P. Two histologically colorectal carcinomas subsets from the serrated pathway show

different methylome signatures and diagnostic biomarkers. *Clin Epigenetics* 2018; **10**: 141 [PMID: 30413173 DOI: 10.1186/s13148-018-0571-3]

- 117 **Nakanishi Y**, Diaz-Meco MT, Moscat J. Serrated Colorectal Cancer: The Road Less Travelled? *Trends Cancer* 2019; **5**: 742-754 [PMID: 31735291 DOI: 10.1016/j.trecan.2019.09.004]



Published by **Baishideng Publishing Group Inc**  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

**Help Desk:** <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

