

Serrated colorectal cancer: Molecular classification, prognosis, and response to chemotherapy

Oscar Murcia, Miriam Juárez, Eva Hernández-Illán, Cecilia Egoavil, Mar Giner-Calabuig, María Rodríguez-Soler, Rodrigo Jover

Oscar Murcia, Miriam Juárez, Eva Hernández-Illán, Cecilia Egoavil, Mar Giner-Calabuig, María Rodríguez-Soler, Rodrigo Jover, Unidad de Gastroenterología, Hospital General Universitario de Alicante, Instituto de Investigación Sanitaria Isabial, 03010 Alicante, Spain

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Correspondence to: Dr. Rodrigo Jover, Unidad de Gastroenterología, Hospital General Universitario de Alicante, Instituto de Investigación Sanitaria Isabial, 12 Pintor Baeza, 03010 Alicante, Spain. rodrigojover@gmail.com
Telephone: +34-965-933468
Fax: +34-965-933468

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Abstract

Molecular advances support the existence of an alternative pathway of colorectal carcinogenesis that is

based on the hypermethylation of specific DNA regions that silences tumor suppressor genes. This alternative pathway has been called the serrated pathway due to the serrated appearance of tumors in histological analysis. New classifications for colorectal cancer (CRC) were proposed recently based on genetic profiles that show four types of molecular alterations: *BRAF* gene mutations, *KRAS* gene mutations, microsatellite instability, and hypermethylation of CpG islands. This review summarizes what is known about the serrated pathway of CRC, including CRC molecular and clinical features, prognosis, and response to chemotherapy.

Key words: Colorectal cancer; Methylator phenotype; Serrated pathway; Chemotherapy; CIMP

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Core tip: Recently, the implication among colorectal cancers with methylator phenotype has burst into the gastroenterology literature. In this review, we analyze the correlation between serrated cancers, the methylator phenotype and other genetic features in order to assess their prognosis and response to adjuvant chemotherapy.

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INTRODUCTION

Colorectal cancer (CRC) is considered a major health issue: it is the most prevalent cancer and the second

GENETIC AND MOLECULAR FEATURES

The serrated pathway is complex and is currently poorly understood. This pathway has two key characteristics, namely aberrant hypermethylation of certain promoter regions in the genome and alterations in MAP kinase signaling pathway genes.

Aberrant hypermethylation

Functions related to the human genome are tightly regulated, and enzymatic systems control processes such as DNA mismatch repair, DNA transcription and replication. DNA transcription is the result of the regulation of genomic expression. Specifically, the transcription machinery interacts with the start codon ATG *via* DNA methyltransferases and forms methyl cytosine^[16]. In humans and other mammals, only cytosine residues that precede a guanosine in the DNA sequence are modified to form CpG dinucleotides. These can change the three-dimensional configuration of the DNA and consequently affect its interaction with transcription factors. Methylation of these CpG dinucleotides usually leads to genetic silencing. CpG dinucleotides are located throughout the DNA sequence, and approximately half of all human gene promoter sequences are embedded in these CpG clusters, which are termed CpG islands^[17]. The CpG island sequence is at least 200 bases long and is usually > 500 bases; the CG content is > 50%, and the ratio of observed-to-expected CpGs is > 60%^[18,19]. In the genomes of cells in healthy tissue, the CpG islands are usually unmethylated, especially those associated with gene promoters. Conversely, about 80% of the CpG dinucleotides that are not part of CpG islands (*i.e.*, that are in DNA non-coding regions) are heavily methylated to prevent the expression of viral sequences that are integrated into the genome as well as the expression of many other DNA elements^[20]. On the one hand, hypomethylation of these CpG dinucleotides could result in harmful gene expression; on the other hand, methylation of the promoter regions could have undesirable adverse effects.

Notably, some methylated promoters do not play any role in tumor development—these genes with methylated promoters are called “methylated in tumor” or MINT genes^[16]. However, other methylated CpG islands in promoter regions silence the expression of known tumor suppressor genes by interrupting the interactions between transcription factors and the start codon. When this happens, it is considered a new phenotype of CRC that is caused mainly by epigenetic alterations rather than by DNA mutations and is called the CpG island methylator phenotype or CIMP. This term was introduced in 1999 when it was first suggested that CRC could be initiated by genetic silencing in the absence of DNA sequence modifications^[21]. There are two types of CIMPs that are closely related and that are sometimes difficult to distinguish. Whereas CIMP-A is more related to

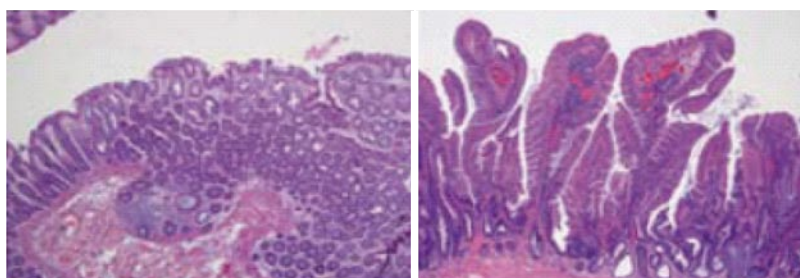
aging, CIMP-C is more related to cancer^[22,23]. In addition, some authors have suggested different ways to categorize CIMPs^[24]. Shen *et al.*^[25] defined three subgroups of CIMP CRCs: CIMP1, CIMP2, and CIMP-negative. KRAS mutations are reported to be the strongest predictor for CIMP2 (92%). Later, Ogino *et al.*^[26] proposed the use of 8 markers rather than 5 to classify methylation. They defined CRCs as CIMP-0 when none of the markers were methylated; as CIMP-low (CIMP-L) when 1 to 5 markers were methylated; and as CIMP high (CIMP-H) when 6-8 markers were methylated. The new cluster of methylated markers proposed by Weisenberger suggests that CIMP-L tumors might have high levels of methylation at other unknown loci rather than at a just a few loci. In fact, more CIMP CRCs were identified when the test panel of methylated markers was expanded^[7]. Although the Weisenberg panel of loci is currently used more often than other panels, it is not yet clear which markers are the most appropriate for establishing the CIMP status of tumors.

The prevalence of CIMP tumors varies in different types of serrated lesions. CIMP-H is present in around 41%-73.3% of MVHPs but only in about 8%-18.2% of GCHPs^[27]. The proportion of CIMP-H in evolved serrated lesions is similar: the proportion is about 44%-76.8% in TSAs and about 80% in SSAs^[28]. Nonetheless, low grade CIMP is more often associated with TSAs than with SSAs (Figures 2 and 3)^[13,27-32].

The MAPK pathway

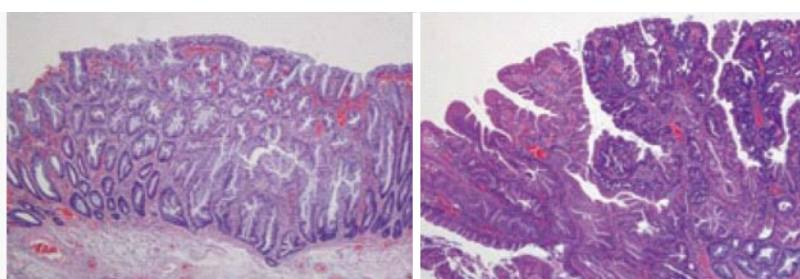
When the serrated pathway was first described, investigators considered it a unique and linear carcinogenesis pathway. However, the current view is that the early events in this pathway include both an alteration of the MAPK pathway plus concomitant DNA epigenetic alterations^[4]. The MAP kinase pathway is a *via* of intercellular signaling transmission. Mutations in their constituent proteins mainly involve the Raf and RAS families (Figure 4). There are 3 types of Raf kinases, termed types A, B, and C. B-Raf (or BRAF) is located on 7p34, is involved in serrated CRC, and is mutated in approximately 10% of CRC cases^[33]. A BRAF mutation is rare in CIN tumors, and its presence almost completely excludes a Lynch syndrome diagnosis^[34,35]. Therefore BRAF can be considered to be specific to CRCs arising *via* the serrated pathway^[7,33,35]. The BRAF V600E mutation, in which valine is substituted for glutamate at codon 600 on chromosome 7^[36], is the most common and the best characterized mutation. V600E leads to constitutive gene activation, thereby inducing cell proliferation and inhibiting apoptosis.

RAS mutations can also lead to dysfunctional MAPK pathway signaling. There are at least three RAS genes, namely H-RAS, N-RAS, and K-RAS or KRAS^[37]. The consequences of KRAS gene mutations are similar to those of BRAF mutations in that the mutations can induce proliferation and inhibit apoptosis. Classically,



Serrated polyp type	MVMP	GCHP
Morphology	Few larger polyps	Many smaller polyps
Preferential location	Right colon	Left colon
CIMP-H phenotype (%)	41-73	8-18
MSI (%)	No reported	8
BRAF mutation (%)	67-88	20-83
KRAS mutation (%)	6-17	8-73

Figure 2 Features of microvesicular hyperplastic polyps and goblet cell hyperplastic polyps. Microvesicular hyperplastic polyps (MVHPs) and goblet cell hyperplastic polyps (GCHPs) are premalignant lesions that differ mainly in their location and morphology. MVHPs are more likely to occur as a few large polyps in the ascendant colon, and GCHPs are more likely to occur as multiple small polyps in the left colon. Each can become senescent or evolve into another type of polyp. Although they can transform into other adenomas, MVHPs more often evolve into sessile serrated adenomas (SSAs), and GCHPs more often evolve into traditional serrated adenomas (TSAs). The prevalence of *KRAS* mutations is higher in TSAs, while *BRAF* mutations are more prevalent in SSAs.



Serrated polyp	SSA	TSA
Prevalence (%)	10-25	1-2
Preferential location	Right colon	Left colon
CIMP-H phenotype (%)	44-77	43-80
MSI (%)	Rarely observed	3
BRAF mutation (%)	32-83	60-76
KRAS mutation (%)	7-25	0-28

Figure 3 Features of sessile serrated adenoma and traditional serrated adenomas. Pathological analysis shows that sessile serrated adenoma (SSAs) are more common than traditional serrated adenomas (TSAs). While TSA is preferentially located on the left colon, SSA is preferentially located on the right side. In addition, both SSAs and TSAs are found in high-grade CIMP tumors, although several studies show a relationship between low-grade CIMP tumors and TSAs. *BRAF* mutations are often observed in SSA, whereas *KRAS* mutations are often observed in TSAs. Contrary to what is found in serrated adenocarcinomas, MSI is almost never present in serrated polyps.

RAS mutation has been linked to the CIN pathway, but RAS is also impaired in some serrated cancers. *KRAS* mutations are found in 30%-40% of CRCs^[38,39]. Activating *KRAS* mutations are most common (up to 80%) in codon 12 but are also found in codon 13; these include the G12D, G12V, and G13D *KRAS* point mutations^[40]. Notably, the combination of *KRAS* mutation plus low grade CIMP in many serrated lesions constitutes an alternative subset of CRC that is established *via* the serrated pathway. For example,

an increase in MGMT methylation rather than MLH1 methylation is associated with CIMP-L and *KRAS* mutations^[41,42].

Serrated pathway

In the serrated pathway, *BRAF* or *KRAS* mutation initially induces a burst of cellular proliferation in the normal colorectal epithelium, and serrated aberrant hyperplastic crypt foci seem to be the earliest histological lesions^[43]. Mutations in both genes,

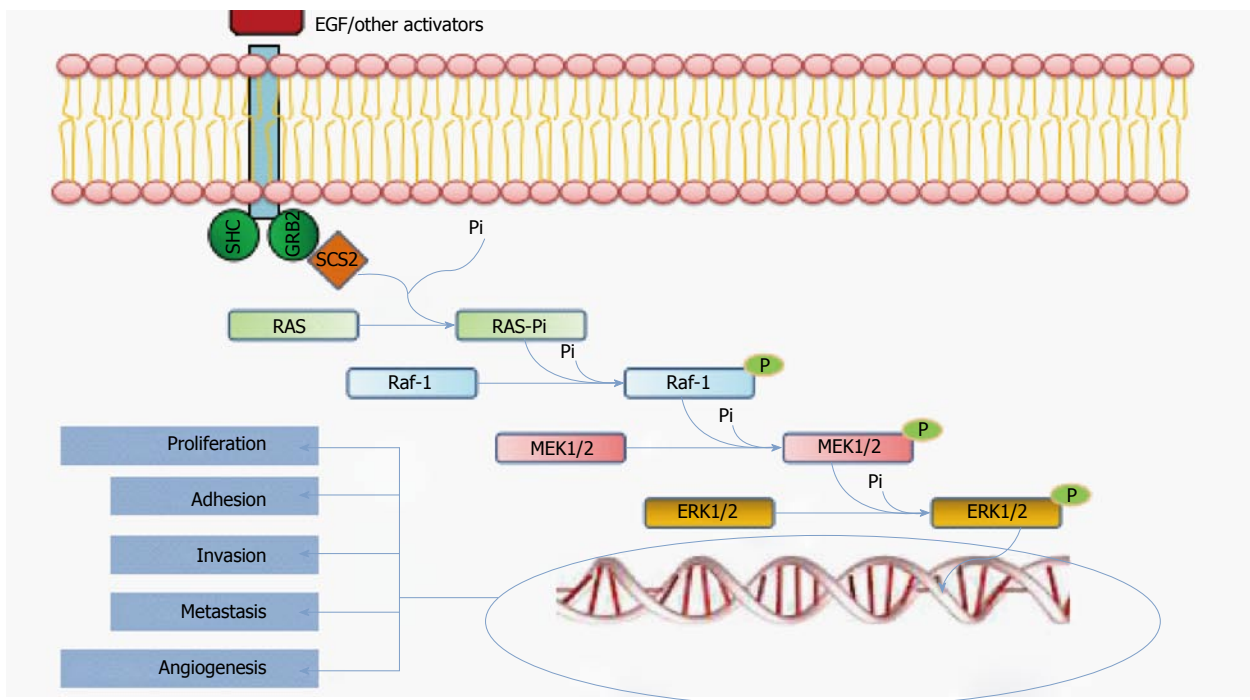


Figure 4 MAPK pathway. This simplified diagram of the MAPK pathway shows the RAS/Raf-1/MEK/ERK pathway. RAS mutations are found in 36% of serrated polyps, and Raf-1 mutations are found in 9%-11%. These mutations promote gene transcription and cellular growth that results in cellular adhesion, invasion, metastasis, and angiogenesis.

although more frequently mutations in *BRAF*, can lead to upregulation of p16INK4a and to an increase in insulin-like growth factor binding protein 7 (IGFBP7) secretion at aberrant crypt foci^[44]. p16INK4a and IGFBP7 are tumor suppressor proteins that prevent polyp growth and that drive these proliferative cells to form small senescent lesions. The silencing of these genes, for example by methylation, allows the cells to escape from cellular senescence and permits the progression to MVHPs or GCHPs and then to serrated polyps^[37]. Some mRNAs have also been linked to serrated carcinogenesis. One example is microRNA-31, located at 9p21.3, which seems to correlate with mutated *BRAF* tumors. MicroRNA-31 may be involved in the progression from HPs to SSAs, since it has been detected in a high proportion of these lesions as well as in the CRCs that evolved from them^[45,46].

Some genes are inhibited in the pathway to malignancy in CRC. Among them, the methylguanine methyltransferase gene, *MGMT*, and the *MLH1* gene are the best characterized. *MGMT* is frequently involved in the progression of TSA^[42]. While *MLH1* silencing is linked to SSA evolution. *MLH1* is one of the main genes in the MMR system, and its inactivation leads to an accumulation of mutations in microsatellite sequences. This results in an MSI phenotype in these tumors, which is a hallmark of CRC in Lynch syndrome. However, only 3% of all CRCs and 20% of MSI CRCs are due to Lynch syndrome^[18]. The remaining 80% of MSI CRCs are considered sporadic CRCs that are caused by the epigenetic inactivation of *MLH1*, which is present in approximately 15% of all CRCs^[47]. These

tumors follow the serrated pathway of carcinogenesis, especially when CIMP is present^[24,48]. In fact, most of the sporadic MSI CRCs are CIMP tumors, although only half of CIMP CRCs show methylation of the MMR genes (Figure 5)^[49,50]. “the natural course from serrated polyps to advanced cancers can be followed in this figure”.

CLINICAL FEATURES

The serrated pathway leads to a broad spectrum of CRCs. It is important to view each CRC as the result of both genetic and epigenetic alterations. In a review of the literature, serrated adenocarcinoma was frequently located on the cecum (52% of the time) and constituted around of 16% of all proximal CRCs^[51-53]. Serrated adenocarcinoma is more common in Caucasians than in Hispanics or African Americans^[54]. Some studies have suggested that there is an association between specific genetic profiles and certain clinical characteristics. Based on this premise, several studies have reported the clinical profiles of serrated adenocarcinoma cases^[8,9].

Some studies distinguish between serrated adenocarcinoma with vs without mutant *BRAF*. Serrated adenocarcinoma with mutant *BRAF* seems to be more frequent in older patients who are heavy smokers and in female patients^[55]. It is more prevalent on the right colon in larger tumors that are usually diagnosed at an advanced stage (either pT4 or N2)^[56,57]. In fact, in the right colon, there seems to be a close relationship between these features and the loss of expression of the *CDX2* gene and increased levels of annexin

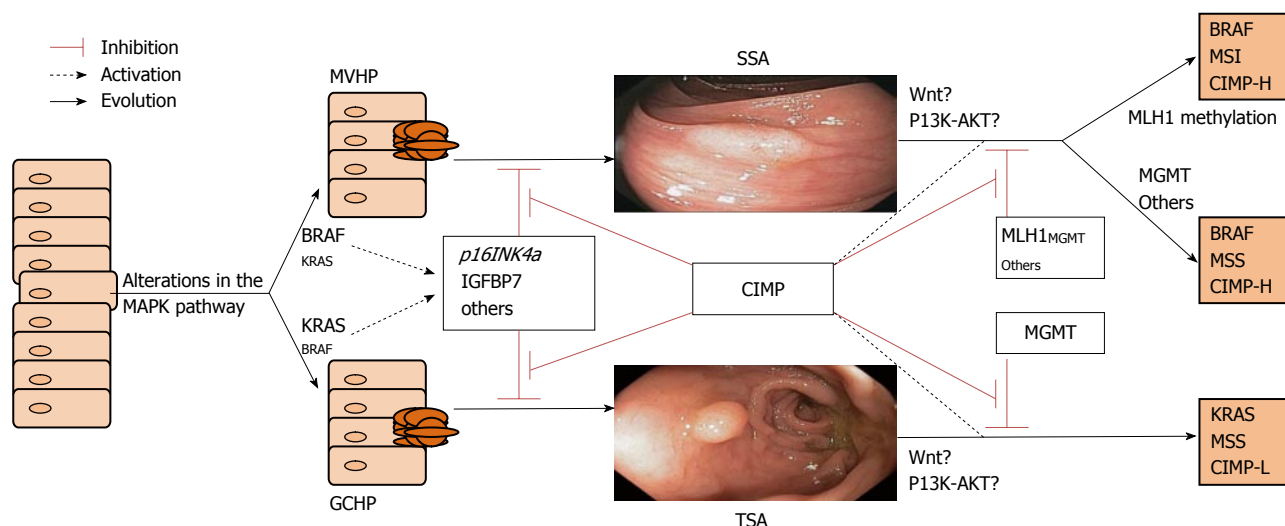


Figure 5 Serrated pathway. This diagram shows the chronological changes that occur during the development of neoplasia. *MAPK* mutations do not affect just one gene; rather, they affect some genes more than others. Gene methylation is successive rather than simultaneous and contributes to the progression of the serrated lesion. Depending on which genes are most affected, serrated cancers can be classified into three subtypes. GCHP: Goblet-cell hyperplastic polyp; MVHP: Microvesicular hyperplastic polyp; SSA: Sessile serrated adenoma; TSA: Traditional serrated adenoma.

A10^[58,59]. In addition, serrated adenocarcinoma cases show a low frequency of liver and lung involvement at the metastatic stage, but there is a higher prevalence of peritoneal involvement at the onset of metastasis^[55,60]. From a pathological viewpoint, *BRAF*-mutated serrated adenocarcinomas are more frequently high grade tumors than other serrated adenocarcinomas^[61].

Mutated *KRAS* tumors, represent a subset of CRCs that arise *via* the serrated pathway. Clinically, these tumors are more variable than tumors with mutated *BRAF*, probably due to the difficulties of differentiating mutated *KRAS* tumors that arise *via* the serrated pathway from those that arise *via* the CIN pathway. *KRAS* mutated serrated tumors are not linked with proximal location, and they are associated with higher body weight, higher body fat percentage, and with female sex^[62]. Although one study found a higher prevalence in men^[63]. *KRAS*-mutated serrated adenocarcinomas frequently show both low grade tumor differentiation and low grade CIMP.

The clinical features of CIMP and MSI tumors frequently overlap. CIMP tumors with MSS have not been described in great detail, but one study showed that they are associated with proximal CRC location, female sex, and the presence of lymph node metastasis and that they tend to present with liver metastasis (Figure 6)^[64].

PROGNOSIS

Until now, the prognosis and management of CRCs has been based on the TNM staging system. However, the genetic and molecular profiles of the different CRC types should be used to improve the classification and management of these tumors. Few studies

have addressed the question of the prognoses of the different CRC subtypes.

The *BRAF* mutation was the first biomarker to be investigated. It has been described as a marker of poor survival, due to its association with remodeling of the extracellular matrix, a process that is important for tumoral invasion and metastasis. Notably, its prognostic role was investigated in a meta-analysis of 26 CRC studies^[65]. In general, the presence of *BRAF* mutation is an independent marker of a poor prognosis and is related to a decrease in 5-year disease-free survival (DFS)^[66]. Its predictive value remains after stratification by age, disease stage, and degree of differentiation. However, other studies found that mutant *BRAF* does not affect the intrinsically good prognosis of MSI CRC. For example, Popovici *et al.*^[67] found *BRAF* to be a marker of poor prognosis only in subpopulations with microsatellite-stable left-sided tumors. Similarly, others report worse prognosis in MSS cancers in men but not in women^[68]. As noted above, there seems to be a strong relationship between the CIMP status and *BRAF* mutations, with the prognostic value of CIMP status being related to the presence of *BRAF* mutations and, less frequently, to the presence of *KRAS* mutations^[69]. Finally, regulation of *SOX2* gene expression and its role as a biomarker of poor survival has been linked to tumors with *BRAF* mutations. This may be related to cellular migration and invasion, since an increase in *SOX2* expression also correlates with CRC liver metastasis^[70].

In contrast to *BRAF*, the MSI status is closely related to good prognosis in CRC, including in serrated tumors. The predictive power of MSI is independent of CIMP, *BRAF*, and *KRAS* status. However, it is clear that MSS tumors are associated with better DFS at any stage when no CIMP, *KRAS*, or *BRAF* mutations

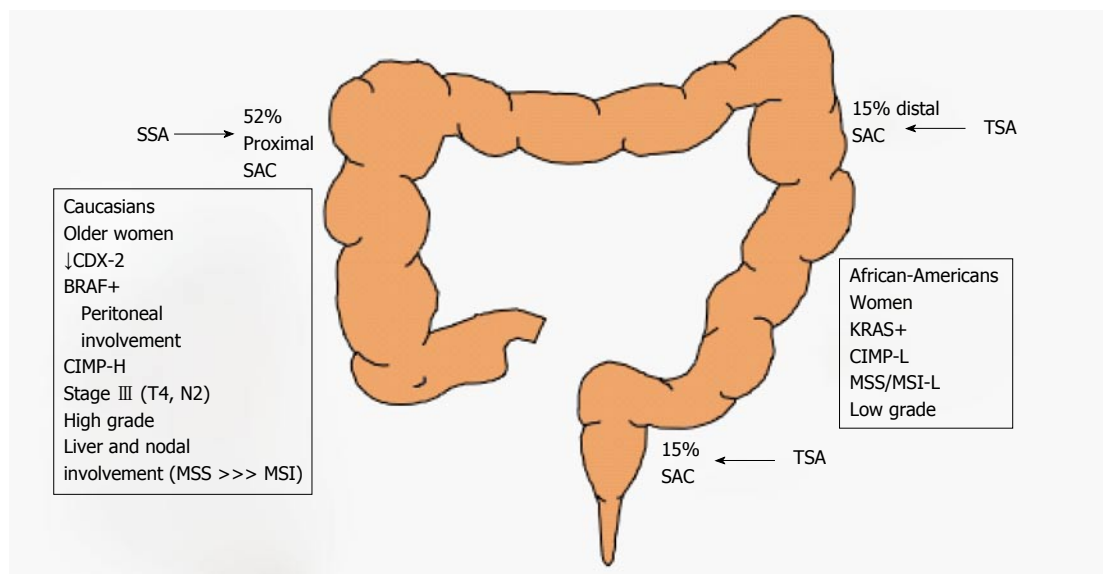


Figure 6 Polyp features according to location. Half of serrated adenocarcinomas (SACs) are located on the right colon. KRAS-mutated polyps are preferentially left-sided. However, polyps with BRAF mutations dominate those found on the right side of the colon. TSA: Traditional serrated adenoma; SSA: Sessile serrated adenoma.

are found; in such cases, DFS is similar to that in familial or sporadic MSI-CRC^[9]. Some clinical trials have reported poor outcomes in patients with CIMP-H MSS CRC. Comparing the two MMR states, stage II and III CRC with MSI has a better prognosis than MSS CRC^[47]. Moreover, combined MSS and CIMP CRC with mutant BRAF or KRAS frequently correlates with liver metastasis at diagnosis and has the worst prognosis of all CRCs^[8,9,64]. The exactly role of CIMP status in metastatic stage has not been elucidated. It's possible that the worst prognosis of those tumors could be due to the presence of BRAS mutations as well.

The prognostic value of KRAS mutations has also been investigated. KRAS mutation is proposed to be a predictor of lower 5-year DFS in almost all studies, without any prognostic differences between right- and left-sided CRCs. However, some point mutations have been described that impact prognosis. For example, a KRAS codon 13 mutant is associated with more aggressive CRC^[68].

The prognostic value of CIMP status remains controversial. There are few doubts about the relatively good prognosis of sporadic CIMP-H MSI CRC. But for sporadic MSS CRC, some studies show no effect of CIMP-H on survival prediction, while others show either a negative or a positive impact^[70]. Ogino *et al.*^[71] described CIMP-H as a specific marker of low CRC mortality, and it appeared to counter the adverse prognostic effect of BRAF mutation in serrated cancers. Perhaps, the hypermethylation of certain genes avoided the proliferative effect of BRAF mutations. One clinical trial found it to be an independent predictor of cancer survival^[72]. Despite this finding, many researchers consider CIMP a predictor of short survival, especially in proximal stage III CRCs^[69,73]. These authors discussed some possible reasons for

these controversial findings, namely differences in cohorts, the use of different panels of methylated markers, the use of different criteria to define CIMP, and confounding factors such as BRAF mutation^[71]. In a recent meta-analysis of CIMP prognostic value, both DFS and overall survival (OS) were shorter in patients with CIMP CRC after adjusting for age, sex, disease stage, and treatment; it was not possible to adjust for BRAF or KRAS mutation or MMR status since not all the studies assessed these possible confounders^[70]. It seems likely that when a CIMP serrated tumor shows MSI, the MSI acts to confer a good prognosis^[71].

Recent studies have revealed a link between some methylated genes and patient prognosis. MLH1 and MGMT methylation have been suggested to be predictors of good prognosis, in part due to the frequent absence of BRAF mutations in these subgroups of tumors. In contrast, hypermethylation of the p14 (CDKN2A^{INK4a}), RASSF1A, and p16 (CDKN2A^{ARF}) genes have been suggested to predict poorer prognosis independent of both stage and histological differentiation grade^[74-76]. Aberrant methylation of genes encoding proteins involved in extracellular matrix remodeling also confers a worse prognosis, as does hypomethylation of *LINE-1* in sporadic MSI CRC^[77]. Hypomethylation of the *IGF-2* gene and RAC1b overexpression are proposed markers of poor prognosis in KRAS/BRAF wild-type metastatic CRCs treated with FOLFOX as first-line therapy^[78]. Finally, a mutated *P1K3CA* gene is associated with a worse survival rate in CRC with wild-type BRAF. Alteration of P1K3CA is found in 10%-20% of cases with CIMP-H. It is currently unknown whether P1K3CA mutation defines a new subset of CRC^[79].

Genetic variability can strongly influence patient outcome, highlighting the need for a new or more fine-tuned CRC classification system. Recently, two

Table 1 Proposed^[80] colorectal cancer classification

	MMR	CIMP	BRAF	KRAS
Phipps				
Subtype 1	MSI-H	+	+	-
Subtype 2	MSS or MSI-L	+	+	-
Subtype 3	MSS or MSI-L	-	-	+
Subtype 4	MSS or MSI-L	-	-	-
Subtype 5	MSI-H	-	-	-
Sinicrope				
Subtype 1	MSI	+	+	+/-
Subtype 2	MSS	+/-	+	-
Subtype 3	MSS	+/-	-	+
Subtype 4	MSS	+/-	-	-
Subtype 5	MSI	-	-	+/-

Based on the classification system proposed by Jass^[80], Phipps *et al.*^[81] and Sinicrope *et al.*^[9] proposed new colorectal cancer (CRC) divisions to better stratify disease based on prognosis. (+): Mutated; (-): Wild-type.

publications established 5 subtypes of CRC and described differences in survival according to the subtypes' phenotypic characteristics. First, based on the classification system of Jass^[80], Phipps *et al.*^[81] divided CRCs into MSI-H and MSS/MSI-L CRC. Later, this group further divided cases according to CIMP status and then by BRAF and KRAS mutation status. The final system describes 5 well-defined CRC subtypes (Table 1). Among the subtypes, the highest survival was found for subtype 5, which is characterized by non-CIMP MSI CRC with wild-type BRAF and KRAS; this is also known as familial MSI CRC or Lynch syndrome. Conversely, the worst prognosis was found for subtype 2, which is CIMP-positive MSS/MSI-L CRC with BRAF mutations and wild-type KRAS. Subtype 2 has the lowest probability of being diagnosed as stage I disease and shows the worst 5-year DFS. Interestingly, subtypes 1 and 2 are very similar, with the MSI status of subtype 1 being the only difference between them.

Sinicrope *et al.*^[9] proposed a similar classification system based on patients with stage III CRC. This group also divided CRC into 5 subgroups, each of which was compared to subtype 4 (MSS CRC with wild-type BRAF and KRAS, representing the classic CIN pathway). The subtypes with MSI have the best prognosis, followed by subtype 5 (which is identical to the Phipps subtype 5) and subtype 1. Regardless of BRAF mutation status, MSI confers relatively good DFS in subtype 1 CRC. In contrast, subtypes 2 and 3 do not differ in terms of prognosis, but their prognosis differs from that of subtype 4. As in Phipps' classification, subtype 2 (CIMP-H MSS CRC with wild-type KRAS and mutant BRAF) shows the worst survival rate. KRAS mutation alone, with no other apparent genetic alterations, is the major alteration in subtype 3, representing an alternative subset of CRC that arises *via* the serrated pathway. Its prognosis is 1.5-times worse than that of subtype 4 (Table 1).

These findings illustrate the importance of classification based on genetic profiling and could improve

Table 2 Summary of the treatment implications of genetic alterations in colorectal cancer

Molecular marker	Implications for CRC treatment
KRAS mutation	Broadly studied in metastatic CRC
	The most predictive biomarker for no response to anti-EGFR, either alone or with CT Worse OS when oxaliplatin is the first-line treatment Irinotecan efficacy is controversial; it may have better effects in stage II and III CRC
BRAF mutation	Overall, no predictive power for CT response
	No predictive power for response to 5-FU plus irinotecan/oxaliplatin or to 5-FU alone in stage II disease A trend toward better survival with 5-FU plus irinotecan in stage III disease Effects of anti-EGFR therapy are controversial, although most studies show a poor response Some studies show no differences in OS/DFS with FOLFOX-panitumumab or with FOLFIRI-cetuximab treatment Resistance to BRAF inhibitors
CIMP	CT results are controversial
	5-FU improves DFS and OS in some studies; in others, survival is reduced One study shows the benefits of 5-FU plus irinotecan in CIMP tumors after stratification by MMR status. CIMP was more strongly associated than MMR status with a better response to irinotecan The use of 5-FU in CIMP tumors is not currently recommended To date, no clinical trials have evaluated the response of CIMP tumors to anti-EGFR therapy
MMR	Prognosis is intrinsically better for MSI CRC, but MSS tumors show a better response to CT
	5-FU improves both DFS and OS in stage II and III MSS CRC but not in MSI CRC CT should only be given for stage II MMS tumors if a high risk factor such as T4 local extension is present MSI CRC shows a good response to irinotecan if BAX expression has been lost

CT: Chemotherapy; DFS: Disease-free survival; anti-EGFR: Epidermal growth factor receptor antibodies; OS: Overall survival; CRC: Colorectal cancer; MMR: Mismatch repair.

our understanding of patient prognosis and disease management.

RESPONSE TO CHEMOTHERAPY

The current trend is towards offering treatment that is individualized according to CRC subtype. The most recent advances in CRC are leading to novel approaches and classification schemes, and it is attractive to think that a particular therapy can be used to target a particular type of CRC, including serrated tumors. Unfortunately, CT is not yet fully guided by the combination of genetic alterations in a CRC, and even the most recent studies have not assessed specific therapeutic management strategies for each subtype. Additional clinical trials are needed to address this. As of now, decisions must be based on studies that evaluated the responses according to individual markers (Table 2).

KRAS mutations have mainly been studied in

metastatic CRCs. In the QUASAR study, KRAS was not a negative predictive factor for the response to standard CT based on 5-Fluorouracil (5-FU) and folinic acid^[81]. However, the major characteristic of mutant KRAS remains its resistance to anti-EGFR monoclonal antibodies: it is the most important predictive marker in terms of the response to panitumumab and cetuximab, either alone or in combination with CT. This finding, which was confirmed in a meta-analysis^[82], is valid for CRC with any inactivating KRAS mutation. It is also possible that the presence of KRAS mutations plus other alterations in NRAS, exons 3 and 4, PIK3CA, PTEN, and BRAF may influence CT resistance. In fact, Tian *et al.*^[83] found that a significant response to anti-EGFR was possible when KRAS, BRAF, and PIK3CA were all wild-type, but not when any one of them was mutated. It is currently standard practice to investigate KRAS status in order to predict the response to anti-EGFR in metastatic CRC.

BRAF mutation has also been widely studied, but its role as a predictive marker remains unclear. BRAF has repeatedly been described as a non-predictive biomarker in CRC treated using standard CT. When CRCs with ^{V600E}BRAF were treated with 5-FU plus irinotecan or oxaliplatin, BRAF did not correlate with either a positive or negative response^[84]. Nonetheless, there was a slightly non-significant trend toward better survival in stage III CRC when irinotecan was added to a 5-FU/leucovorin regimen^[85]. Notably, regarding the response to other drugs, both a null predictive value and a poor response to anti-EGFR have been reported for CRC with mutant BRAF^[65]. BRAF did not show predictive power in the OPUS or CRYSTAL trials, which used FOLFIRI ± cetuximab^[86,87]. The PRIME study reported similar results with FOLFOX4 ± panitumumab treatment^[88]. Regarding the poor response, Di Nicolantonio *et al.*^[89] found that KRAS-/BRAF+ CRC showed a 0% response rate to anti-EGFR in second or subsequent lines of treatment; in contrast, the response rate was 32% in KRAS/BRAF CRC. Loupakis *et al.*^[90] reported a similar response rate with irinotecan plus cetuximab. A meta-analysis that included these last two studies as well as other studies comparing metastatic CRC with mutated vs wild-type BRAF concluded that the response to anti-EGFR could be considered poor in tumors with mutant BRAF^[91]. However, other studies did not find differences in the responses of CRCs with BRAF mutation vs wild-type BRAF after treatment with cetuximab, capecitabine, oxaliplatin, and bevacizumab^[92,93]. Thus, to summarize, the use of BRAF mutation status as a predictive marker of the response to EGFR-targeted treatment remains controversial.

BRAF inhibitors such as vemurafenib and dabrafenib show good efficacy in melanoma. Silencing of ^{V600E}BRAF correlates with an increase in epithelial differentiation, in CDX-2 and claudin-1 expression^[94,95], and in cellular adhesion. However, experiments in cell lines demonstrate that CRCs are intrinsically resistant

to BRAF inhibition. This resistance may be dependent or independent of the ERK MAPK signaling pathway. Activating PIK3CA and AKT1 mutations and the loss of PTEN function have been proposed as possible mechanisms that underlie this resistance^[82,96,97]. CRAF may also be involved. Recently, better growth inhibition was observed when BRAF inhibitors were used in combination with anti-EGFR monoclonal antibodies. Treatment with an anti-EGFR agent (like cetuximab) or a tyrosine-kinase inhibitor (such as erlotinib or gefitinib) plus a BRAF inhibitor results in sustained MAPK pathway suppression^[98,99]. Different combinations of vemurafenib, erlotinib, capecitabine and/or bevacizumab, and cetuximab and/or irinotecan have shown better responses than vemurafenib alone^[100]. Moreover, using vemurafenib plus anti-EGFR could improve clinical efficacy^[101]. In fact, a pilot trial with 15 patients is ongoing, and the results look promising^[102]. Simultaneous inhibition of the BRAF and PIK3 cascades shows a trend towards tumor regression in both mouse and human cell cultures. Ongoing studies are assessing triple combination therapy (*i.e.*, inhibition of BRAF, EGFR, and PIK3CA).

A different question regarding serrated CRCs concerns the response of CIMP tumors to the different CT options. To date, several studies have reported conflicting results. The hypothesis that CIMP cancers inhibit gamma-glutamyl hydrolase, thus enhancing intracellular folate levels and modulating a better response to 5-FU, suggested that these CRCs would benefit from adjuvant CT regimens^[103-107]. Juo *et al.*^[70] published the first meta-analysis to address this matter. The meta-analysis identified 7 studies that evaluated the response of serrated CRC to CT as adjuvant therapy after surgical resection. Some of the studies described the benefits of 5-FU in both stage II and III CRC. Van Rijnsoever *et al.*^[108] found higher DFS in patients treated with 5-FU compared with patients that received surgical resection alone, as did Donada *et al.*^[109] who looked at stage II CRC. However, Jover *et al.*^[110] described a significant response to 5-FU in terms of DFS in stage II and III non-CIMP CRCs but not in CIMP CRCs. Two studies found that CIMP status had non-significant predictive value in terms of 5-FU treatment. Kim *et al.*^[111] compared a FOLFIRI regimen in non-CIMP vs CIMP metastatic CRC, while Han *et al.*^[112] investigated patients with stage II and III disease treated with a FOLFOX combination. A total of 4 studies reported that CT was beneficial in these patients, whereas 2 found non-significant results and 1 concluded the opposite. In a study that was not part of the meta-analysis, Wang *et al.*^[113] also found worse DFS rates when 5-FU was given to CIMP-positive tumors, in agreement with the findings of Jover's study. Shiovitz *et al.*^[114] also analyzed OS with irinotecan therapy. They compared 5-FU plus leucovorin with and without irinotecan in stage III CRCs and found a non-significant trend towards better survival with the

triple combination. In addition, patients with CIMP tumors seemed to benefit more after the tumors were stratified according to MSI status. It could be due to the better prognosis associated to unstable tumors, but further explanations regarding this are needed. Moreover, in this study, CIMP status was more strongly associated with the response to irinotecan than was MMR status.

These conflicting results may be due in part to differences in definitions, gene panels, CIMP marker thresholds, and laboratory techniques used to assess CIMP status. Currently, the National Comprehensive Cancer Network (NCCN) treatment guidelines for colon cancer (version 3) do not recommend the use of CT for stage II (T2N0M0) CIMP cancers^[115]. Because of these controversial results, the predictive value of CIMP status regarding treatment with 5-FU remains unclear, and more randomized clinical trials are needed.

Finally, the role of MMR status in predicting the response to CT has been studied extensively^[116,117]. Several studies have established that MSI tumors have an intrinsically better prognosis than MSS tumors. Jover *et al.*^[118] analyzed OS and DFS in a cohort of 505 patients with stage II or III CRC according to their MMR status after receiving 5-FU. MSS CRCs showed improved DFS and OS, but no improvement was seen in MSI CRCs^[102,119]. These differences remained after multivariate analysis controlled for the TNM stage, age, and sex. A prospective study by Sargent *et al.*^[119] had similar findings and suggested that cancers with MSI do not benefit from 5-FU adjuvant CT. However, the decision to treat stage II MSS or MSI-L cancers should be based on high risk factors such as T4 tumor status, perforation, and obstruction, among others. The response to irinotecan was also assessed in MSI CRC. There was a better response when the tumor had lost BAX expression, and the author proposed that this was by far the best criterion for predicting efficacy^[120]. Current management may include the addition of irinotecan or oxaliplatin, but more studies are needed to evaluate different combinations of CT for MSI CRC.

FUTURE DIRECTIONS

This review enhances the current change of mind about CRC. The concept of a unique model of carcinogenesis is obsolete and the consideration of pathogenesis of CRC as only three possible pathways is changing. In the recent years, a lot of publications about several aspects, from molecular and genetic discoveries to pathologic classifications, have seen the light. Current trends seem to guide to new approaches of CRC in many aspects. Great disparity in clinical trials in prognosis and response to treatment has taken place among last years. In fact, it seems to have more than twenty-five genetic alterations to date, and increasingly. Some authors have understood the problem and are making new classifications basing on prognostic implications. Even, it's possible that

CRCs following the serrated pathway are divided into more genotypes in the near future. The complete understanding of molecular pathways is the first necessary step.

Many efforts are necessary to get many consensus, like about the definition and method of assessing CIMP phenotype and the possibility of treating CRCs with mutant BRAF when no lymph nodes are found. Randomized clinical trials evaluating response to different CTs, drugs as EGFR-ab and other biologic treatments should take place to clarify their paper on serrated cancers. After that, assessing response to each subtype, it shall be possible to establish a more individualized prognosis and therapy to each patient.

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