Review Article

Genetic and epigenetic changes in aberrant crypt foci and serrated polyps

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Aberrant crypt foci (ACF) in colorectal mucosa are the earliest known morphological precursors to colorectal cancer and can be subclassified as dysplastic, heteroplastic (non-dysplastic), and mixed types. Serrated adenoma (SA) is a polyp with serrated architecture and dysplasia, and can be subclassified as traditional SA or sessile SA. Sessile SA is thought to be preneoplastic and differs from most lesions in the traditional SA category because of their flat morphology and general lack of cytological dysplasia. Serrated polyps include hyperplastic polyps (HP), SA, and admixed hyperplastic-adenomatous polyps and are considered a morphological continuum encompassing heteroplastic ACF, HP, admixed hyperplastic-adenomatous polyps, and SA. Recent studies have uncovered other developmental pathways including a heteroplastic ACF-HP/SA-carcinoma sequence and a heteroplastic ACF–adenoma–carcinoma sequence. Heteroplastic ACF histopathologically resemble HP and SA. Sporadic HP are usually present in the left colon, are small, and are considered benign. However, adenocarcinoma arising in the setting of colorectal HP or SA, especially in patients with hyperplastic polyposis, has been described. The relationship between heteroplastic ACF, HP, and colorectal cancer is less certain than that of dysplastic ACF. Here, we discuss the current understanding of genetic and epigenetic alterations in the development of colorectal cancer. Our goal is to provide a conceptual framework for understanding the heteroplastic ACF–HP/SA–carcinoma sequence. (*Cancer Sci* 2008; 99: 1071–1076)

Precursor lesions for colorectal cancer

In general, colorectal polyps are classified as adenomas or hyperplastic polyps (HP).[1] Most colorectal cancers (CRC) develop from adenomatous polyps (tubular adenoma) and show morphological and genetic progression through an adenoma–carcinoma sequence, even in hereditary colorectal cancer syndromes.[2–4]

Sporadic HP are usually present in the left colon, are small, and are generally regarded as harmless lesions with no potential for malignancy.[5,6] However, adenocarcinoma arising in the setting of colorectal HP or serrated adenomas (SA), especially in patients with hyperplastic polyposis (HPP), which is characterized by the presence of numerous HP or large HP, have been described.[7–11] Indeed, patients with HPP have an increased risk of CRC.[11–18] Serrated polyps (SP) include HP, SA, and admixed hyperplastic-adenomatous polyps (AHAP),[19,20] and are considered a morphological continuum encompassing non-dysplastic (heteroplastic) aberrant crypt foci (ACF), HP, AHAP, and SA.[21] SA are composed of dysplastic epithelium but with the sawtooth configuration that is typical of HP.[20] SA are subclassified as traditional SA and sessile SA.[22,23] Sessile SA is thought to be preneoplastic and differs from most lesions in the traditional SA category because of its flat morphology and general lack of cytological dysplasia.[22,23] The incidence of SA is reported to be 0.8–4.9% of colorectal tumors.[24,25] In addition, the incidence of carcinoma in SA is reported to be 1.5–19.2%, which is equal to or lower than the incidence in tubular adenoma.[24]

Recent studies have proposed a heteroplastic ACF–HP/SA–carcinoma sequence as an alternative pathway to the typical adenoma–carcinoma sequence.[8–11,21] In addition, another pathway, a heteroplastic ACF–adenoma–carcinoma sequence, has also been proposed.[26,27] In some pathways in colorectal tumorigenesis, genetic and epigenetic alterations have been well postulated.[28–30] However, genetic and epigenetic changes related to morphological progression, including the heteroplastic ACF–HP/SA–carcinoma sequence and the heteroplastic ACF–adenoma–carcinoma sequence have not been well characterized because of the complexity of the genetic and epigenetic events underlying these pathways. In the present review, we discuss the current knowledge regarding genetic and epigenetic alterations in the development of CRC. The goal is to provide a conceptual framework to understand the heteroplastic ACF–HP/SA–carcinoma sequence.

Molecular pathways

In the adenoma–carcinoma sequence, a series of tumor-suppressor genes on chromosomes 5q (*APC*), 17p (*p53*), and 18q (*DCC*, *SMAD2*, and *DPC4*/*SMAD4*) are inactivated by mutations and chromosomal deletions.[2,31,32] Deletions of chromosome 1p are found in early as well as advanced stages of colorectal tumorigenesis.[33–35] Frequent activating mutations in the *K-ras* oncogene are thought to arise in large preneoplastic adenomas.[2] *BRAF* mutations tend to occur in a mutually exclusive relationship with *K-ras* mutations.[19,36–38] *BRAF* mutations have been found in HP, SA (including some from patients with HPP) and colorectal carcinomas.[39–41]

A subset of CRC show a characteristic mutator phenotype that leads to microsatellite instability (MSI) and mutations of other genes, such as *TGFβRII*[42] and *BAX*.[43] This mutator phenotype usually results from inactivation of mismatch repair genes such as *hMSH2* and *hMLH1*.[44–47] A subset of familial CRC (hereditary non-polyposis CRC) result from germline mutations in the mismatch repair genes.[2,47] However, approximately 15–20% of non-familial (sporadic) CRC have MSI because of epigenetic inactivation of *hMLH1*.[48–50]

Aberrant methylation of CpG islands occurs in genetic diseases such as fragile-X syndrome,[51] in aging cells,[52] and in neoplasia.[53,54] Examples of this process in CRC include inactivation of the cell cycle regulator *p16*,[55] the DNA repair gene *MGMT*,[56] the mismatch repair gene *hMLH1*,[57] and *APC*.[58] A distinct pathway for colorectal tumorigenesis has been described, termed CpG

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island methylator phenotype (CIMP).[29] Tumors with this phenotype are characterized by a high degree of concordant CpG island methylation, which affects most of the genes known to be methylated in this tumor type.[29]

Given that genetic (mutations) and epigenetic (CIMP) changes are common in CRC, both could arise independently of each other, such that CRC might contain an accumulation of genetic and epigenetic changes that occurred randomly and were selected for stochastically during progression. Alternatively, epigenetic changes may mark a distinct group of tumors that have a distinct etiology and molecular profile.

Dysplastic ACF

A dysplastic ACF–adenoma–carcinoma sequence is well recognized.[59] ACF in colorectal mucosa are the earliest known morphological precursors to CRC.[26,60–64] The histopathology of human ACF varies but can be subclassified as dysplastic, heteroplastic (non-dysplastic), and mixed type.[59,60] Dysplastic ACF resemble adenomas and are more common in familial adenomatous polyposis (FAP), which is the result of germline mutation of the *APC* gene, than in patients with sporadic colorectal neoplasia (Table 1). In addition to dysplasia, these ACF are characterized by abnormal epithelial proliferation in the upper aspects of the crypts, lack of methylation and mutations of *K-ras*, and the presence of *APC* mutations in dysplastic ACF from FAP patients but not patients with sporadic CRC (Fig. 1; Table 1).[19,26,59,64] As shown in Table 1, mutation of *APC* was found in 8/8 (100%) dysplastic ACF from FAP but in only 5/28 (18%) and 0/5 (0%) dysplastic ACF from sporadic CRC. In contrast, mutation of *K-ras* was observed predominantly in dysplastic ACF from sporadic CRC compared with dysplastic ACF from FAP (2/4 [50%] and 18/28 [64%] vs 0/24 [0%]; Table 1). Although CpG island methylation of the *MINT1*, *MINT2*, *MINT31*, *p16*, *MGMT*, and *hMLH1* loci tended to be more frequent in dysplastic ACF from sporadic CRC than in dysplastic ACF from FAP (3/4 [75%] vs 2/24 [8%]; Table 1), further analyses are needed because the number of dysplastic ACF from sporadic CRC analyzed was too small.

Heteroplastic (non-dysplastic) ACF

Dysplastic ACF are recognized precursors of CRC, but the relationship of heteroplastic ACF to CRC is less certain.[26,60–64] The possibility that heteroplastic ACF are precursors of a subset of CRC is supported by several lines of evidence: (1) heteroplastic ACF are clonal;[65] (2) genetic alterations that are common in CRC, such as *K-ras* mutation,[26,60,61,63,64] chromosome 1p loss,[59] and CpG island methylation,[59] are present in heteroplastic ACF; (3) ACF, adenomas, and carcinomas share similar incidences and anatomical distributions;[66–68] and (4) ACF with mixed heteroplastic and dysplastic components have been described.[69] These findings have suggested the presence of a heteroplastic ACF–adenoma–carcinoma sequence in which *K-ras* mutation precedes *APC* mutation.[26,27]

An alternative pathway for colorectal carcinogenesis with a heteroplastic ACF–HP/SA–carcinoma sequence has also been proposed.[8–11,21] Because heteroplastic ACF resemble HP histopathologically,[26,60,64] heteroplastic ACF may be precursors of serrated lesions that progress to CRC. Chan *et al*. reported that CpG island methylation of the *MINT1*, *MINT2*, *MINT31*, *p16*, *MGMT*, and *hMLH1* loci is present in approximately half of the heteroplastic ACF in patients with sporadic CRC (Fig. 2; Table 2), whereas CpG island methylation is rare in dysplastic ACF associated with FAP (Table 1).[59] Thus, CpG island methylation may be an early event in colorectal carcinogenesis via a heteroplastic ACF–HP/SA–carcinoma sequence.

Interestingly, serrated hyperplastic ACF has a higher frequency of *BRAF* mutations than non-serrated hyperplastic ACF (Table 2).[70] In contrast, the frequency of *K-ras* mutations was

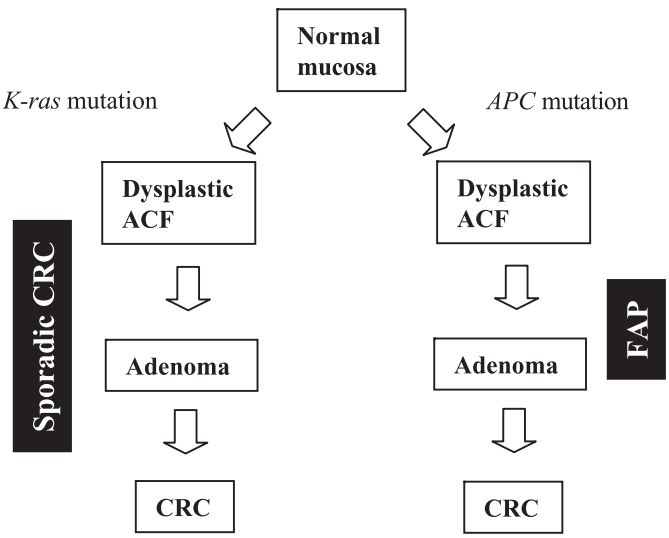

Fig. 1. Schematic diagram of the dysplastic aberrant crypt foci (ACF)–adenoma–carcinoma sequence. CRC, colorectal cancer; FAP, familial adenomatous polyposis.

Table 1. Reported frequencies of genetic and epigenetic alterations in dysplastic aberrant crypt foci (ACF)

Alteration	Dysplastic ACF		Author
	From FAP (%)	From sporadic CRC (%)	
APC mutation		18 (5/28)	Otori *et al*.[64]
	100 (8/8)	0 (0/5)	Takayama *et al*.[26]
K-ras mutation	0 (0/24)	50 (2/4)	Chan *et al*.[59]
		64 (18/28)	Otori *et al*.[64]
	13 (1/8)	64 (7/11)	Takayama *et al*.[26]
BRAF mutation	0 (0/21)	0 (0/3)	Beach *et al*.[19]
Chromosome 1p loss	0 (0/24)	0 (0/3)	Chan *et al*.[59]
MGMT methylation	5 (1/20)	25 (1/4)	Chan *et al*.[59]
CIM	8 (2/24)	75 (3/4)	Chan *et al*.[59]
CIMP-high	4 (1/24)	25 (1/4)	Chan *et al*.[59]

CIM, CpG island methylation of *MINT1*, *MINT2*, *MINT31*, *p16*, *MGMT* and/or *hMLH1*; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; FAP, familial adenomatous polyposis.

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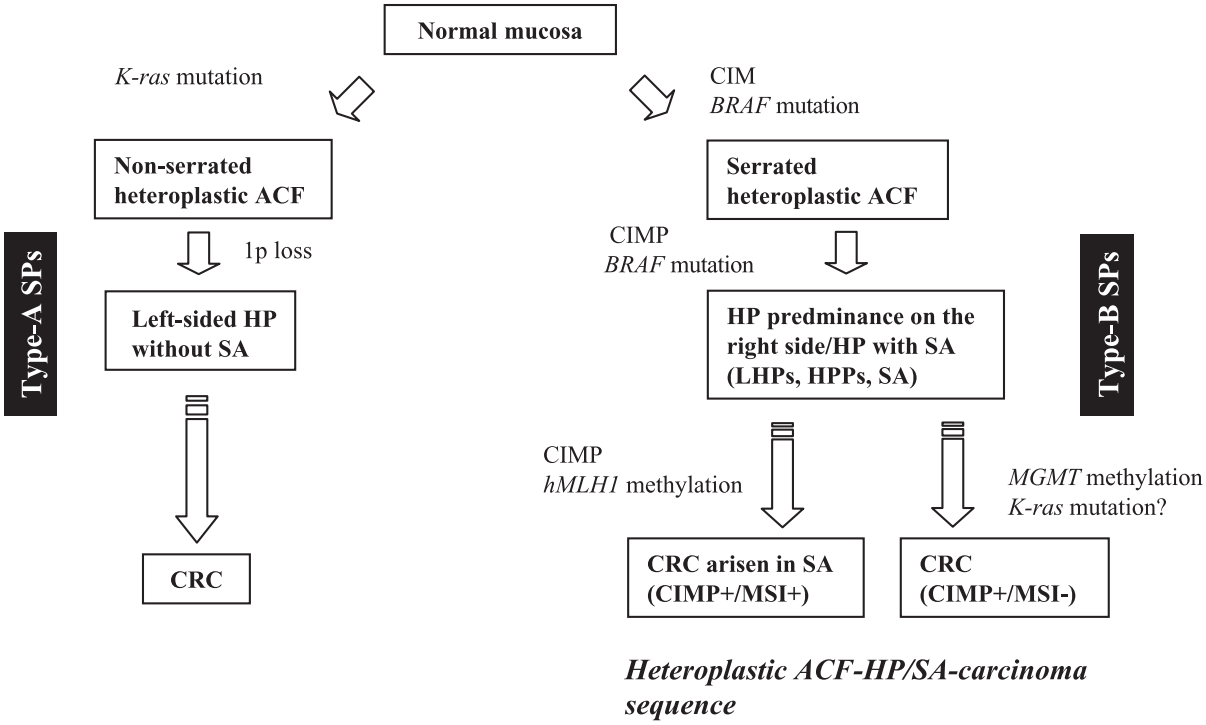

Fig. 2. Diagram of pathways to colorectal cancer (CRC) via hyperplastic aberrant crypt foci (ACF). CIM, CpG island methylation; CIMP, CpG island methylator phenotype; HP, hyperplastic polyp; HPP, hyperplastic polyposis; LHP, large hyperplastic polyp; MSI, microsatellite instability; SA, serrated adenoma; SP, serrated polyps.

Table 2. Reported frequencies of genetic and epigenetic alterations in heteroplastic aberrant crypt foci (ACF)

Alteration	Heteroplastic ACF			Author
	With sporadic CRC (%)	Serrated	Non-serrated	
APC mutation	0 (0/56)			Otori *et al*.[64]
	0 (0/8)			Takayama *et al*.[26]
K-ras mutation	36 (10/28)			Chan *et al*.[59]
	88 (28/32)			Takayama *et al*.[26]
	93 (50/56)			Otori *et al*.[64]
		19 (3/16)	42 (14/33)	Rosenberg *et al*.[70]
BRAF mutation	4 (1/26)			Beach *et al*.[19]
		63 (10/26)	3 (1/33)	Rosenberg *et al*.[70]
Chromosome 1p loss	10 (2/20)			Chan *et al*.[59]
MGMT methylation	13 (3/24)			Chan *et al*.[59]
CIM	50 (14/28)			Chan *et al*.[59]
CIMP-high	11 (3/28)			Chan *et al*.[59]

CIM, CpG island methylation of *MINT1*, *MINT2*, *MINT31*, *p16*, *MGMT* and/or *hMLH1*; CIMP, CpG island methylator phenotype; CRC, colorectal cancer.

higher in non-serrated hyperplastic ACF than in serrated hyperplastic ACF (Table 2).[70] This mutually exclusive relationship between *K-ras* and *BRAF* mutations suggests the existence of distinct pathways to hyperplastic ACF as shown in Figure 2.

Morphological and molecular associations

Previous studies have shown that genetic and epigenetic alterations detected frequently in CRC are present in sporadic HP and in HP, SA, AHAP, tubular adenomas, and carcinomas of patients with HPP.[10,11,60,71–78] These alterations include *APC* and *K-ras* mutations, chromosome 1p loss, MSI, and CIMP (Table 3). There is evidence for two CIMP-positive pathways for colorectal carcinogenesis. One is characterized by methylation of *MGMT* associated with the MSI-low phenotype, and the other is characterized by the CIMP-high phenotype, paucity of *K-ras* mutations, and with or without MSI-high dependent on the methylation status of *hMLH1*.[29,57,79–81] Jass *et al*. described the importance of serrated lesions, low levels of MSI, *K-ras* mutation, and *MGMT* hypermethylation in colorectal carcinogenesis (Fig. 2).[21,82] Other investigators have reported that *MGMT* methylation correlates positively with *K-ras* mutation in the traditional pathways but negatively in the SP neoplasia pathways.[83] Further studies are needed to elucidate the association between *MGMT* methylation and *K-ras* mutations in SP.

Hyperplastic polyps in the right colon differ morphologically from HP in the left colorectum.[84] In addition, Rashid *et al*. reported differences in topographic expression of $p21^{Waf1/Cip1}$

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Table 3. Reported frequencies of genetic and epigenetic alterations in hyperplastic polyps (HP), serrated polyps (SP), serrated adenoma (SA), admixed hyperplastic-adenomatous polyps (AHAP) and adenocarcinoma (AC)

Alteration	HP			SP			SA (%)	AHAP (%)	AC arising in SA (%)	Authors
	Predominantly right side (%)	Predominantly left side (%)	*P*-value	With HP predominantly right side/HP with SA (type-B SP) (%)	With HP predominantly left side without SA (type-A SP) (%)	*P*-value				
APC mutation							20 (7/35)			Fogt *et al*.[77]
							4 (1/26)			Dehari *et al*.[76]
K-ras mutation	0 (0/34)	17 (6/36)	**0.025**	6 (3/51)	19 (5/26)	0.111	0 (0/4)	67 (2/3)		Beach *et al*.[19]
	4 (2/45)	11 (9/83)	0.326				0 (0/6)	67 (2/3)		Rashid *et al*.[11]
	2 (1/44)	14 (8/58)	**0.041**	5 (3/64)	17 (8/46)	**0.049**	25 (2/8)			Chan *et al*.[73]
							0 (0/9)			Chan *et al*.[41]
							24 (7/29)		0 (0/11)	O'Brien *et al*.[83]
BRAF mutation	68 (23/34)	19 (7/36)	**<0.0001**	61 (31/51)	12 (3/26)	**<0.0001**	75 (3/4)	33 (1/3)		Beach *et al*.[19]
							100 (9/9)			Chan *et al*.[41]
							62 (18/29)		82 (9/11)	O'Brien *et al*.[83]
Chromosome 1p loss	4 (2/45)	17 (14/83)	**0.051**				0 (0/6)			Rashid *et al*.[11]
	5 (2/44)	17 (10/58)	**0.064**	3 (2/64)	22 (10/46)	**0.004**	0 (0/8)			Chan *et al*.[73]
MGMT methylation							45 (13/29)		20 (2/10)	Toyota *et al*.[79]
CIMP-high	76 (26/34)	19 (7/34)	**<0.0001**	76 (38/50)	4 (1/25)	**<0.0001**	75 (3/4)	100 (3/3)		Beach *et al*.[19]
	77 (34/44)	19 (11/58)	**<0.0001**	77 (49/64)	4 (2/46)	**<0.0001**	75 (6/8)			Chan *et al*.[73]
							68 (15/22)			Park *et al*.[78]
							79 (23/29)		90 (9/10)	O'Brien *et al*.[83]
MSI-high	0 (0/45)	5 (4/83)	0.297				0 (0/6)	0 (0/3)		Rashid *et al*.[11]
	0 (0/34)	0 (0/36)	NA	6 (1/51)	0 (0/26)	1.000	0 (0/4)	33 (1/3)		Beach *et al*.[19]
	0 (0/44)	0 (0/58)	NA	2 (1/64)	0 (0/46)	0.500	13 (1/8)			Chan *et al*.[73]
							9 (2/22)			Park *et al*.[78]
							0 (0/29)		82 (9/11)	O'Brien *et al*.[83]

Values in bold indicate presence of statistical significance. Serrated polyps include HP, SA, and AHAP. CIMP, CpG island methylator phenotype; MSI, microsatellite instability; NA, not applicable.

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cyclin-dependent kinase inhibitor and Ki-67 proliferation marker in right- and left-sided HP.[11] The genetic and epigenetic alterations in HP from patients with HP predominantly in the left colorectum without SA and patients with SP without SA (defined as type-A SP) also differ from HP from patients with HP predominantly in the right colorectum and SP from patients with SA (defined as type-B SP). Type-A SP have more *K-ras* mutations and loss of 1p but fewer *BRAF* mutations,[39–41] and lower CpG island methylation than Type-B SP.[39,73,85] In contrast, type-B SP have frequent *BRAF* mutations and CIMP but infrequent *K-ras* mutations and loss of chromosome 1p.[11,39,73,85] *BRAF* mutations were detected more frequently in tubular adenoma in patients with multiple or large HP and HPP (predominant in type-B SP) than in those with sporadic adenomas.[37] In addition, the fact that colon cancers derived from SA contain frequent *BRAF* mutations and MSI[83] (Table 3) suggests that CIMP-high and *BRAF* mutation precede MSI in the heteroplastic ACF–HP/SA–carcinoma sequence (Fig. 2). These data provide additional evidence that progression of colorectal carcinogenesis in patients with type-B SP is distinct from that in patients with type-A SP.

Conclusion

Although the vast majority of HP never progress to cancer, the molecular and morphological observations described in this review suggest that carcinogenesis may occur via a heteroplastic ACF–HP/SA–carcinoma sequence. Identification of additional molecular markers to predict the malignant potential of HP is needed. A better understanding of molecular carcinogenesis of the colorectum may provide insights that will improve diagnosis and therapy of colorectal tumors.

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