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Aberrant Crypt Foci as Precursors in Colorectal Cancer Progression

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Abstract

Colorectal cancer progression originates when accumulated genetic and epigenetic alterations cause genomic instability and a malignant phenotype. Subsequent molecular pathway deregulation leads to histopathologic changes that are clinically evident as aberrant crypt foci (ACF) and visualized by high-magnification chromoscopic colonoscopy. ACF are biomarkers of increased colorectal cancer risk, particularly those with dysplastic features. Genetic profiling using genomic instability, loss of heterozygosity, and methylation analysis has revealed a minority population of ACF genotypically analogous to cancer.

Keywords

crypt; gene; colorectal; colonoscopy; methylation

I. Introduction

Aberrant crypt foci (ACF) are considered the earliest neoplastic lesions in colorectal cancer progression [1–3]. The evolution of carcinoma from normal colonic mucosa begins when genetic alterations cause deregulated differentiation and uncontrolled proliferation. Major theories of colon tumorigenesis include the progression from exophytic adenoma to carcinoma [4,5] and a *de novo* malignant pathway arising from grossly normal mucosa [6–8]. Human ACF were identified following their discovery in mice treated with the colonotropic carcinogen azoxymethane (AOM) [9,10]. A minority fraction of ACF is proposed to attain the malignant phenotype and develop into colorectal cancer (CRC), potentially bypassing polyp formation [1–3].

Dysplastic ACF are postulated premalignant lesions synonymous with microadenoma because of their histologic resemblance to adenomatous polyps, related genomic profile, and clinical association with adenomas and CRC [1,11]. Dysplastic ACF are significantly larger

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and contain more apoptotic bodies than hyperplastic ACF [12], and they likely arise from hyperplastic ACF that acquire certain genetic and/or epigenetic alterations [13]. While left colon ACF are more prevalent [11,12,14,15] and much denser [16] than right colon ACF, dysplastic ACF are more frequently associated with right colon tumors [16] and have a higher grade dysplasia than left colon ACF [17]. These studies suggest that ACF, particularly large dysplastic ACF, are precursor lesions of adenomas and CRC.

ACF prevalence and number are significantly greater in patients with adenoma than in normal patients and greatest in those with CRC irrespective of age [3]. Analysis of *dysplastic* ACF prevalence and number elicits an even stronger association to adenoma and CRC patients [3]. Patients with a greater number of adenomas have significantly more ACF, and these ACF tended to be larger (crypt/focus) and dysplastic [3,18]. Interestingly, adenomas diagnosed during high-magnification chromoscopic colonoscopy (HMCC) have been observed with confluent margins to adjacent ACF [3], a phenomenon similar to carcinomas arising directly from adenomas in surgical specimens [19]. A greater ACF density (ACF/cm²) is observed in resected colons with sporadic CRC than in those with benign disease such as diverticulitis [1,2,20–22]. In familial adenomatous polyposis (FAP) patients, most ACF are dysplastic [11,18] and significantly more dense than in sporadic CRC patients [1].

ACF are known biomarkers of CRC risk and chemopreventive response to diet [23,24] and drugs such as sulindac [3], deoxycholic acid [25], and aspirin [25,26]. The high-animal fat, low-fiber Western diet is an independent risk factor for CRC [27,28]. In rats fed a high-fat Western-style diet, wheat bran supplementation as a source of dietary fiber prevents both ACF and colon tumors, while vitamin E and beta-carotene demonstrates a potential to inhibit the progression of ACF to CRC [23]. Specific phytochemicals including flavonoids and glucosinolate breakdown products, certain polyunsaturated fatty acids, and the short-chain fatty acid butyrate have all been reported to suppress ACF formation in animal carcinogenesis models by modulating cell proliferation and apoptosis [24,29].

Smoking is a common environmental risk factor for developing ACF, adenomas, and CRC [30–32]. In an age-corrected group of mostly African-Americans, ACF were found in significantly greater numbers after 15 pack-years and were significantly larger after 30 pack-years, indicating a dose-response relationship [37]. This illustrates ACF as biomarkers of carcinogen exposure and genomic damage.

II. High-Magnification Chromoscopic Colonoscopy

The efficacy of colonoscopy to identify and remove visible polypoid adenomas has resulted in a reduction of CRC incidence in high-risk patients and the general population [33,34]. However, the lesions commonly missed are flat rather than exophytic, and this contributes to the false-negative rate of screening. The screening prevalence of flat colonic lesions in the general population is reported as 23% in North America and other western European countries but remarkably increases up to 45% in Japan [35]. The false-negative rate for polyps and CRC is reported as high as 25% and 4%, respectively [33]. Both flat adenomas and dysplastic ACF are easily missed during conventional colonoscopy, and this may contribute to the significant number of cases where polypectomy fails to prevent cancer progression [34]. Although operator inexperience and/or technical errors contribute to false-negative endoscopic screening, flat or depressed adenomas are elusive without magnification and/or chromoscopic technology [35].

HMCC is an endoscopic technique developed to enhance diagnostic accuracy and guide therapeutics. This technique improves the sensitivity and specificity of colonoscopy for flat

and depressed adenomas [33,35,36]. Endoscopic magnification ranges up to 1125-fold and provides real-time visualization of crypt morphology and mucosal abnormalities not seen with standard endoscopy. Stains applied to the mucosal surface further augment morphology, allowing for the detection of flat neoplasms and ACF endoscopically rather than histologically in surgical specimens [33,37].

Various stains can be used for chromoendoscopy, and it is important for the endoscopist to be familiar with stain characteristics and features on specific tissue. The three main categories of dyes are contrast, absorbing, and reactive [33,36,37]. Contrast dyes such as indigo carmine and cresyl violet enhance mucosal groove patterns and accentuate lesions causing surface pattern disruption. When using absorptive dyes such as methylene blue, it is mandatory to wash the mucosa with a proteolytic solution prior to application. Because areas of dysplasia and active inflammation poorly absorb methylene blue, dysplasia is highlighted based on the extent of cellular absorption [36]. Reactive dyes are used less commonly and currently have less diagnostic relevance [36].

Once the endoscopist identifies a region of colon requiring detailed examination, the mucosal surface must be carefully cleaned to remove feces and excess mucus. The common techniques for topical dye application use either a syringe or diffusion catheter [33,35,36]. Some clinicians report prescribing oral dye capsules, such as indigo carmine, that are taken with bowel preparation; however, this method has been unsuccessful due to insufficient distal colon staining [35].

Topographic features are used to make a presumed tissue diagnosis *in vivo* during HMCC. The Kudo pit-pattern classification system helps determine a lesion's malignant potential based on the degree of crypt architecture irregularity [37]. Endoscopic biopsy removes the entire lesion for histologic and molecular analysis.

The efficacy of HMCC to detect flat or depressed lesions has been validated in different parts of the world [37,38]. In patients at high-risk for CRC, such as those with inflammatory bowel disease, HMCC has been incorporated into the guidelines for CRC surveillance [35,36,39]; nevertheless, the use of this technique still remains uncommon in the U.S. This is partly due to a lack of training, additional time compared to conventional colonoscopy, and the introduction of narrow-band imaging, spectroscopy, and confocal coherence tomography [35,36]. Despite its limited use, HMCC has been shown to enhance the diagnostic capability of standard colonoscopy for the detection of malignant and premalignant lesions such as ACF [3,40]. Encouraging results demonstrate the benefit of using HMCC to identify ACF during cancer surveillance in ulcerative colitis patients [39,41,42].

III. Histopathology of ACF

A. Topographic Features

ACF have two major features: topographic and histologic [43,44]. Compared to adjacent normal colonic mucosa, ACF are elevated, deeply stained, and have larger crypts with shape polymorphism (rounded, serrated, and elongated). Some cases of ACF containing a single crypt abnormality can be subtle [45]. Nevertheless, by definition, aberrant crypt size should be at least twice that of its normal surrounding counterparts, and the epithelial lining should be thicker than in normal adjacent crypts. In addition, the luminal opening should be elliptical rather than circular as seen in normal colonic mucosa.

B. Histologic Classification

There are multiple proposed classification schemes regarding ACF [11,12,14,17,22,43–46] ranging from two-tier classification (hyperplasia or neoplasia) [12] to four-tier classification (simple, hyperplasia, mild/moderate dysplasia, or severe dysplasia) [44]. Taking into account all previous work, we believe ACF are better classified into hyperplastic and dysplastic subtypes for prognostic significance and simplicity as recommended by the World Health Organization (Table I) [47].

1. Hyperplastic ACF—Sporadic ACF generally have histopathologic features that resemble hyperplastic polyps [11]. Hyperplastic crypts are larger and longer than normal crypts and sometimes show apical branching. The luminal opening is serrated and slightly elevated from the surrounding normal mucosa but without dysplasia. Epithelial cells staining positive for proliferating cell nuclear antigen (PCNA) show an upward expansion of the proliferative crypt compartment when compared to normal crypts [48]. Nuclei are enlarged and sometimes crowded without stratification. Goblet and absorbing cells are mixed with partial mucin depletion. Although an absence of adjacent lymphoid follicles is considered a histologic feature of ACF [43], submucosal lymphoid aggregates or follicles may be present [13].

2. Dysplastic ACF—Crypts and crypt cells both have different degrees of abnormality. The histologic characteristics of dysplasia (adenomatous epithelium) are epithelial hypercellularity with abnormal nuclear features (stratification, elongation, enlargement, polymorphism, hyperchromatism, loss of polarity, and irregular outline), reduced cytoplasmic mucin content, and disordered maturation with involvement of the upper region of crypt epithelium. Since epithelial proliferation in hyperplastic polyps is generally confined to the crypt base as in hyperplastic ACF, similar appearing ACF should not be interpreted as dysplastic [11,48]. In this regard, dysplastic crypt cells staining positive for PCNA and Ki-67 extend more prominently to the upper and middle crypt compartments than do hyperplastic crypt cells [11,12,17,48].

IV. Genomic Profile of ACF

A. Gene Mutations

Most ACF remain dormant or even regress and disappear, suggesting that additional genetic changes are required for malignant transformation [4,5]. *KRAS* [3,11,13,14,18,46,49–53], *APC* [46,50,51], *TP53* [14,54], and *BRAF* [52,55] mutations have been identified within ACF. *KRAS* and *APC* mutations are important early molecular events in colon carcinogenesis, while *DCC* and *TP53* appear to be altered at later stages [14,50,54].

Certain ACF contain identical mutations to the corresponding adjacent tumor, and these ACF may have a greater malignant potential [50,54]. Conversely, a high degree of mutation heterogeneity also exists between individual ACF as well as ACF and tumors from the same colon for *KRAS* [14,49,50,54], *BRAF* [52], and nonrandom X chromosome inactivation [56,57]. Therefore, although ACF do not appear histologically neoplastic, a high percentage of ACF are monoclonal; they are genetically neoplastic rather than merely hyperplastic mucosal lesions [14,49,50,52,54,56,57].

ACF in patients with FAP are genetically distinct from sporadic ACF [51]. FAP ACF are predominantly dysplastic and invariably contain somatic *APC* mutations similar to FAP adenomas. Interestingly, *KRAS* mutations are much less common in dysplastic FAP ACF than in dysplastic sporadic ACF (13% and 82% respectively) but similar for FAP and sporadic adenomas (73% and 65% respectively) [51]. Alternately, *APC* mutations are not

detected in sporadic ACF, but 78% of sporadic adenomas have mutated *APC* [51]. Assuming the ACF-adenoma-carcinoma sequence, these findings suggest that somatic *APC* mutation occurs first in FAP ACF, followed by *KRAS* mutation during adenoma and tumor formation. In cases of sporadic tumorigenesis, *KRAS* mutation appears to initiate ACF pathogenesis with subsequent *APC* mutation occurring during adenoma and tumor formation.

A model of sporadic colon cancer in AOM-exposed mice has been used to help define the underlying genetic features that predict the malignant potential of ACF [58]. A cDNA microarray was constructed to decipher discrepancies in genetic signature between low-risk (low tumor sensitivity to AOM) and high-risk (high tumor sensitivity to AOM) mouse strains [58]. Many alterations are common to both strains, but six gene clusters are significantly altered (upregulated or downregulated in at least one histologic group) and allow for complete strain segregation. Dysplastic ACF are rare in low-risk mice compared to high-risk mice, and although it is difficult to segregate dysplastic and hyperplastic ACF by clustering because genetic differences are subtle, dysplastic ACF have a divergent risk potential based on distinct molecular signatures. *Smpd3a* (alias *Asml3a*), *Pdzk1ip1* (alias *Map17*), *Rab24*, *Slpi*, *Thbs4* (thrombospondin 4), and *Ndufa13* (formerly *Grim19*) are differentially down-regulated in the low-risk mice and up-regulated in high risk mice, and this data has been validated by quantitative PCR [58]. Overexpression of these genes is implicated in murine tumor progression, and future human studies may elucidate a potential role for analogous genes in human colorectal tumor progression.

Nos2 (alias iNos) upregulation, *Ctnnb1* mutations, and altered cellular localization of Ctnnb1 are all observed in dysplastic ACF, adenomas, and carcinomas, but not in hyperplastic ACF of AOM-exposed rats [59]. These findings occur early in AOM-induced rat colon carcinogenesis, implicating Nos2 and Ctnnb1 with causal roles in ACF dysplasia and tumorigenesis. Ctnnb1 alterations are involved in Nos2 upregulation, and its product, nitric oxide, causes DNA damage, neovascularization, and enhanced Ptg2 (alias Cox-2) activity [59–61]. Ptg2 overexpression in AOM-exposed rats is found mainly in the large and medium colon tumors and promotes tumor growth via known mechanisms of apoptosis inhibition and tumor angiogenesis augmentation [59]. PTGS2 overexpression in human CRC is associated with cytoplasmic CTNNB1, which may function to stabilize PTGS2 mRNA [62].

AOM-induced colon carcinogenesis in animal models is not only practical for studying tumor suppressor gene expression in ACF but also for testing the chemopreventive properties of drugs targeting such genes. Exposing rats to AOM significantly induces Nos2, Ptg1, and Ptg2 enzyme activities in colonic mucosa, but this effect is more pronounced on Ptg2 than Ptg1 [61]. Combination of the selective Ptg2 inhibitor celecoxib with the selective Nos2 inhibitor SC-51 suppresses Ptg2 activity greater than celecoxib alone, in accord with the abovementioned nitric oxide mechanism. SC-51 reduces crypt multiplicity of four or more aberrant crypts per focus in a dose-dependent fashion, and the highest effective dose significantly suppresses ACF incidence and crypt multiplicity comparable to the nonselective cyclooxygenase inhibitor sulindac (positive control). A combination of the minimally effective doses of SC-51 and celecoxib significantly reduces total colonic ACF and aberrant crypt multiplicity to the maximal effect achieved by sulindac [61]. PTGS2 inhibition is also believed to be one of the chemopreventive mechanisms of phytochemicals in CRC [24,29].

B. Genomic Instability

Genomic instability (GI) is a fundamental feature of solid tumors that develops as genomic damage accumulates over time from inappropriate chromosomal segregation, inaccurate

DNA replication, excessive environmental damage saturating intact repair pathways, and/or defective repair mechanisms. Tumor progression occurs when GI surpasses a certain threshold. As a cell's genome becomes increasingly unstable from acquired oncogenic mutations and silenced tumor suppressors, a malignant cell phenotype emerges and leads to clonal expansion. Tumor heterogeneity thus exists between different tumors, although GI can potentially generate intratumor genomic heterogeneity via expansion of multiple malignant clones. Measuring GI is a valuable additional tool for stratifying the malignant transformation potential of ACF, and various methods exist [63]. Chromosomal instability is used generally to describe aneuploidy, while intrachromosomal instability results from insertions, deletions, inversions, translocations, amplifications, and point mutations.

Point mutation induced GI rarely causes malignancy except in cancers exhibiting the replication error phenomenon, such as hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome). Patients with HNPCC carry a germline mismatch-repair (MMR) gene mutation most often in *MLH1* or *MSH2*, a mutation rarely found in sporadic tumors [64]. Microsatellite instability (MSI) is a molecular marker for MMR gene mutations [64]. Microsatellites, also known as short tandem repeats or simple sequence repeats, usually consist of two, three, or four nucleotides repeated 10 to 100 times and randomly distributed throughout the genome mainly within non-coding introns. A common example is a (CA)_n repeat which is often used to construct primers for inter-(simple sequence repeat) PCR (ISSR-PCR). This class of polymorphism is evolutionarily divergent because of DNA polymerase's propensity for counting mistakes when replicating 10 or more tandem repeats. MMR gene mutations lead to accumulated DNA replication errors resulting in tumor suppressor and oncogene mutations as well as greater variability (expansion or contraction) in microsatellite length (*i.e.* MSI). A subset of ACF is known to contain MSI [65], and a graduated increase in MSI has been observed along the ACF-adenoma-carcinoma progression, implicating ACF as CRC precursor lesions [64].

An ideal method for measuring intrachromosomal instability would calculate the number of genomic events per cell generation that altered coding or transcriptional regulation. This is difficult because of genetic diversity and evolving tumor cell heterogeneity. GI resolution can be limited because late-stage genomic events occurring in a minority cell population are indistinct by gel electrophoresis of million-cell tumor analysis. Reasonable sampling alternatives include laser capture microdissection (LCM), high performance liquid chromatography (HPLC), and matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry.¹ Current methods in CRC utilize sampling techniques to estimate intrachromosomal instability in defined portions of the genome: flow cytometry [66], array comparative genome hybridization (aCGH) [67–69], randomly primed PCR [70], ISSR-PCR [71–74], and allelotyping for loss of heterozygosity (LOH) [54,71–73].

Randomly primed PCR reveals altered DNA fingerprints in nearly all colon tumors analyzed (96%) and in a minority population of ACF (23%) [70]. Although no amplifications or deletions are observed in ACF using bacterial artificial chromosome (BAC) aCGH [69], 78% of ACF contain band alterations via ISSR-PCR, and 25% show *moderate* instability by the GI index (GII) [69]. GII is the percent of electrophoretic bands altered in migration or intensity as compared to the total band number in normal mucosa from the same patient. Failure of BAC aCGH to detect GI in ACF indicates that early genomic alterations are usually less than 150 kb, the approximate size of a BAC clone insert [69]. On the other hand, ISSR-PCR amplification of DNA between microsatellites creates relatively short products (900 bp) that vary much in length [69,73,74]. The sensitivity of ISSR-PCR allows it to detect amplifications or deletions (band intensity) if present in a relatively large

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percentage of tumor cells, but genetic alterations leading to new bands (band migration) requires a smaller fraction of tumor cells for detection.

GI is likely a cause rather than effect of malignancy, since colonic crypts acquire GI early in tumor progression [69,73,74]. When individual crypts are isolated from normal colonic mucosa, no band alterations are observed by ISSR-PCR [72]. Conversely, mean GII in sporadic adenomas and colorectal tumors has been reported as essentially identical at approximately 4% [72–74]. Although the ACF mean GII reported in one study is significantly lower (1.5%), a 25% subset of ACF with *moderate* GI had a similar calculated mean GII (3.9%) [69], and this minority population appears to correspond to the 23.3% reported above by Luo *et al.* [70]. The aforementioned data suggests that a population of ACF exists with a degree of GI resembling neoplasia, and these ACF may have a high likelihood of progressing to malignancy.

C. Loss of Heterozygosity

Methods detecting LOH typically unveil instability occurring later in tumorigenesis than ISSR-PCR. LOH denotes the loss of either a paternal or maternal allele and can arise by several pathways, including deletion, gene conversion, mitotic recombination, or chromosome loss. If a necessary tumor suppressor allele is lost and the remaining allele is mutated or epigenetically silenced, a cell would theoretically acquire a malignant phenotype. Patient prognosis has been shown to decline independent of tumor stage when LOH is present [75]. Because LOH of tumor suppressor genes is a relatively late event in colon carcinogenesis, ACF with LOH are proposed to have greater malignant potential [57,72].

Genome-wide allelotyping with amplification of 348 microsatellite markers has been used to quantitate intrachromosomal instability in sporadic adenomas and carcinomas by providing high-resolution of LOH events [72]. The mean fractional allelic loss (FAL) rate, or the fraction of assayed alleles exhibiting LOH, is over 3 times greater in carcinomas (0.095) than in adenomas (0.03) [72]. Genome-wide FAL rates do not significantly correlate with the smaller genomic events detected by ISSR-PCR and MSI, but loci on chromosomes 3, 8, 11, 13, 14, 18, and 20 demonstrate significantly elevated instability by GII and/or FAL rate in LOH+ tumors [72]. A recent study from the same laboratory amplified 28 markers from microdissected tumors arising directly from villous adenomas and report a carcinoma mean FAL rate (0.135) significantly higher than that of the adjacent adenoma (0.052) [19]. The FAL rates reported for CRC and adenomas in this study were nearly twice that in their former study because the 28 markers chosen were already linked to LOH [19,72]. These LOH markers in adenomas and CRC provide loci for further genetic profiling of ACF relative to tumor progression.

DCC is a putative tumor suppressor gene deleted in 70% of CRC but only infrequently in adenomas and not in ACF [76]. LOH has been reported in ACF at locus D18S47 on chromosome 18q, a microsatellite linked to *DCC* and *SMAD4* (formerly *DPC4*) [49,73]. One patient with D18S47 LOH had an identical *TP53* mutation in both an ACF and the adjacent tumor [54]. Another ACF and its adjacent adenoma in the same patient were both without LOH or *TP53* mutation, suggesting tumor evolution from a genetically unique ACF population [54]. In light of these findings, future research can include LOH to help characterize an ACF subset with greater malignant potential. Identifying high-risk ACF biomarkers will allow patients to receive personalized screening strategies.

GSTM1 is a glutathione S-transferase isozyme present in about half the population. Absence of *GSTM1* may preclude detoxification of colon carcinogens, thereby contributing significantly to GI and CRC risk [71]. Sporadic CRC patients with a *GSTM1*-null genotype have tumors with significantly elevated GII but no associated LOH [71]. A significant

association has been observed between *GSTM1*-null genotype and ACF dysplasia (unpublished observations). Further analysis of *GSTM1* genotype, GI, and ACF histopathology in patients with and without CRC can be used to characterize ACF malignant potential and assess cancer risk.

D. Methylation and Epigenetic Silencing

When dense CpG dinucleotides occur within a tumor suppressor gene promoter (CpG island), aberrant methylation of these CpG cytosine residues can epigenetically silence transcription. Methylation is a common, early event in sporadic CRC evident by detection in ACF [53,77–80] and polyps [81–83] via DNA bisulfite conversion and methylation-specific PCR (MSP). CpG island methylator phenotype (CIMP) in CRC is characterized by methylation of multiple CpG dinucleotides including those within the promoters of *MLH1* and tumor suppressor *CDKN2A* [42,55,62,81,83–85]. Sporadic CRC with MSI usually results from *MLH1* silencing by promoter methylation rather than somatic MMR mutation [55,86]. Tumor suppressor promoter methylation contributes to GI, and CIMP is associated with high MSI in CRC [55,62,84]. Not all CIMP cancers, however, contain MSI or intrachromosomal instability [72,85].

Epigenetic transcriptional silencing by methylation appears to emerge *de novo* during cancer progression and can eventually lead to lesions with CIMP. Although concordant CIMP has been observed in colonic lesions from the same CRC and hyperplastic polyposis patients [81,83], methylation status has also been shown to be discordant among adenomas from non-cancer patients [82]. Similarly, even though methylation of MINT31 (cloned CpG island differentially methylated in CRC) [87], *CDH13* (H-cadherin), and *RBPI* (alias *CRBPI*) has been demonstrated in the majority of ACF (54%) and tumors (68%), discordant methylation profiles have also been observed when comparing ACF and tumors from the same patient [79].

Interestingly, *PTGS2* is overexpressed in most colorectal tumors and a subset of adenomas [62,88]; however, a minority population of sporadic tumors (13%) and adenomas (14%) has been identified with *PTGS2* methylation correlating significantly with CIMP [89]. Therefore, although sulindac significantly decreases and even eradicates ACF in some cases, an unresponsive population may still exist that can progress to tumor [3]. Elucidating the epigenetic profile of ACF by determining CIMP and *PTGS2* methylation status has the potential to predict clinical response to prophylactic and therapeutic pharmacotherapy for CRC.

Histology has been correlated with gene methylation in both adenomas and ACF. Tubulovillous/villous adenomas carry a higher malignancy risk and are also associated with more frequent tumor suppressor methylation [82]. In a study examining the methylation status of *CDKN2A*, *APBA1* (formerly *MINT1*), *APBA2* (formerly *MINT2*), MINT31, *MGMT*, and *MLH1* using MSP, methylation was more characteristic of ACF in patients with sporadic CRC (53%) than with FAP (11%), and dysplastic ACF in particular were more frequently methylated in sporadic CRC patients (75%) than patients with FAP (8%) [53].

Tumor-suppressor *CDKN2A*, a cyclin-dependent kinase inhibitor frequently mutated or deleted in cancer, is transcriptionally silenced by methylation in a subset of CRC [90–93]. *CDKN2A* arrests G1 progression and invokes cellular senescence by targeting downstream RB1, CDK4, CDK6, and CCND1, while the transcript variant commonly known as p14ARF (alternate reading frame) functions through MDM2 and TP53 [93,94]. These *CDKN2A* pathways have relatively few known mutations in CRC. This has been previously explained by inactivated APC leading to *MYC* activation, because *MYC* expression is known to circumvent *CDKN2A* and *CDKN2B* (alias p15INK4B) mediated growth arrest [90,95,96].

However, recent data from inducible cell lines suggest that a MYC-ARF interaction can inhibit MYC independent of the ARF-TP53 axis, implicating *CDKN2A* deregulation in CRC tumorigenesis [97].

In a study of sporadic CRC, *CDKN2A* methylation correlated with negative *CDKN2A* immunohistochemistry and was more frequent in poorly differentiated adenocarcinoma, demonstrating its prognostic value [93]. Low *CDKN2A* expression has also been associated with lymph node metastasis and larger tumor size [92]. Although the degree of *CDKN2A* expression correlates significantly with methylation density, CRC tissues display a wide variety of methylation densities [92]. Moreover, neoplasms display heterogeneous *CDKN2A* immunostaining, with higher expression bordering normal tissue and correlating inversely with Ki-67, RB1, and CCNA2, markers of cell-cycle progression [98].

CDKN2A upregulation has been observed in a population of human ACF, adenomas, primary carcinomas, and metastatic tumors [91,98,99], as well as in AOM-exposed mouse preneoplastic lesions, compared to nearly undetectable levels in normal mucosa [100]. This suggests that *CDKN2A* overexpression occurs early, with epigenetic silencing commencing later in tumor progression. Consequently, it is important to note that *aberrant* expression of *CDKN2A* indicates a poor prognosis in CRC [99].

Both *CDKN2A* methylation and dysplastic ACF have been linked to right-sided colon tumors [16,17,91], and *CDKN2A* methylation in ulcerative colitis patients suggests an ACF-dysplasia-cancer sequence in colitis-associated tumor progression [42]. As a result, dysplastic ACF in conjunction with *CDKN2A* methylation have considerable potential as screening biomarkers of cancer progression in sporadic right-sided colon cancer and for surveillance of colitis cancer by HMCC.

V. Conclusion

ACF represent the earliest identifiable mucosal abnormality of the colon and rectum and are biomarkers of CRC risk. HMCC is a valuable clinical tool with the potential to decrease false-negative colonoscopy-screening rates by augmenting the diagnosis of flat colorectal lesions and microradenomas. Dysplastic ACF with similar genetic alterations to CRC may have greater potential to develop into malignant tumors than hyperplastic ACF. During the early transformation of normal glandular epithelium to ACF, the genome and epigenome become increasingly unstable, leading to changes in the expression of key tumor suppressors. Genetic and epigenetic profiling has characterized a malignant genotype in a minority population of ACF and has the potential to be combined with HMCC in the clinical arena. ACF are also associated with colitis cancer, and while HMCC has been successfully used as a surveillance technique in ulcerative colitis patients, large-scale studies are needed before it is accepted for routine use in certain high-risk patients.

Abbreviations

ACF	aberrant crypt foci
AOM	azoxymethane
CRC	colorectal cancer
HMCC	high-magnification-chromoscopic-colonoscopy
FAP	familial adenomatous polyposis
PCNA	proliferating cell nuclear antigen

GI	genomic instability
HNPCC	hereditary nonpolyposis colorectal cancer
MMR	mismatch repair
MSI	microsatellite instability
ISSR-PCR	inter-(simple sequence repeat) polymerase chain reaction
aCGH	array comparative genome hybridization
LOH	loss of heterozygosity
BAC	bacterial artificial chromosome
GII	genomic instability index
FAL	fractional allelic loss
MSP	methylation-specific PCR
CIMP	CpG island methylator phenotype

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TABLE I
 Histologic Characteristics of Hyperplastic and Dysplastic Aberrant Crypt Foci (ACF)

	Hyperplastic ACF	Dysplastic ACF
Architecture		
Crypt shape	Larger and longer, apical branching	Generally smaller
Serration	Present	Generally absent
Mucin depletion	Partially present	More prominent
Maturation	Disordered, upper crypt not involved	Disordered, upper crypt involvement
Nuclei		
Stratification	Absent	Present
Shape	Round to oval	Elongated
Size	Enlarged	Larger than hyperplastic
Polymorphism	Absent	Present
Hyperchromatism	Absent	Present
Proliferation nuclear index	Upward expansion of proliferative crypt compartment	More prominent extension to upper and middle crypt compartments