

Dysplastic Aberrant Crypt Foci: Biomarkers of Early Colorectal Neoplasia and Response to Preventive Intervention



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ABSTRACT

The discovery of aberrant crypt foci (ACF) more than three decades ago not only enhanced our understanding of how colorectal tumors form, but provided new opportunities to detect lesions prior to adenoma development and intervene in the colorectal carcinogenesis process even earlier. Because not all ACF progress to neoplasia, it is important to stratify these lesions based on the presence of dysplasia and establish early detection methods and interventions that specifically target dysplastic ACF (microadenomas). Significant progress has been made in characterizing the morphology and genetics of dysplastic ACF in both preclinical models and humans. Image-based methods have been

established and new techniques that utilize bioactivatable probes and capture histologic abnormalities *in vivo* are emerging for lesion detection. Successful identification of agents that target dysplastic ACF holds great promise for intervening even earlier in the carcinogenesis process to maximize tumor inhibition. Future preclinical and clinical prevention studies should give significant attention to assessing the utility of dysplastic ACF as the earliest identifiable biomarker of colorectal neoplasia and response to therapy.

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Introduction

Significant progress has been made in identifying both natural and synthetic agents that are efficacious in preventing the formation of colorectal tumors in preclinical animal models. Unfortunately, translation of these data to a clinical setting has been challenging due in part to the lack of an early intestinal event, either morphologic or genetic, that can serve as a robust and reliable biomarker of response to chemopreventive intervention. In theory, an ideal biomarker would arise very early during inception of the neoplastic process and prior to any evidence of grossly identifiable disease. Because the genetic alterations associated with colorectal tumor formation are highly heterogeneous, the search for such predictive biomarkers has focused on the earliest histologic alterations arising in a background of nonneoplastic colonic mucosa. The following review focuses on the ability of a small foci of dysplastic crypts (microadenoma) to serve as a biomarker of the earliest sign of colorectal neoplasia and response to preventive intervention.

Aberrant crypt foci (ACF) are the earliest histologic alterations to arise during the multi-step formation of colorectal

neoplasia. These lesions were first reported in 1987 by Bird (1) who, with the use of a dissecting scope, observed clusters of differentially stained crypts with an abnormally thick epithelial lining in the colonic mucosa of C57BL/6 and CF1 mice treated with the colon carcinogen azoxymethane (AOM). The clinical significance of this finding was confirmed in 1991, when similar atypical crypts were identified in whole mounts of normal-appearing human colon stained with methylene blue (2, 3). The grossly observed aberrant foci (1 to more than 30 crypts) were 3-fold larger in diameter than normal human colon crypts and exhibited atypical oval to slit-shaped luminal openings (2).

Histology of ACF

ACF can be classified as hyperplastic (enlarged and elongated) or dysplastic lesions (4). Dysplastic ACF (microadenomas) exhibit epithelial changes that are neoplastic in nature and identical to those seen in tubular and villous adenomas, including hypercellularity with enlarged hyperchromatic nuclei, varying degrees of nuclear stratification, loss of polarity, high nuclear/cytoplasmic ratio, nuclear crowding, and increased mitotic index (3). In contrast to hyperplastic ACF and normal mucosa, proliferation (Ki67 and PCNA positivity) in dysplastic ACF extends to the epithelial surface. By definition, dysplastic ACF are microadenomas and precursors of grossly visible neoplastic lesions/tubular adenomas.

Dysplastic ACF (microadenomas) often exhibit accumulation of nuclear β -catenin and/or mucin depletion. β -Catenin accumulated crypts were first identified in AOM-treated rats (5) and are found frequently in other carcinogen-induced models of colorectal tumorigenesis (6). Dysplastic

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ACF are also present in the human colon and show nuclear localization of β -catenin. Although these lesions exhibit the histologic features of dysplasia, most cannot be identified grossly on the mucosal surface without magnification or staining with methylene blue (7). On the basis of an increased frequency of β -catenin mutations and enhanced proliferative activity as compared with classic ACF, β -catenin-accumulated crypts are more likely to progress to neoplasia (6). Mucin-depleted crypts exist as focal lesions and contain scant if any mucin on topography and positive staining with Alcian blue-periodic acid Schiff on histologic analysis (8). The presence of mucin-depleted crypts in humans was first observed by Femia and colleagues (9) in colon specimens surgically resected from individuals at high risk for colorectal cancer. Detection was accomplished by staining the tissues first with methylene blue and then with high-iron diamine Alcian blue. All mucin-depleted crypts identified harbored some degree of dysplasia. Mucin-depleted crypts were heterogeneous in size (some only 3–6 crypts) and present at a density 30-fold less than that of ACF in patients with familial adenomatous polyposis (FAP), a heritable syndrome where individuals develop hundreds to thousands of polyps and ultimately colorectal cancer due to a mutation in the adenomatous polyposis coli (APC) gene. Mucin-depleted crypts were located much less frequently in patients with colorectal cancer with sporadic disease as compared with patients with FAP. Interestingly, mucin-depleted crypts were found more often adjacent to colorectal cancers in the normal colonic mucosa of fresh colectomy specimens (8). Although these data imply a temporal association between β -catenin-accumulated crypts, mucin-depleted crypts, and adenoma formation, additional studies are needed to establish this relationship and eliminate the impact of a potential field effect.

Biomarkers of Colorectal Cancer Risk

Several studies have documented an association between the presence of dysplastic ACF and risk for colorectal cancer. In the case of FAP, the vast majority of patients (93.6%) had dysplastic ACF, while these lesions were detected in only 7.0% of patients who lacked a germline APC mutation (10). The relative risk ratio (RR) for developing dysplastic ACF was higher (RR, 18.14) than that for developing nondysplastic ACF (RR, 1.29) in patients with colorectal cancer (11). The average number of ACF per cm^2 was highest among FAP patients (19.9/ cm^2), as compared with patients with colon cancer (0.37/ cm^2) and normal individuals (0.18/ cm^2) (3). In contrast, Cho and colleagues (12) did not observe any dysplasia among 655 ACF from 45 subjects with sporadic adenomas; 78% of the subjects possessed nondysplastic ACF. In an independent study, only 2.0% of patients with adenocarcinomas also had dysplastic ACF in the rectum, while 38% of the rectal ACF identified by chromoendoscopy were histologically confirmed to be hyperplastic (13).

Although the role of dysplastic ACF in predicting colon cancer risk and/or recurrence remains controversial, several

groups report an increase in the prevalence of ACF that parallels the stepwise progression of disease (normal colon to adenoma/dysplasia to cancer). This has been observed in both patients with colon and rectal cancer (14–16) and in individuals with colitis-associated dysplasia (17). Anderson and colleagues (18) reported a direct relationship between a high number (>6) of ACF in the distal colorectum and the development of advanced neoplasia over the next 5 years (adjusted odds ratio, 12.27). To our knowledge, no association has been established between environmental and demographic factors and the risk of developing dysplastic ACF in humans. Irrespective of dysplasia, the total number of ACF increases with age; single ACF are found predominantly in younger patients (below the age of 40), whereas the highest numbers are observed in subjects 50 to 70 years of age (19). An association has been observed between smoking long-term and number of ACF (18, 20). Individuals with a smoking history of ≥ 20 pack years had a significantly higher number of ACF detected during endoscopy than never-smokers (age adjusted odds ratio, 3.16) (20).

Spontaneous Regression

Despite their recognition as very early lesions, ACF are known to regress over time as part of their natural history (21–24). Use of confocal endomicroscopy in mice with conditional deletion of the *Apc* and/or *K-ras* gene revealed the appearance of colonic dysplastic ACF (microadenomas) within 3 weeks of AdenoCre delivery. However, most of these small lesions regressed spontaneously by 10 weeks of age, while a few progressed to larger adenomas (21).

Although adult *Apc*^{Min/+} mice have approximately 20 dysplastic ACF (microadenomas) per colon, only 1 to 3 gross adenomas develop, indicating that most dysplastic ACF do not progress (22). These dysplastic ACF are less than 300 μm in diameter and lack mucin. Oyama and colleagues (23) reported that the size of dysplastic ACF was stable in *Apc*^{Min/+} mice, with no change in diameter observed between 5 and 35 weeks of age. These self-limiting features appear to mimic those of human ACF. In a clinical study to monitor ACF over time, 60% of the population had at least 1 rectal ACF at baseline colonoscopy. Less than 50% of the ACF identified originally could be reidentified one year later (24). However, more than 50% of the subjects developed new ACF. Although these data clearly demonstrate the dynamic growth characteristics of ACF within the colon, the potential ability of even a single small dysplastic lesion to progress to colorectal cancer is of clinical significance and cannot be overemphasized.

Early Detection in Humans

Establishment of a standardized and reliable method for the endoscopic detection of human ACF *in vivo* remains challenging, primarily due to their small size (<5 mm in diameter). Numerous attempts to deviate from routine macroscopic surveillance via white-light endoscopy have been met with limited enthusiasm, primarily due to the additional time

needed to accurately detect ACF during colonoscopy. Despite this challenge, ACF, including β -catenin-accumulated crypts and mucin-depleted crypts, can be identified in humans using magnifying chromoendoscopes and narrow-band imaging techniques (25). High-definition magnifying colonoscopy, when used in combination with indigo carmine or methylene blue spray, is providing new insight into the growth characteristics of ACF and has facilitated the detection of ACF within the proximal colon of 39% of healthy adults during routine surveillance (26). The percentage of dysplastic ACF was 4-fold higher in the proximal colon (52%) than in the distal region (13%). Dysplasia was 7 times more likely to occur in ACF in the proximal versus distal colorectum. Although it is recognized that not all studies have yielded a strong correlation between ACF and cancer development (12, 27), the current data provide support for continued investigation of ACF, in particular dysplastic ACF, as an early surrogate biomarker of risk for colorectal cancer.

Despite demonstrated successes in identifying human ACF using high definition/magnification chromoendoscopy, a need exists to develop methods that are less specialized and more compatible with routine clinical care. A few endoscopic techniques in particular show great promise for detecting dysplastic ACF in humans in the future. First, by placing a high magnification, flexible endocytoscope in direct contact with the stained bowel wall, Cipolletta and colleagues (28) were able to distinguish the histologic features of colonic crypts with low-grade dysplasia from those without dysplasia in real-time with high sensitivity (91.4%). The resulting *in vivo* images of the gastrointestinal mucosa were of high quality and comparable to those of conventional histology. Dysplastic ACF displayed polygonal crypt contours and irregular and elongated cell nuclei with pseudostratification toward the lumen of the crypt. Such histologic approaches, although promising, are compromised by significant barriers that will need to be overcome prior to routine clinical use. Consistent with strategies developed by others, endocytoscopy was time-consuming (average, 44 min) and interrogated only a small area of the colon. Clinical implementation of such technology would be paradigm shifting for gastrointestinal endoscopists, as the operator would be required to obtain specialized training in histology.

Results from recent studies indicate that ACF can be detected in the human colon without using dye spray or methylene blue. In a prospective study, patients with colorectal neoplasms were examined using either narrow-band imaging or blue-laser imaging in combination with magnifying endoscopy (29). This so-called image-enhanced endoscopy accentuates the microvasculature and surface features of colorectal lesions, leading to improvements in detection (30, 31). Detection rates for dysplastic and nondysplastic ACF in the prospective study were 84.4% and 80.3%, respectively. Use of image-enhanced endoscopy led to reductions in both the time required for bowel preparation and ACF detection. Muguruma and colleagues (32) successfully identified ACF within the normal-appearing

colorectum of patients with colorectal cancer postoperatively using a novel fluorogenic probe (DNAT-Me) activated by glutathione S-transferase P1-1. Direct comparison of DNAT-Me with conventional methylene blue staining revealed DNAT-Me was superior, as it exhibited a significantly higher signal-to-noise ratio, was faster to use, and did not require high magnification endoscopy for ACF detection due to its strong fluorescence intensity. Unfortunately, no attempts were made in this study to distinguish between dysplastic and nondysplastic ACF. Clearly, the approaches outlined above hold great promise for the future and warrant further investigation.

Detection of Dysplastic ACF in Mice

As in humans, dysplastic ACF arise early in a unique strain of multiple intestinal neoplasia ($Apc^{+/Min-FCCC}$) mice established by this group (Fig. 1). $Apc^{+/Min-FCCC}$ mice develop colorectal adenomas at a higher multiplicity (3.8 ± 0.3 , mean \pm SEM) than that reported by others for the C57BL/6J strain (1.1 ± 0.1) (33). At 7 weeks of age, the incidence of dysplastic ACF is 45% and increases to 65% by 21 weeks of age (34). The average multiplicity of dysplastic ACF is 0.63 per mouse. Of note, based on our experience, full-length colons that have been “bread loafed” are best suited for the histologic identification of dysplastic ACF, as compared to “jelly rolls.” Unfortunately, few mouse models of colorectal carcinogenesis have been evaluated for the presence of dysplastic ACF.

Although stains can be used to accentuate the irregular topography of ACF (enlarged crypts, thickened epithelial lining, elliptical luminal openings), detection of dysplastic regions is much more challenging. Decolorization of carcinogen-treated rat colons with 70% methanol following incubation with 0.2% methylene blue led to a differential staining pattern and facilitated the detection of aberrant crypts harboring dysplasia (35). Dysplastic regions were more likely to retain methylene blue after destaining than normal or nondysplastic crypts, most likely due to a higher DNA content and cell density. This demonstrated success dictates further investigation of this method for identifying small dysplastic lesions without the need for histology.

Identification of areas within the colonic mucosa that harbor aberrations in RNA or protein expression, prior to gross evidence of tumor formation, remains a promising strategy for the earliest detection of dysplasia. However, selection of an optimal cellular alteration that can be monitored in real time with great specificity and sensitivity has been challenging. This group (36) and others (37) have focused on the overexpression of MMPs as an early event in colorectal tumorigenesis. In Apc^{Min} mice, matrilysin (MMP-7) mRNA was detected in 88% of adenomas (not present in the normal colonic mucosa) and localized to the luminal surface of dysplastic glands instead of in the extracellular matrix as expected (38). Knockout of MMP-7 in Apc^{Min} mice caused a 58% reduction in the multiplicity of adenomas, as well as a decrease in tumor size. The ability of MMP-7 to impact the rate of tumor growth, by

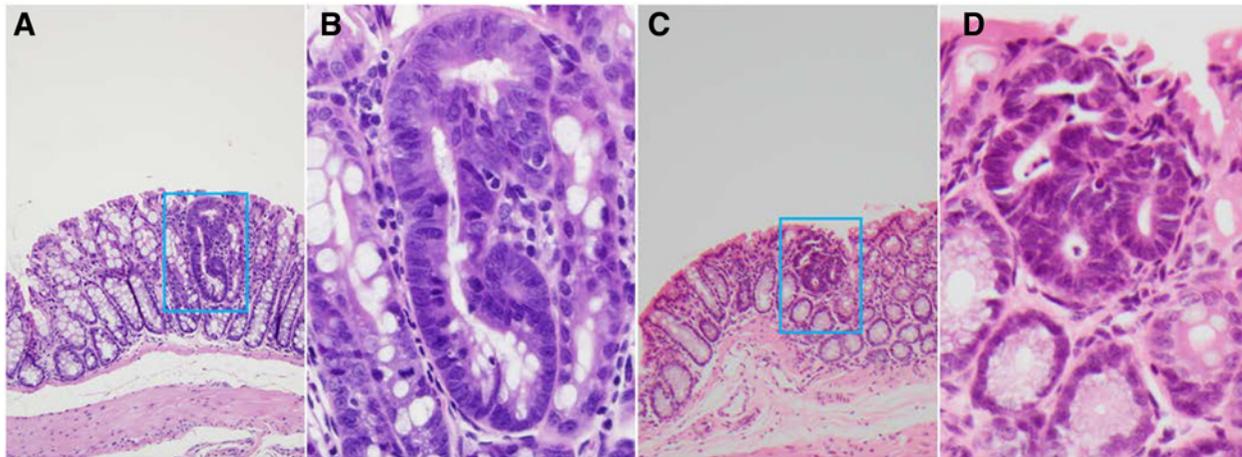


Figure 1. Dysplastic ACF (microadenoma) within the colonic mucosa of $Apc^{+/Min-FCCC}$ mice. Panels show a representative single crypt (A) and a three-crypt (C) microadenoma (100 \times). B and D, A high-power view (400 \times) of the same microadenoma, respectively.

activating luminal or membrane-bound cytokines and growth factors or as the result of tumor-induced changes in its secretion and access to potential substrates, has been postulated. In humans, MMP-7 was upregulated very early in dysplastic colonic epithelial cells, including low-grade dysplastic adenomas, and its concentration increased with grade of dysplasia (39). When combined, these data suggest that MMP-7 plays an important role in the earliest stages of colon tumor development.

Dysplastic ACF have been detected in tumor-prone $Apc^{+/Min-FCCC}$ mice by this group using bioactivatable MMP probes that employ near-infrared fluorophores as optical sensors (Visen Medical/PerkinElmer) (36). In these mice, transcript levels of *MMP-7*, a downstream target of TCF signaling, are 100-fold higher in colorectal adenomas as compared with those of the nonneoplastic colonic mucosa (40). Injection of $Apc^{+/Min-FCCC}$ mice with MMPsense 680, a near-infrared bioactivatable probe, resulted in its proteolytic cleavage in discrete areas of the colon by a wide array of MMP isoforms and the detection of fluorescent signal. In addition to detecting all grossly visible polypoid lesions, the probe identified 50% of the earliest nonpolypoid lesions (1–2.4 mm) and 25% of the dysplastic ACF (0.05 mm), as confirmed by histology (Fig. 2) (36). All dysplastic ACF (≤ 4 crypts) exhibited cytoplasmic localization of MMP-7 in the epithelial compartment (Fig. 3). These data demonstrate the feasibility of using molecular image-based probes to detect dysplastic ACF *in vivo* within the murine colonic mucosa. The current need for intravenous delivery of the probe and the unknown long-term safety of the agent remain barriers to the clinical translation of this approach.

Genetic Aberrations in ACF

The genetic alterations that arise in ACF vary depending upon the transgenic (e.g., Apc^{Min}) or carcinogen-induced

model being studied. For example, AOM induces mutations in both β -catenin and *K-ras* (41). Thus, the resulting mutational spectra may not mimic the natural course of ACF formation and progression. For this reason, the following section focuses on only human ACF.

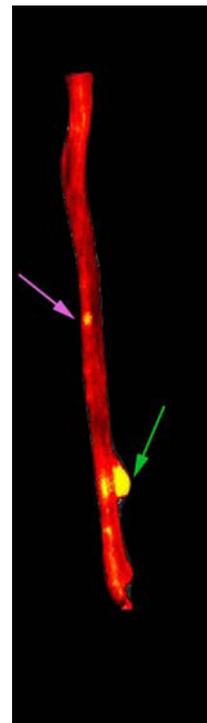


Figure 2. Fluorescent image of a colon excised from an $Apc^{+/Min-FCCC}$ mouse, generated using the IVIS Spectrum. The upper colon lesion (purple arrow) was not visible grossly, while the lower lesion (green arrow) was detected at the time of necropsy.

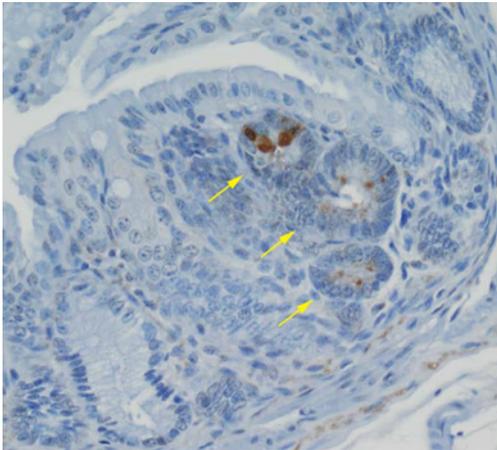


Figure 3.

IHC staining of MMP-7 of a colonic microadenoma in an *Apc^{+/Min-FCC}* mouse. The microadenoma (arrows) exhibits expression of MMP-7, while the nonneoplastic crypts are negative for MMP-7 (400 \times).

Dysplastic and hyperplastic ACF from subjects with heritable (FAP) or sporadic colorectal cancer possess distinct profiles of *APC* and *K-RAS* mutations (**Table 1**). *K-RAS* mutations are found in the majority of hyperplastic ACF (67%–100%) irrespective of the population, while FAP patients harbor dysplastic ACF with *K-RAS* mutations less frequently than patients with sporadic colorectal cancer (0%–13% vs. 40%–67%, respectively) (10, 42–45). *APC* mutations are rarely found in hyperplastic ACF (0%–11%) from patients with either FAP or sporadic colorectal cancer, and are completely absent in dysplastic ACF from those with sporadic disease. As expected, all dysplastic ACF from patients with FAP possess *APC* mutations. Similar to *K-RAS*, mutation of *BRAF* is not a common event in ACF (0%–12.5%), irrespective of the lesion subtype or patient population (46, 47).

Even subjects who are not at high risk for colorectal cancer develop ACF that bear mutations in key tumor suppressor genes and oncogenes. Mutations in *APC*, *BRAF*, and *K-RAS* were found in dysplastic ACF from “healthy” adults at a frequency of 75%, 12.5%, and 12.5%, respectively. Although these subjects did not have a history of colorectal cancer or meet the Amsterdam criteria for FAP or HNPCC, 25% had a history of colorectal polyps (26). Synchronous polyps were

Table 1. Profile of *K-RAS* and *APC* mutations in human ACF^a.

ACF	Mutation			
	<i>K-RAS</i>		<i>APC</i>	
Dysplastic	FAP 0%–13%	Sporadic CRC 40%–67%	FAP 100%	Sporadic CRC 0%
Hyperplastic	Abundant (67%–100%)		Rare (0%–11%)	

Abbreviation: CRCF, colorectal cancer.

^aBased on refs. 10, 42, 43, 44, 46, 47.

identified in approximately half (54.7%) by high-definition chromoendoscopy. Analysis of ACF for a large panel of driver mutations revealed mutations in *BRAF*, *K-RAS*, *N-RAS*, and *ERBB2* (8.3%, 16.7%, 8.3%, and 4.2% respectively), but not *APC* in hyperplastic ACF. In summary, *APC* mutations are common in dysplastic ACF from healthy adults, while *K-RAS* mutations are the more frequent mutation in hyperplastic ACF. Collectively, these data indicate that *APC* and *K-RAS* are the key genetic drivers of ACF formation.

With the advent of genomic technologies has emerged new opportunities to characterize ACF with respect to methylation status, microsatellite instability (MSI) and loss of heterozygosity (LOH). Methylation of *p16*, *MINT1*, *MINT2*, *MINT31*, *MGMT*, and *MLH1* was found more frequently in dysplastic ACF from patients with sporadic colorectal cancer than in patients with FAP (45). Methylation of *PTCH1* was present in 64.8% of dysplastic ACF and 19.8% of nondysplastic ACF from healthy subjects and subjects with colon polyps, adenomas, or cancer (48). Although the frequency of MSI and LOH of *APC*, *PTPRJ*, *TP53*, and *DCC* were similar in dysplastic and nondysplastic ACF (49), MSI analysis using five mononucleotide repeat targets (BAT-25, BAT-26, NR-21, NR-24, and NR-27) showed a larger percentage of hyperplastic versus dysplastic ACF were MSI-high (35% vs. 13.8%, respectively) (26).

Biomarkers of Therapeutic Response

Preclinical models

Numerous preclinical studies have used ACF as a surrogate endpoint when evaluating the efficacy of a chemopreventive agent. However, most studies report the total number of ACF without confirming the presence (or degree) of dysplasia, either by histology or using stains specific for mucins. Such evaluations are critical for comprehensive interpretation of the data, as most ACF do not progress to colorectal adenomas.

The ability of various dietary interventions to modulate the growth of dysplastic ACF has been documented in several studies. Administration of a high-fat diet (20%), a known risk factor for colorectal cancer, to AOM-treated rats increased the number of animals with dysplastic ACF by 53%, as compared with controls maintained on a standard AIN93M diet (50). As anticipated, a corresponding increase in tumor incidence was also observed in the same animals. In contrast, dietary supplementation of a high-fat diet with polyphenol polyphenon E (PPE), a purified extract of green tea catechins, for 8 weeks caused a dose-dependent reduction in the number of histologically confirmed dysplastic ACF within the colon of AOM-treated rats (51). Most impressive was the ability of PPE to inhibit ACF with high-grade dysplasia by more than 50% ($P < 0.05$). These data are consistent with clinical, epidemiologic, and preclinical studies that have established an association between consumption of green tea and a decreased risk of colorectal cancer (52–54). The number of mucin-negative ACF

was 65% lower in dimethylhydrazine (DMH)-treated animals fed a diet containing lycopene (300 ppm), a carotenoid found in tomatoes, as compared with those on a diet without lycopene (55). Recently, the effect of apiaceous and cruciferous vegetables on sialomucin-expressing and mucin-depleted ACF was examined in DMH-treated rats (56). Rats fed apiaceous vegetables (celery and parsnips) for 10 weeks developed approximately 20% fewer total dysplastic (sialomucin-expressing plus mucin-depleted) ACF, as compared with those fed a basal diet ($P < 0.05$). Likewise, the density of dysplastic ACF (number/cm²) was reduced, although not significantly, in animals consuming the cruciferous vegetable diet, as compared with untreated controls ($P = 0.075$). These data provide additional support for the use of dysplastic ACF as surrogate biomarkers of the preventive or promotional properties of dietary interventions *in vivo*.

Preventive agents have been identified that specifically target dysplastic ACF (microadenomas) in mice. This discovery is most exciting, and holds great promise for (i) intervening very early in the carcinogenesis process; and (ii) using these lesions as biomarkers of early drug efficacy in preclinical tumor models. However, it should be noted that the ability of an agent to inhibit the formation of dysplastic ACF is predicated, in part, by the presence or absence of adenomas at the time of treatment initiation. Chronic exposure of *Apc*^{+/*Min*-FCCC} mice to atorvastatin (100 ppm for 14 weeks) completely inhibited the development of dysplastic ACF in mice that did not have gross colon tumors at the time of study enrollment, as confirmed by colonoscopy. As expected, colon tumor incidence was also reduced significantly (32%) in mice treated with atorvastatin as compared to untreated controls (34). Interestingly, a similar reduction was not observed in *Apc*^{+/*Min*-FCCC} mice that received the same regimen of atorvastatin (100 ppm for 14 weeks) but possessed colorectal adenomas at baseline colonoscopy. Thus, the chemopreventive activity of an agent can vary depending on the tumor status of the animal at treatment initiation. This finding not only stresses the underlying importance of knowing the tumor status of all animals at baseline, but suggests that the inhibitory activity of specific agents may have been missed in the past due to the lack of attention given to dysplastic ACF as an endpoint and/or the characteristics of the population being treated. These data underscore the importance of tailoring preventive interventions based on the risk profile of the target population to achieve maximal protection from cancer and ensure safety.

Administration of a vaccine against MASH2, the murine ortholog of the basic helix-loop-helix transcription factor Human achaete scute homolog 2 (HASH2), to tumor-prone *Apc*^{+/*Min*-FCCC} mice led to significant inhibition of dysplastic colorectal ACF (57). HASH2 plays a critical role in controlling stem cell fate in the non-neoplastic intestine and is overexpressed in the majority of colorectal cancers (58, 59). Similar to the atorvastatin intervention described above, tumor inhibition was observed only when the

immunotherapy was introduced prior to the formation of gross adenomas. Prophylactic use of recombinant MASH2 protein combined with AS15 immunostimulant caused an approximate 3-fold reduction in the multiplicity of dysplastic ACF in *Apc*^{+/*Min*-FCCC} mice, as compared to controls injected with only buffer (57). No significant effect was apparent when the immunotherapy was delivered to animals with established colorectal adenomas. Thus, although dysplastic ACF most likely continue to develop within the colorectum throughout the life of *Apc*^{+/*Min*-FCCC} mice, it appears a small window of opportunity exists during which their growth can be interrupted successfully.

Clinical chemoprevention trials

Although several therapeutic trials have been conducted in humans using ACF as an endpoint, very few have specifically evaluated the response of dysplastic ACF to the intervention. Shpitz and colleagues (60) assessed the effect of chronic administration of aspirin on the histologic characteristics and distribution of ACF in patients with colorectal cancer. Samples of normal colon, at least 2 cm from the tumor margin, were collected from 59 patients who had been treated with aspirin (56 subjects, 100 mg/day; 3 subjects, 325 mg/day) on a regular basis for at least one year (median exposure 48 months) and 135 patients who were not taking aspirin or any other NSAID (control group). The overall incidence of histopathologically confirmed ACF was reduced 47% in patients treated with aspirin as compared with controls. In the left colon, aspirin not only reduced the percentage of samples with ACF by 52.5% ($P < 0.0001$), but significantly decreased the density of ACF by 82% ($P < 0.01$) as compared to those in the control group. Most importantly, the percentage of ACF that were dysplastic was reduced 48% from that of the control group. This result did not achieve statistical significance most likely due to the small sample size. A similar trend was observed in the right colon.

Our inability to readily identify which ACF are dysplastic *in vivo* has severely compromised their use as a biomarker of colorectal cancer risk in humans. Likewise, few studies have assessed the impact of therapy on the development of dysplastic ACF retrospectively in banked specimens. In light of this technical challenge, numerous preclinical and clinical studies have employed total ACF as a surrogate biomarker of cancer risk and therapeutic response. As summarized in **Table 2**, the impact of preventive agents on total ACF and colon tumors in animal studies is highly consistent. Such a correlation is less clear when comparing the effect of the same agent/drug on (i) total ACF in preclinical versus clinical studies or (ii) total ACF versus colorectal adenomas following a clinical intervention. Although patient characteristics, drug dose, treatment length, and agent bioavailability could contribute to these inconsistent findings, the total ACF, irrespective of presence of dysplasia, may not be sufficiently robust to serve as a biomarker of tumor response, especially in humans.

Table 2. Effect of select agents/drugs on total ACF and colon tumors in preclinical and clinical studies.

Agent/drug	Preclinical studies		Clinical studies	
	Total ACF (model)	Adenoma/cancer (model)	Total rectal ACF (subject population)	Adenoma (subject population)
Aspirin	↓ AOM (61-65) ↓ DMH (66)	↓ DMH (67) ↓ AOM (65, 68, 69) ↓ AOM-DSS (70) No effect (AOM) (71)	↓ CRC (60)	↓ CRC (72) ↓ adenomas (73) ↓ Nonsmokers with adenoma or adenocarcinomas (74)
Celecoxib	↓ DMH (66, 75)	↓ AOM (68) ↓ Apc ^{Min} (76, 77)	No effect (adenomas) (12)	↓ Adenomas (78)
DFMO	↓ AOM (79, 80)	↓ AOM (62, 71, 80, 81) ↓ Apc ^{Min} (77)		
Sulindac	↓ AOM (82)	↓ Apc ^{Min} (77) ↓ Pirc (83) ↓ Size only (Apc ^{Min-FCCC}) (34)	↓ No history of polyps (84) No effect (CRC or advanced adenomas) (13)	↓ FAP (85-88) ↓ No history of polyps (84)
DFMO + Aspirin		↓ AOM (62)	↓ CRC or advanced adenomas (89)	No effect (CRC or advanced adenomas) (89)
DFMO + Sulindac		↓ Apc ^{Min} (77)		↓ Adenomas (90) Ongoing (FAP) (91)
DFMO + Celecoxib		↓ Apc ^{Min} (77)		No better than celecoxib alone (FAP) (92)
Etodolac (COX-2 inhibitor)	↓ AOM (82)	↓ AOM-DSS (93)	No effect (no history of polyps) (84)	No effect (no history of polyps) (84)
Atorvastatin	↓ Dysplastic ACF (baseline tumor-free Apc ^{Min-FCCC}) (34)	↓ AOM (68) ↓ Apc ^{Min} (76) ↓ Incidence (baseline tumor-free Apc ^{Min-FCCC}) (34)	No effect (CRC or advanced adenomas) (13)	
Curcumin	↓ AOM (94, 95) ↓ DMH (75, 96)	↓ AOM (97, 98) ↓ AOM-DSS (99)	↓ Smokers, no history of polyps (100)	No effect (FAP) (101)
Metformin	↓ DMH & diabetic (102) ↓ DMH (103) ↓ AOM (104)	↓ DMH & diabetic (102) ↓ AOM (104)	↓ Nondiabetic, no CRC (105) ↓ Impaired glucose tolerance, no polyps (106)	↓ Nondiabetic adults with adenomas (107)

Abbreviations: CRC: colorectal cancer; DFMO: difluoromethylornithine; DMH: dimethylhydrazine; DSS: dextran sulfate sodium; Apc^{Min-FCCC}: Fox Chase Cancer Center strain of Apc^{Min} mice.

In summary, few preclinical efficacy studies have utilized dysplastic ACF as a primary experimental endpoint. Emerging data from this group and others suggest that dysplastic ACF/microadenomas represent a promising biomarker of very early response to preventive intervention. Successful identification of agents that target dysplastic ACF holds promise for intervening even earlier in the carcinogenesis process, prior to the formation of gross lesions. The feasibility of using dysplastic ACF as an intermediate endpoint of drug efficacy in a clinical setting will rely on the development of new image-based strategies with which to detect their presence in the nonneoplastic colonic mucosa.

Future Perspectives

Recent advances in next-generation sequencing have provided new insight into the genetic basis of colorectal tumors. Application of this technology to colonic epithelial cells microdissected from dysplastic ACF will reveal the earliest events associated with tumor initiation and the preneoplastic state. Future studies should also focus on identifying those genes that are differentially expressed in dysplastic ACF versus adjacent nonneoplastic colonic mucosa using whole

transcriptome profiling (RNASeq). Such alterations are anticipated to inform the (i) development of chemopreventive strategies with which to disrupt the earliest molecular alterations that lead to tumor formation and (ii) selection of potential biomarkers of the responsiveness of early lesions to therapeutic regimens. Our success in translating these data to a clinical setting relies heavily on the generation of a non-invasive molecular imaging approach for the accurate identification of early dysplastic lesions *in situ*. Although chromoendoscopy has become an effective method for detecting ACF during routine endoscopy, further development of an image-based method to detect dysplastic ACF without a tissue biopsy is anticipated to revolutionize colorectal cancer screening and significantly reduce the morbidity and mortality associated with this prevalent disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Dysplastic Aberrant Crypt Foci: Biomarkers of Early Colorectal Neoplasia and Response to Preventive Intervention

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