

HISTOLOGIC AND MOLECULAR PROGRESSION IN COLONIC CARCINOGENESIS

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INTRODUCTION

Sporadic colorectal adenocarcinomas can arise via two well-known genetic pathways. In about 85% of patients tumors develop as a result of genetic alterations in the APC/B-catenin/Tcf-4 signaling pathway, which ultimately results in the development of chromosomal instability. The multistep sequence of genetic alterations in this pathway of carcinogenesis, first elucidated by Fearon and Vogelstein, involves sequential genetic alterations involving the APC/ β -catenin, KRAS, TP53, and DCC genes (among others). These molecular events have been conceptually correlated with histologic progression from early adenoma to carcinoma in situ to invasive carcinoma (1).

The second pathway of tumorigenesis, which is responsible for about 15% of sporadic colon cancers, involves damage to the DNA mismatch repair gene system, resulting in microsatellite instability and the accumulation of mutations in a distinct group of genes. However, the corresponding histologic progression from early precursor lesion to invasive tumor has not been well established for this pathway. Published data from a variety of studies suggesting that a distinct multistep histologic progression does occur in this setting is presented in the following discussion.

ABERRANT CRYPT FOCI (ACF)

Occurrence in Human Colons:

Aberrant crypt foci are defined by their macroscopic appearance in colonic mucosa stained with methylene blue. They consist of groups of crypts with a distinctive configuration, due to an increase in diameter and sometimes slit-like or serrated luminal openings. They occur in clusters of 3-4 to more than 250. It has been suggested that ACFs form as a result of impairment of

the normal process of crypt fissioning (2). Histologically they most often have features reminiscent of a hyperplastic polyp, with increased mucin content and a serrated crypt configuration. However, a subset of crypts exhibits dysplastic change indistinguishable from that of an adenoma (3). There is also some data to suggest transition (? progression) from a hyperplastic to a dysplastic appearance (3,4).

The occurrence of ACFs in human colons has been well-documented (5,6). In two studies of colon resection specimens (7,8) ACFs were identified more frequently in the left colon than in the right colon. In one of the studies (7) ACFs were actually more common in non-neoplastic colons than in colons resected for cancer. However, in both studies the subgroup of *dysplastic* ACFs were more common in tumor bearing colons, and the ACFs were of greater size. ACFs have also been detected in the colons of patients with familial adenomatous polyposis, and are almost always dysplastic in that setting.

ACFs can be detected *in vivo* utilizing a magnifying colonoscope to closely examine the mucosa after it has been sprayed with methylene blue. The prevalence and number of ACFs increased with the age of the patient in one such study (9). Moreover, treatment with sulindac led to a significant decrease ($P < 0.001$) in the number of ACFs (with complete disappearance in 7 of 11 treated patients).

Molecular Pathology of ACFs

Over the past 20 years data has accumulated that suggest that ACFs, or at least a subset of them, are precursor lesions in the development of colonic adenocarcinoma. ACFs have been demonstrated to be clonal by the HUMARA analysis, although only 11 ACFs were analyzed, and all showed either "atypia" or dysplasia (9). It must be emphasized, however, that small groups of cells in normal human tissues may be of clonal origin. Several groups have also documented a higher proliferative labeling index in ACFs when compared to surrounding normal mucosa (10,11).

Several groups of investigators have examined the incidence of APC and K-*ras* mutation in ACFs in humans (12-17). In the most recent study (18) K-*ras* codon 12 mutations were identified in 82% of "sporadic" non-dysplastic ACFs, with no significant difference in the rate for ACFs collected from colons that were normal, had adenomas, or carcinomas. Interestingly, the

rate of *K-ras* mutation in sporadic *dysplastic* ACFs was actually lower, at 63%, which was about the same as for sporadic small (< 5 mm) adenomas (64%). None of the sporadic ACFs exhibited APC or β -catenin mutations (compared to rates of 71% and 8.3 % respectively for small adenomas). However, abnormal cytoplasmic and (rarely) nuclear accumulation of β -catenin has been identified in a minority of ACFs (particularly dysplastic ones) by immunohistochemical means (19).

Microsatellite instability is a common finding in ACFs from HNPCC patients but is detected in a minority (10-22.5%) of ACFs in colons with sporadic cancers (20-22). Loss of hMLH1 or hMSH2 protein expression was not evident, however, in the nine ACFs examined (22).

HYPERPLASTIC POLYPS

Although generally considered innocuous by surgical pathologists, a significant rate of *K-ras* mutation (22-47%) has been documented in apparently ordinary hyperplastic polyps (10,23,24). A variety of other genetic alterations have been described in these polyps, including mutation of the TGF-B RII gene and deletions on chromosome 1p (25-27). The proliferation rate in the epithelium of hyperplastic polyps is greater than normal colonic mucosa (11). In addition, microsatellite instability has been identified in 29% of hyperplastic polyps in one study (28).

The significant rate of genetic abnormalities of various types in hyperplastic polyps has led to a reconsideration of their premalignant potential. It has also led to a careful re-examination of the histologic features of these polyps, in the hopes of identifying characteristics that would allow for the separation of innocuous lesions from the (presumably smaller) subset that might represent a precursor lesion in colon carcinogenesis (29,30). Because of the similarity of the architectural configuration of some ACFs, hyperplastic polyps, and serrated adenomas, it has been postulated that this may represent a sequence of precursors for colon tumor development (see discussion below). Nonetheless, the high prevalence of hyperplastic polyps in the human colon in comparison to the much lower incidence of colon cancer is *de facto* evidence that the majority of hyperplastic polyps are indeed non-progressive lesions of no clinical consequence.

SERRATED ADENOMA

Serrated adenomas were first recognized as a distinct entity in 1990 (31). They are most common within the left colon, particularly the rectosigmoid (32,33). The presence of foci of carcinoma in situ, intramucosal adenocarcinoma, and invasive adenocarcinoma in examples of these polyps provides incontrovertible evidence of their malignant potential (31-34). Although the histologic features of serrated adenomas have been well documented, there is still considerable confusion regarding the precise separation from hyperplastic polyps and traditional adenomas. It appears, for instance, that at least some cases of so-called "hyperplastic polyposis" actually represent "serrated adenomatous polyposis". (35). *Mixed polyps* containing separate areas with typical features of hyperplastic polyp, serrated adenoma, and conventional adenoma, are well documented.

There are significant differences in the rates of some molecular alterations in serrated adenomas and hyperplastic polyps. For instance, p53 mutations are frequent (about 50%) in serrated adenomas (particularly in areas of high grade dysplasia or carcinoma), and are virtually never present in hyperplastic polyps (34,36,37). The data regarding the rate of K-*ras* mutation in serrated adenomas is conflicted, with reported incidences of 58%, 8.3% and 5% (34,37,38). The cause of this wide range is unclear. As is true for hyperplastic polyps, APC mutations are extremely uncommon (<5%) in serrated adenomas (37,39). Microsatellite instability was identified in 53% of serrated adenomas in one study, and was most often MSI-L (28). Mixed polyps in that study had an even higher rate of MSI (83%).

COLON CANCER - Microsatellite instability phenotype

While 85% of all colonic cancers develop as a result of alterations of the APC/B-catenin/TCF-4 pathway, the remainder appear to arise via defects in the mismatch repair gene system, resulting in microsatellite instability. Tumors with MSI can develop due to a germline mutation in a mismatch repair gene (HNPCC), or as a result of somatic alterations (primarily hypermethylation of the promotor region of a mismatch repair gene). While early studies suggested that HNPCC was responsible for 3-6% of colon cancers (40), more recent studies have reported a prevalence of no more than 1-2% (41-44). Thus, the overwhelming majority of MSI-H tumors occur as

a result of somatic alterations in a mismatch repair gene, virtually always hMLH1 (45). There are characteristic histologic features that allow identification of MSI-H tumors (as opposed to MSS tumors), including medullary, mucinous or signet ring cell differentiation, intraepithelial lymphocytosis, and poor differentiation (46). While these features are fairly specific for MSI-H tumors the sensitivity is poor, and thus confirmation with MSI testing is required (46). A study has also been performed to identify clinical and histologic features that might allow distinction between HNPCC and sporadic MSI-H tumors. In a multivariate analysis advanced age at diagnosis and presence of tumor heterogeneity were independently associated with sporadic MSI-H tumors, and peritumoral and tumor infiltrating lymphocytes were associated with HNPCC tumors. In addition, the adenomatous component adjacent to sporadic MSI-H tumors more often has a serrated appearance, while HNPCC tumors are bordered by conventional adenomatous mucosa (45,47).

Serrated Pathway of Colonic Carcinogenesis - Jass Hypothesis

JR Jass has proposed a distinct pathway of colonic cancer progression from ACF to hyperplastic polyp (HP) to serrated adenoma (SA) to sporadic MSI-L (and some MSI-H) tumors (48-50). Besides the common element of serrated architecture across this spectrum, there are a number of histologic and molecular features that support such a paradigm. First, transitional histologic forms exist between all of these lesions. Hyperplastic ACFs and HPs are histologically indistinguishable except for the larger size and more polypoid configuration of the polyps. In a comprehensive study (10) comparing these two lesions the patterns of lectin binding and PCNA expression were quite similar, and distinct from those of conventional adenomas. However, on the basis of a significant difference in the rate of *K-ras* mutation (40% for HP and 67% for hyperplastic ACF) the authors concluded that they were distinct entities, and proposed the term "*heteroplastic ACF*" to avoid any implication of a direct relationship between these lesions (10). On the other hand, the rates of MSI in ACFs and HP are quite similar (28).

SAs commonly contain a component of pure appearing hyperplastic polyp (28). Also, the pattern of mucin gene expression in HPs and SAs is quite similar (MUC 2+, MUC5AC+, MUC4-), and is distinct from that expressed in conventional adenoma (33,51,52). Identical patterns of mutation in MSI

markers have been demonstrated in the hyperplastic and adenomatous components of mixed polyps (28). Moreover, one study of sporadic MSI cancers revealed a significantly higher incidence of contiguous SA than was evident in the control group of MSS tumors (12% vs. 2.4%). In addition, the adjacent invasive component always had a similar serrated architecture (47).

Intriguing patterns of molecular alterations can be discerned from the data presented herein which provide evidence for this serrated pathway of histologic progression. Alterations in the APC/ β -catenin/TCF-4 are very uncommon in sporadic hyperplastic ACF, HPs, SAs, and MSI tumors. Conversely, there is a significant rate of MSI (usually MSI-L) in all of these lesions [ACF (10-22%), HPs (29%) SAs (53%) and mixed polyps (83%).]. In addition, the incidence of *K-ras* mutations is high in all of these lesions [88 % in sporadic ACFs, 22-47% in HPs, 5-58% in SA and 54% in MSI-L tumors (54)]. However, MSI-H tumors exhibit a significantly lower rate of *K-ras* mutation (7%), suggesting an alternative pathway of initiation and early progression for these tumors (53).

Clearly much more work must be done to clearly elucidate the genetic pathways responsible for the development of MSI tumors in the colon. The participation of surgical pathologists in these studies will be essential in order that the genetic events are correlated with precursor lesions that precisely defined histologically. It is likely that there are subtypes of ACFs, HPs and SAs that heretofore have not been recognized as separate entities. For instance, it has been suggested that large sessile serrated lesions in the proximal colon may be the precursors for MSI-H tumors and smaller serrated lesions in the rectum may progress to MSI-L tumors (28,53).

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