Serrated Pathway and APC (Conventional)-Type Colorectal Polyps

Molecular-Morphologic Correlations, Genetic Pathways, and Implications for Classification

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Abstract

This review addresses the genetic mutations and cell signaling pathway alterations in colorectal premalignant polyps, focusing on the link between molecular changes and morphologic features. Biallelic APC (adenomatous polyposis coli) mutations are directly responsible for the specific and characteristic cytologic features of dysplastic cells in conventional tubular adenomas. Sessile serrated adenomas (SSAs) are the precursor lesions of the serrated neoplasia pathway. The BRAF activating mutation and hypermethylation of SLC5A8, which mediates short chain fatty acid transport, may be the important events in the genesis of SSAs. Intracellular butyrate inhibits histone deacetylase, allowing histone hyperacetylation and, eventually, transcriptional activation of specific genes. Decreased p21(WAF1/CIP1) and activation of the mitogen-activated protein kinase pathway may be the key intermediary alterations. Progressive loss of cell cycle control and decreased and altered cytoplasmic differentiation produce the characteristic constellation of morphologic changes of SSAs and traditional serrated adenomas.

Most colorectal adenocarcinomas arise via the tumor suppressor (chromosomal instability) or serrated neoplasia (mutator) molecular pathway. The tumor suppressor (chromosomal instability) pathway was first proposed by Vogelstein et al and Eide as a model of progression and transformation of adenomas to adenocarcinoma. Conventional tubular and tubulovillous adenomas are well-recognized, premalignant, precursor lesions of the tumor suppressor (chromosomal instability) pathway. The serrated neoplasia (mutator) pathway develops by the accumulation of insertion or deletion mutations throughout the genome, leading to genomic instability and resulting in microsatellite unstable-high (MSI-H) adenocarcinomas. Kambara et al first suggested that some hyperplastic-like polyps were precursors lesions in this pathway. A sufficiently large body of evidence has since accrued showing that 2 subtypes, the sessile serrated adenoma (SSA) and the serrated adenoma are bona fide premalignant lesions.

Some authors have used the term traditional before the term serrated adenoma to designate the Longacre and Fenoglio-Preiser described lesion.

Many of the genetic mutations and cellular pathway alterations underlying the development and progression to invasive adenocarcinoma have been delineated. Links between these specific molecular alterations and the morphologic features of colorectal precursor polyps have not been studied extensively. Examining the current body of knowledge in an attempt to link molecular changes with morphologic features may offer insight and a better understanding of their morphologic features and assist in the classification of serrated neoplasia pathway precursor polyps. This article attempts to link genetic mutations and alterations of cell signaling pathways with specific morphologic features of premalignant polyps of the tumor suppressor and...
serrated neoplasia (mutator) pathways. In addition, a molecular mechanism to explain many of the morphologic features of serrated neoplasia pathway polyps is proposed.

Discussion of these issues is divided into the following sections: (1) dysplasia molecular model: adenomatous polyposis coli (APC)-type (conventional) tubular adenomas; (2) dysplasia molecular model: serrated neoplasia pathway polyps; (3) p21(WAF1/CIP1) and BRAF and serrated neoplasia morphologic features; (4) high-grade serrated dysplasia; and (5) early and advanced serrated neoplasia pathway polyps.

Dysplasia Molecular Model: APC-Type (Conventional) Tubular Adenomas

Most practicing pathologists have a strong visual image of the cytologic appearance of conventional tubular and tubulovillous adenomas. The cytologic features of dysplastic epithelial cells constituting conventional tubular adenomas are similar in familial adenomatous polyposis–associated and solitary sporadic lesions. The morphologic similarity of tubular adenomas in these 2 disparate clinical settings is due to a similar set of genetic events. Although the mechanisms of mutation in familial adenomatous polyposis and sporadic tubular adenomas differ, adenomatous cells in both clinical settings have biallelic APC mutations as the key genetic event that produces the distinctive nuclear and cytoplasmic alterations termed adenomatous. In the context of linking molecular changes with morphologic features, the cytologic features of adenomatous dysplasia can be viewed as APC-type dysplasia. In both clinical settings of APC-type dysplasia, a truncated APC protein, via the Wnt/β-catenin/Axin pathway, results in altered apoptosis and cell-cycle control, which drives the neoplastic cell proliferation.12-17

The APC protein also controls microtubule function in the nucleus and cytoplasm. In the nucleus, microtubules attach to the kinetochore during mitoses. The spindle checkpoint surveillance mechanism monitors this attachment and ensures that all chromosomes are arranged linearly on the metaphase plate before allowing mitosis to proceed.18 Truncated APC protein produces abnormal microtubule attachment, resulting in a defective spindle checkpoint system that allows the cell to prematurely exit out of mitosis into anaphase.19-23 Abnormal chromosome segregation resulting from the premature exit, before each chromosome pair can segregate evenly, produces dicentric chromosomes that are potent initiators of chromosome instability.18,19 In the cytoplasm, truncated APC alters microtubule formation, bundling, and transport. β-catenin/TCF-4 also mediates the shift from progenitor to differentiation status and direction of differentiation in colonic epithelial cells.24-28 Perturbations of these cytoplasmic components produce an inappropriate stem cell phenotype with defects in enterocyte migration, abnormal junctional complexes, and decreased mucin production.29-31

The nuclear and cytologic features of APC-type dysplasia can be linked directly to APC alterations in the previously mentioned cell cycle control and cytoplasmic differentiation signaling pathways.32-38 A distinctive feature of APC-type dysplasia is its narrow range of nuclear and cytoplasmic alterations. The singular cytology that is APC-type dysplasia suggests that a single or few key regulatory and control genes directly result in its characteristic cytologic features. A single gene–single cytology relationship would explain the repetitive, consistent morphologic features of sporadic tubular adenomas in the numerous patients undergoing screening colonoscopies and in different types of clinical settings.

Each colonic mucosal crypt is a discrete clonal unit.39 In conventional tubular adenomas, APC-type dysplastic transformation occurs within the basilar stem cell population.40 Proliferative and survival advantages of the APC-type dysplastic cells result in a monoclonal cell population or so-called unicryptal adenoma.39 Cell proliferation in tubular adenomas has been shown conclusively to be a bottom-up growth process, with crypt fission the primary mechanism of polyp growth.39,41 Small conventional tubular adenomas are composed of a cluster of monoclonal APC-type dysplastic crypts.39

Dysplasia Molecular Model: Serrated Neoplasia Pathway Polyps

Right colon serrated neoplasia pathway polyps arise via the mutator mechanism. As a result, there is no direct association between specific genetic mutations and unique cytologic features. As in other studies, neither APC mutations nor elevated nuclear β-catenin levels were found in a study of serrated polyps associated with early MSI-H adenocarcinomas.42-48 Instead, several key genetic mutations seem to initiate, facilitate, and/or actively enhance progressively greater perturbations in cell-signaling pathways, resulting in a spectrum of specific cytologic features in the cells that form serrated neoplasia pathway polyps. It is this spectrum of cytologic features that accounts for the different types of serrated polyps.

The most common early genetic changes in right colon serrated neoplasia pathway polyps are the BRAF activating mutation and DNA cytosine-guanine base pair (CpG island) hypermethylation.5,49-53 One of the first genes linked to the serrated neoplasia pathway was the DNA mismatch repair complex component, hMLH1. Hypermethylation of its promoter gene causes decreased efficiency of the DNA mismatch repair complex.50,51,54-58 Mutations progressively accrue throughout the genome, eventuating in genetically unstable MSI-H adenocarcinomas.56,57,59,60 Although hMLH1 was one of the first to be studied, hypermethylation of the MLH1 promoter and subsequent...
microsatellite instability is predominantly a late-stage, advanced lesion event and is rare in small early SSAs. All nonmalignant SSA lesions reported in a study of serrated polyps associated with early MSI-H adenocarcinomas were microsatellite-stable, which is similar to the findings of previous studies.

Right colon serrated neoplasia pathway polyps have lacked a unifying conceptual model that links molecular and cell signaling alterations to specific morphologic features and progression to adenocarcinoma. Although Jass et al proposed a model of SSA, mixed hyperplastic/adenomatous polyp, traditional serrated adenoma, and adenocarcinoma that included hypermethylation and BRAF mutation as early events and hMLH1 down-regulation as a late event, the intervening genetic alterations and their association with specific cytotologic features or polyp morphologic features are unknown.

A recently characterized gene, SLC5A8, may be the potential key, early genetic alteration, along with BRAF, necessary for the development of right colon serrated neoplasia pathway polyps. The protein product coded by SLC5A8 mediates short chain fatty acid uptake into colonic epithelial cells. Butyrate, the most potent and prevalent short chain fatty acid in the colon, is produced by anaerobic bacterial fermentation of ingested fiber. Intracellular butyrate inhibits histone deacetylation, which allows the histones, primarily H3, to undergo hypermethylation and thereby facilitates transcription and increases in crypts as they leave the proliferating compartment and move up the crypt.83

Features Serrated Neoplasia Morphologic

p21(WAF1/CIP1) is a cyclin-dependent kinase inhibitor protein that regulates cell cycle control, apoptosis, and cell maturation. It induces cell arrest in the G1 phase of the cell cycle. Normal levels of β-catenin and TCF-4 protein expression allow p21(WAF1/CIP1) to be expressed, which inhibits crypt proliferation and causes mature colonicyte and goblet mucin cell differentiation. In the normal colon, p21(WAF1/CIP1) induces goblet cell differentiation and increases in crypts as they leave the proliferating compartment and move up the crypt. p21(WAF1/CIP1) causes colonicyte and goblet cell differentiation by activating extracellular signal-regulated kinase (ERK), which in turn, up-regulates trefoil factor (TFF)3 and MUC2 expression.

Evidence that supports SLC5A8 and BRAF as the key genes related to the development of serrated neoplasia morphologic features comes from several avenues. Concurrent SLC5A8 silencing and BRAF gene abnormalities do not seem to be a chance association. In thyroid papillary carcinomas, in which the BRAF activating mutation is a common early event, neoplasms with the BRAF mutation had a mean 63-fold lower than average SLC5A8 transcript level, whereas carcinomas without the BRAF mutation had a mean 5-fold lower than average transcript level. The authors suggested that methylation-associated SLC5A8 silencing most likely resulted from BRAF activation of the mitogen-activated protein kinase (MAPK) pathway and that SLC5A8 gene silencing may be a key factor in determining whether neoplastic transformation occurs.

Similar events and processes may be occurring in colonic serrated neoplasia pathway lesions. In the colon, CpG island hypermethylation is an early event in a subset of aberrant crypt foci lesions and in a spectrum of serrated polyps, including hyperplastic polyps, SSAs, and traditional serrated adenomas. CpG island methylation has been shown to increase along this pathway of histologic polyp progression. SLC5A8 seems especially sensitive to hypermethylation. Decreased or silent SLC5A8 expression due to hypermethylation is a frequent early event in a subset of aberrant crypt foci lesions, SSAs, conventional serrated adenomas, and microsatellite-unstable adenocarcinomas with chromosomal instability. Decreased or absent SLC5A8 transcription results in decreased H3 histone acetylation by inhibition of histone deacetylase, which in turn causes down-regulation of p21(WAF1/CIP1) transcription and decreased p21 protein. Decreased butyrate alone seems to be an insufficient down-regulator of p21(WAF1/CIP1), suggesting that SLC5A8 inactivation is a key factor in this process. In biallelic, APC-inactivated mouse colonic adenocarcinomas, decreased expression or complete inactivation of p21(WAF1/CIP1) results in increased cell proliferation and decreased apoptosis.

**p21(WAF1/CIP1) and BRAF May Account for Serrated Neoplasia Morphologic Features**

Decreased p21(WAF1/CIP1) transcription and the BRAF mutation–induced constitutive activation of the RAS/RAF/MEK/ERK pathway seem to produce β-catenin/TCF-4 switch-independent alterations in the colonic mucosa similar to the morphologic features of SSAs. Both genes act via different cell signaling pathways to inhibit p53-dependent apoptosis. In colonic mucosa, disruption of apoptosis leads to rapid accumulation of too many crypt epithelial cells. The limited surface area in the crypts causes cells to pile up and internally enfold, producing a serrated appearance when cross-sectioned. Dilation of crypt bases and internal serration that becomes apparent in the basilar region of crypts are 2 characteristic features of small sessile serrated pathway polyps (SSAs), suggesting that interruption of apoptosis is an early event. The early occurrence of the BRAF activating mutation in SSAs and its direct effect on apoptosis inhibition support this temporal relationship.
Decreased p21 protein may be the underlying cause of the expanded proliferation zone in serrated pathway polyps. p21\(^{WAF1/CIP1}\) functions to maintain cells in G\(_0\) and decreased p21 protein permits cells to remain in a state of continuous proliferation.\(^{96,97,99}\) This proliferating cell population would rapidly expand upward and replace preexisting nonproliferating cells. A characteristic feature of SSA dysmaturational crypts is an abnormal expanded proliferation zone, consisting of mitotically active, immature-appearing cells with minimal cytoplasm, large oval nuclei, and fine chromatin that extend into the mid or superficial crypt regions (dysmaturational crypts).\(^{10,98,100}\) These morphologic changes seem remarkably similar to the changes induced by down-regulated p21\(^{WAF1/CIP1}\) or absent p21 protein, in my opinion. The appearance of delayed cytoplasmic maturation in dysmaturational crypts may reflect the extent of expansion of proliferating zone cells.

Studies have shown that crypt epithelial cells of hyperplastic polyps, SSAs, and traditional serrated adenomas show gastric foveolar columnar mucin cell differentiation.\(^{101-103}\) Biochemically, these cells express TFF1 or TFF2 and MUC5AC mucin and have decreased expression of the colonic mucin cell marker, TFF3.\(^{101,102,104,106}\) The extent of gastric foveolar columnar mucin cell differentiation increases in more advanced lesions, with coexpression of gastric and colonic differentiation occurring at all levels of the crypt in traditional serrated adenomas, suggesting there is progressive loss of control over stem cell differentiation associated with morphologic progression.\(^{101}\)

Experimentally, cells that do not express TFF3 colonic-type mucins express gastric-type TFF1 and TFF2, and, conversely, cells that express colonic TFF3 protein stop producing TFF1 or TFF2.\(^{85,107,108}\) Increased TFF3 protein results in increased goblet cell differentiation.\(^{85}\) Decreased p21\(^{WAF1/CIP1}\) transcription results in decreased TFF3 and MUC2 expression and goblet cell differentiation in the colon.\(^{93,94}\) Constitutive activation of BRAF activates the MEK/MAPK pathway, which, in turn, results in decreased TFF transcription.\(^{109}\) It is possible that decreased colonic goblet cell differentiation and the variable extent of gastric columnar cell differentiation and mucin production may be due to a complex interplay of decreased p21\(^{WAF1/CIP1}\) and BRAF mutation–MEK/MAPK pathway activation.

Similar to the processes occurring in small conventional tubular adenomas, early-stage serrated neoplasia pathway polyps may arise via stochastic growth and crypt fission producing a small cluster of crypts lined by a monoclonal serrated-type dysplastic cell population.\(^{11}\) In larger conventional tubular adenomas, multiple monoclonal APC-type dysplastic cell populations develop, with each subclone populating a small region of adjacent crypts.\(^{39,110,111}\) The boundaries of each monoclonal cryptal region are not morphologically apparent because of the singular cytologic appearance of APC-type dysplastic cells.

A similar process may occur in larger serrated neoplasia pathway polyps, including SSAs and traditional serrated adenomas. Multiple monoclonal populations of serrated-type dysplastic cells, each with a distinctive and unique set of cytologic features, populate small regions of adjacent crypts. This model would account for the patchwork-like pattern of cytoplasmic differentiation in dysmaturational crypts within the nonmalignant serrated polyps associated with early MSI-H adenocarcinomas.\(^{42}\) Each of these regions was composed of a small group of dysmaturational crypts lined by similar-appearing cells that differed in their cytologic features from the cells lining the adjacent cluster of dysmaturational crypts. This model would also account for the focality of the high-grade dysplasia seen in the polyps.\(^{42}\)

### High-Grade Serrated Dysplasia

High-grade serrated-type epithelial dysplasia developed from crypt bases or within the mid-crypt region of the mucosa in the study of serrated polyps associated with early MSI-H adenocarcinomas that appears in this issue of the *Journal*.\(^{42}\) This has been observed by other authors.\(^{11}\) In normal gastric mucosa, the proliferative compartment is located in the mid-level foveolar region, resulting in bidirectional differentiation of cells.\(^{103,112}\) SSAs and traditional serrated adenomas undergo progressively greater degrees of gastric-type differentiation, including a shift in the location of the dominant proliferative compartment from basilar to the mid-crypt region and bidirectional cell differentiation toward the surface and basilar crypt regions.\(^{101,105,113}\)

In the cervix, high-grade dysplasia arises in the transition zone where the most active proliferation facilitates acquisition of the genetic mutations necessary for malignant transformation. It is possible that high-grade dysplasia arises from the basilar or mid-crypt regions in serrated polyps for a similar reason. High-grade dysplasia that develops from the mid-crypt region and extends upward to involve predominantly the superficial region of the serrated neoplasia pathway polyp might be arising in the most active proliferation region of the mucosa, and its mid-crypt location may reflect an advanced state of gastric foveolar cell differentiation with a shift in the proliferative zone compartment. The specific genetic mutations and cell cycle alterations, aside from hMLH1 hypermethylation, that lead to malignant transformation are unknown. Jass et al.\(^{59}\) raised the possibility that HPP1 was related in some way to neoplastic transformation. It is interesting that in experimental situations, p27\(^{kip1}\) inactivation can induce colonic adenocarcinomas independent of p21\(^{WAF1/CIP1}\) and APC\(\beta\)-catenin mutations or pathway activation.\(^{114}\) Other candidate pathways include the TGF-\(\beta\) pathway via SMAD mutations, hTERT, and p53.\(^{115,116}\)
Early and Advanced Serrated Neoplasia Pathway Polyps

Some dysmaturational crypt epithelial cells immediately adjacent to high-grade epithelial dysplasia in SSA polyps associated with early MSI-H adenocarcinomas resemble low-grade APC-type dysplasia.42 However, close inspection revealed that their nuclei were cuboidal rather than pencillate and basilar rather than pseudostratified, and their chromatin pattern was finely granular rather than uniformly coarse.

Longacre and Fenoglio-Preiser117 initially proposed the division of mixed colonic polyps into mixed hyperplastic/adenomatous polyps, in which the 2 histologic patterns were distinct and separate, and (traditional) serrated adenoma, in which serrated glands were lined by adenomatous epithelium. Although conceptually straightforward, distinguishing between hyperplastic/adenomatous mixed polyps and traditional serrated adenomas often is subjective and arbitrary (personal experience). Furthermore, deciding whether the epithelium of a serrated polyp is sufficiently “adenomatous” to warrant a diagnosis of traditional serrated adenoma or SSA if insufficiently “adenomatous” also often is arbitrary, especially if the cytologic definition of APC-type dysplasia is loosely applied.

A broad range of lesions fall under the diagnostic umbrella of traditional serrated adenoma. The cytologic features of the adenomatous epithelium in some traditional serrated adenomas are less than those in classic APC-type dysplasia, whereas other lesions appear to be SSAs with a component of dysplastic epithelium.118 The nonmalignant regions of the serrated polyp cases reported were morphologically similar, if not identical, to SSAs.98 By adding the areas of high-grade epithelial dysplasia, the studied lesions were morphologically identical to descriptions of traditional serrated adenomas.117 A recent study found that 85% of SSAs were hypermethylated compared with 70% of morphologically similar polyps with foci of dysplasia (small traditional serrated adenomas).51 A large body of evidence has accrued showing that the phenotypic expression, cell signaling pathway perturbations, and genetic mutation profiles are accrued showing that the phenotypic expression, cell signal-

Furthermore, these 3 lesions seem to be fundamentally the same morphologic lesion when areas of high-grade dysplasia are excluded from the comparison.

These observations and study results support the opinion of previous authors that SSAs, mixed hyperplastic/adenomatous polyps, and traditional serrated adenomas are related lesions at different stages along the serrated neoplasia pathway.11,50,54,58,121-123 An alternative possibility is that traditional serrated adenomas may be a divergent lesion within the serrated family of precursor lesions. In 1 study, BRAF mutations were found in 75% of lesions the authors regarded as SSAs, 20% of lesions the authors regarded as traditional serrated adenomas, approximately 19% of hyperplastic polyps, and in 0% of traditional tubular adenomas.5

The placement of SSAs and traditional serrated adenomas on the same molecular pathway should not be construed to mean they should be treated identically. The optimal clinical management of each lesion has not yet been determined. A morphologic classification system of serrated polyps has been suggested based on computer-assisted definition of distinctive histologic features.10 In my opinion, histologic subdivision of these lesions (microvesicular serrated polyp, goblet cell–type serrated polyp, mucin poor–type serrated polyp, and mixed microvesicular serrated polyp/goblet cell–type serrated polyp) should be based on outcome differences or therapeutic factors. Morphologic classification systems that are devoid of clinical significance are meaningless and often highly subjective.

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