"Microsatellite Instability and Serrated Adenomas in Common Practice"

United States and Canadian Academy of Pathology
Annual Meeting
Boston, Massachusetts
March 8, 2009

Robert E. Petras, M.D., FCAP, FACG
Associate Clinical Professor of Pathology
Northeastern Ohio Universities
College of Medicine

National Director for Gastrointestinal Pathology Services
AmeriPath, Inc.
7730 First Place, Suite A
Oakwood Village, Ohio
Phone: (866) 4GI-PATH
Fax: (440) 703-2155
Email: rpetras@ameripath.com
SERRATED POLYPS AND COLORECTAL ADENOCARCINOMA
Genetic Considerations, Microsatellite Instability and Lynch Syndrome
R.E. Petras, M.D.
Ameripath, Inc.

There may be at least five separate but overlapping molecular pathways to colorectal cancer (1). Approximately 20% of colorectal carcinomas appear to have a genetic basis (2,3). This latter group includes the 3% of cases related to Lynch syndrome (Hereditary Nonpolyposis Colon Cancer Syndrome [HNPCC]) and the 1% associated with familial adenomatous polyposis (FAP) and its variants. About 85% of colorectal cancers are thought to originate through the chromosomal instability pathway. These tumors typically demonstrate DNA aneuploidy and have abnormalities of chromosomes 5, 17, 18 and contain mutational changes in APC gene, K-ras proto-oncogene, DCC tumor suppressor gene and p53 tumor suppressor gene (4). Familial adenomatous polyposis colorectal carcinomas arise via this pathway. Approximately 15% of colorectal carcinoma appears to arise in the so-called “mutator phenotype”. These cancers tend to be DNA diploid and are associated with microsatellite instability. The Lynch syndrome cancers are associated with the “mutator phenotype”.

DNA integrity is essential for normal cell function. DNA insults can occur due to the direct effects of chemicals or radiation and are usually corrected through the excision repair system. DNA replication errors are of two types; 1) simple mispairing of nucleotides, the most common type, and 2) “slipping” errors, in which genes may contain too many or too few copies of repeat short DNA nucleotide sequences known as “microsatellites”. Normally, these errors are recognized, the cell cycle arrested and the mismatched segment corrected. For those errors not immediately corrected by DNA polymerase, the mismatch repair (MMR) system acts as a back-up for additional proofreading of DNA. Failure to repair mismatches allows the error (mutation) to persist and to become the template for subsequent DNA replication (5). The known mismatch repair genes and their relative frequency in Lynch syndrome are presented in Table 1.

TABLE 1

<table>
<thead>
<tr>
<th>GENE</th>
<th>FREQUENCY</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMLH1</td>
<td>49%</td>
<td>3p21</td>
</tr>
<tr>
<td>hMSH2</td>
<td>45%</td>
<td>2p15</td>
</tr>
<tr>
<td>hPMS2</td>
<td>4%</td>
<td>7p22</td>
</tr>
<tr>
<td>hPMS1</td>
<td>1%</td>
<td>2p32</td>
</tr>
<tr>
<td>hMSH6</td>
<td>1%</td>
<td>2p15</td>
</tr>
<tr>
<td>hMSH3</td>
<td>0%</td>
<td>5q11-13</td>
</tr>
</tbody>
</table>
Microsatellite instability (MSI) is best viewed as an epiphenomenon found in colorectal tumor DNA but not in non-neoplastic tissues. It indicates that extensive mutation exists in the non-encoding repetitive DNA sequences that are particularly prone to replication error, the microsatellites. The majority of MSI is linked to somatic inactivation of \textit{hMLH1} through hypermethylation inactivation of the promotor region; but it can also be detected in persons with germline mismatch repair gene mutations, the definition of Lynch syndrome (5,6). MSI is detected in 15% of colorectal cancers overall and is present in over 95% of the cancers found in patients with Lynch syndrome.

Lynch syndrome patients, because they have a germline mutation of a mismatch repair gene, are at increased lifetime risk for colorectal (up to 80%) and other cancers (3,6). These cancers develop at significantly younger ages (e.g., average age for colorectal carcinoma = 44 years) (6). Other Lynch syndrome related tumors include cancers of the endometrium, ovary, stomach, biliary tract, urinary tract, kidney, central nervous system, small bowel, and skin tumors (6).

Lynch syndrome patients and families can sometimes be identified by taking a careful patient and family medical history, can be suggested from the pathologic findings of excised tumors, and can be detected by direct evaluation of the mismatch repair system. Pathologic features of colorectal cancer that suggest MSI/Lynch syndrome include right-sided location, synchronous or metachronous large bowel cancers, large bulky polypoid tumors with circumscribed pushing margins, tumors showing prominent lymphoid infiltrate, cancers of poor differentiation (medullary or undifferentiated carcinoma) or mucinous and signet ring cell histology (2,3,7).

The diagnosis of Lynch syndrome is evolving. Originally, the Amsterdam criteria were used to clinically identify HNPCC including the Lynch syndrome patients (8). The original Amsterdam criteria include; a) three or more relatives with a colorectal cancer with at least one a first-degree relative; b) colorectal carcinoma in two generations; and c) one or more colorectal carcinomas occurring in a person less than 50 years of age. In order to increase the sensitivity, the Amsterdam criteria were modified (Amsterdam II criteria) to include; a) three or more relatives with any Lynch syndrome related cancer; b) colorectal carcinoma in two generations; and c) and one or more Lynch syndrome related carcinomas in a person younger than 50 years of age (9). There are many problems with detecting Lynch syndrome based upon the Amsterdam criteria alone. Patient histories are less useful now than in the past because of smaller family sizes. Excision of colorectal adenomas interrupts the adenoma-carcinoma sequence. Patients in whom the family history is unknown or incomplete limit the utility of these criteria. Physician history taking is often not thorough. More importantly, depending upon the cohort, up to 33% of persons having a germline mutation of a mismatch repair gene are Amsterdam criteria negative and only 60% of Amsterdam criteria positive kindred have a detectable mutation (9-16). These Amsterdam positive/gene mutation negative kindred are often referred to as familial colorectal cancer syndrome type X.

Special testing (MSI testing by polymerase chain reaction [PCR] or immunohistochemical stains) now augments the clinical criteria. Controversy over the use of MSI analysis has led to the development of the Bethesda guidelines for testing colorectal tumors for microsatellite instability. The latest iteration, the \textit{revised Bethesda guidelines} (12) requires than just one of the following criteria be met: colorectal cancer before age 50, synchronous or metachronous colorectal or other Lynch-related tumor regardless of age, colorectal cancer with MSI-high pathology in a patient less than 60, person with colorectal cancer and a first-degree relative with colorectal adenoma or carcinoma or other Lynch-related tumor (cancer less than 50; adenoma less than 40), colorectal cancer with two or more relatives with colorectal or other Lynch-related tumor regardless of age.

The American Gastroenterological Association (AGA) position states that genetic testing should be performed on families meeting Amsterdam criteria, on any affected person meeting the modified Bethesda guidelines, and on any first-degree relative of those with known mutations of mismatch repair
genes (3). They suggest that following pre-test genetic counseling and written informed consent, immunohistochemistry for MMR gene products and/or MSI testing by PCR be performed on tumor tissue. The international guidelines for evaluation of MSI by PCR recommend use of consensus markers; BAT25, BAT26, D5S346, D2S123, D17S250. If two or more markers are abnormal, the carcinoma is considered MSI-High (MSI-H). If one marker is abnormal, the tumor is classified as MSI-Low (MSI-L). If no markers are abnormal the cancer is referred to as MSI-Stable (MSS). Laboratories using more than 5 loci modify this classification with ≥ 30% - 40% abnormal defined as MSI-H, < 30% - 40% as MSI-L and none abnormal as MSS. Immunohistochemistry can be used to detect MSI. Almost all MSI-H cancers can be identified if the antibody panel includes MLH1, MSH2, PMS2 and MSH6 (13,16). Immunohistochemistry and MSI analysis by PCR each have advantages and limitations. PCR requires a molecular laboratory and usually requires normal tissue for comparison. Immunohistochemistry is more widely available but can be limited by poor tissue fixation or poor technique rendering interpretation difficult. Immunohistochemistry may be superior because the findings can direct gene sequencing and MSI is not always seen in Lynch syndrome kindred with MSH6 germline mutation (14). Patients with MSI-H cancer should undergo additional genetic testing including gene sequencing. MSS and MSI-L tumors require no further testing (3). Additional genetic evaluation may be considered if the clinical history is compelling.

The clinical significance of identifying Lynch syndrome is that affected individuals and at risk persons are identified and can be screened and treated with correct surgery. Subtotal colectomy is usually recommended to treat Lynch related colon cancer because of the high likelihood of synchronous/metachronous cancers. Partial colectomy with colonoscopy every 1-2 years is a reasonable alternative (6). Furthermore, clinicians can institute proper screening such as colonoscopy at a young age, (beginning at age 25 or 5 years younger than the youngest cancer in the family), periodic endometrial sampling (every 1-2 years starting at age 25), pelvic ultrasound, CA125 serum testing and urine cytology or molecular testing for urinary tract carcinoma. Many experts screen all resected colorectal cancers for MSI initially by PCR. Immunohistochemistry is a useful alternative and some prefer this as the initial test because an abnormality in protein expression correlates almost invariably with MSI-H by PCR. In cases showing normal MMR proteins or equivocal staining by immunohistochemistry, MSI testing by PCR should be done in clinically suspicious cases to exclude a germline mutation that can yield an antigenic protein that is biologically inactive.

MSI testing in sporadic colorectal carcinoma is a subject of considerable contemporary interest and debate. Much like their Lynch syndrome counterparts, sporadic MSI-H carcinomas have a predilection for the right colon, mucinous histology and a prominent lymphoid infiltrate (17). There are strong arguments for routine testing for MSI in all resected colorectal carcinoma including the lower mortality rate independent of tumor stage (18). Sporadic MSI-H cancer can also be associated with an increased rate of metachronous tumors with subsequent clinical implications for cancer surgery, surveillance and follow-up. MSI status may also have implications for chemotherapy. There is improved survival in MSS and MSI-L stage II and stage III cancers treated with fluorouracil-based regimens (19,20). Finally, routine MSI testing could increase the detection of Lynch syndrome because 44% of probands were over age 50 and up to 22% of patients in Lynch syndrome did not fulfill Amsterdam or Bethesda guidelines (16).

**COLORECTAL SERRATED POLYPS AND THE SERRATED PATHWAY TO COLORECTAL CANCER**

**Colorectal Hyperplastic Polyps and Hyperplastic (Serrated) Polyposis Syndrome**

Hyperplastic polyps are the most common benign polyp of the large intestine (2,21). These polyps are usually small (less than 5 mm), sessile and are often about the same color as the surrounding colonic
mucosa. Histologically, evenly distributed absorptive and goblet cells line crypts that are elongate and dilated. Inhibition of normal apoptosis is thought to be the underlying mechanism for polyp formation and because there are more epithelial cells per unit area than normal, the cells must pseudostratify, imparting a serrated or micropapillary appearance. Characteristically, the basement membrane under the surface epithelium is thickened and hyalinized. Regenerative epithelial changes, mitoses figures and active inflammation can be quite prominent at the crypt bases. This regenerative area can occasionally cause diagnostic confusion with dysplasia and carcinoma, especially in a variant referred to as inverted hyperplastic polyp (22,23). In this inverted variety, the regenerative epithelium of the crypt base is misplaced into or beneath the muscularis mucosae. Most examples of inverted hyperplastic polyp are now probably best classified as a sessile serrated polyp (see below) and are easily recognized if one is cognizant of its existence and also notes the overall architectural and cytologic similarity to hyperplastic polyp/sessile serrated polyp. The entity is distinguished from invasive adenocarcinoma by the lack of infiltration and tumor desmoplasia.

The differential diagnosis between hyperplastic polyp and tubular adenoma can be difficult, especially in a diminutive polyp that has been treated by hot biopsy (so-called “Thermal Polyp”). Useful features in the differential are found in Table 2.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hyperplastic Polyp</th>
<th>Tubular Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regenerative Zone</td>
<td>Basal</td>
<td>Surface</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Usually No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyalinized basement membrane</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

In a “tight call”, as long as an adenoma diagnosis is not going to result in a surgical resection (e.g., right colonic adenoma incompletely excised), I error on the side of adenoma to insure that the patient will receive more frequent surveillance. Mixtures of hyperplastic polyp, sessile serrated polyp and adenoma occur (24,25). Mixed polyps and serrated adenomas are considered in more detail below.

**Hyperplastic (Serrated) Polyposis Syndrome**

Rare examples of patients with colons carpeted by hyperplastic-like polyps (so-called hyperplastic polyposis) have been described (2). The WHO defines hyperplastic polyposis as individuals with: a) 5 or more hyperplastic polyps proximal to the sigmoid colon of which 2 are ≥ 1 cm, b) any number of hyperplastic polyps proximal to the sigmoid colon if the person has a first degree relative with hyperplastic polyposis and c) more than 30 hyperplastic polyps of any size and any location (26). The form with 30 or more small hyperplastic polyps without sessile serrated polyp morphology (see below) has been called type 2 and probably does not predispose to adenocarcinoma (27). The type 1 associated with large (> 1 cm) polyps with sessile serrated polyp morphology is associated with MSI-H cancers in which there is methylation-induced loss of expression of hMLH1 (27,28). Indeed, hyperplastic polyposis may be a marker for the so-called “mutator phenotype”. Colectomy specimens typically show a spectrum
of serrated polyps with typical hyperplastic polyps, traditionally defined serrated adenomas (see below) and unusual hyperplastic polyps (sessile serrated polyps – see below). Serrated polyposis may be a better name for this syndrome. Hyperplastic polyposis patients are prone to colorectal carcinoma with a reported prevalence of up to 50%. Once diagnosed, careful consideration should be given to the clinical follow-up and prophylactic colectomy may be indicated (29). Some cases have shown evidence of inheritance presumably caused by a genetic predisposition to hypermethylation. The type and order of methylated genes varies and may account for MSS, MSI-L and MSI-H cancers described. Some patients with MYH-associated polyposis (MAP) also met criteria for hyperplastic polyposis syndrome and MAP should be considered when adenomas coexist with hyperplastic polyposis (30). When several cancers in hyperplastic polyposis syndrome families are MSI-H, the distinction from Lynch syndrome can be difficult. Features that favor hyperplastic polyposis include; background serrated adenomas and sessile serrated polyps, presence of some MSS or MSI-L cancers in the kindred, older age at onset of cancer, limited numbers of affected family members, methylation of \textit{hMLH1} and failure to detect germline mutation of mismatch repair genes.

\textbf{Serrated Polyps and Colorectal Adenocarcinoma}

Several lines of evidence link “hyperplastic polyps” with colorectal carcinoma. Investigators have reported individual cases and small series of carcinoma complicating “hyperplastic polyps” (31-39). The association between colorectal cancer and hyperplastic polyposis has already been noted above. There is a high rate of co-existing hyperplastic polyps but not adenomas in patients with MSI-H carcinoma (31). A large series of MSI-H colorectal carcinoma predated by biopsy proved “hyperplastic polyps” at the same site has been reported (36).

Molecular events involved in the serrated polyp family are now recognized. Methylation-induced inactivation of mismatch repair genes occurs in both hyperplastic polyps and carcinoma. As shown in Table 3, methylation inactivation of genes and certain gene mutations (especially BRAF) appear to be involved in the serrated pathway to carcinoma (40,41). These molecular events have been verified (42-47).

\begin{table}
\centering
\caption{Methylation/Mutations in Serrated Polyp Family}
\begin{tabular}{llll}
\textbf{} & \textbf{HP (\%)} & \textbf{SSP (\%)} & \textbf{Mixed (\%)} \\
\textbf{MINT 1} & 23 & 30 & 100 \\
\textbf{MINT 2} & 32 & 70 & 100 \\
\textbf{MINT 31} & 23 & 70 & 100 \\
\textbf{hMLH1} & 0 & 13 & 70 \\
\textbf{MGMT} & 36 & 57 & 60 \\
\textbf{KRAS (mutation)} & 18 & 13 & 0 \\
\textbf{BRAF (mutation)} & 19 & 75 & 89 \\
\end{tabular}
\end{table}

HP = hyperplastic polyp; SSP = sessile serrated polyp; Mixed = mixed polyps and serrated adenomas.
“Hyperplastic polyps” associated with carcinoma have been unusually large and right-sided. They have been reported under a number of synonyms including giant hyperplastic polyp, sessile serrated adenoma, sessile serrated polyp, inverted hyperplastic polyp, and polyp with epithelial serrated proliferation. It is becoming clear that there are several different pathological entities that have been called “hyperplastic polyps” in the past. This serrated polyp family includes conventional hyperplastic polyp, mixed hyperplastic/sessile serrated polyp/adenoma, serrated adenoma (epithelial dysplasia defined, usually pedunculated and left sided, having eosinophilic cytoplasm and showing gastric foveolar change and often referred to as the traditionally defined serrated adenoma) and hyperplastic-like polyps with unusual features that have been referred to as sessile serrated polyps or sessile serrated adenomas (29,32,34,37). Sessile serrated polyps appear related to serrated adenomas and mixed polyps and could be the specific precursor lesion to sporadic MSI-H carcinoma. Transitions from sessile serrated polyps to areas of dysplasia and carcinoma with loss of hMLH1 protein expression have been described (38,39).

Sessile serrated polyps as the name implies are sessile, large (frequently 1 cm or more), right-sided, and often show poor endoscopic circumscription. A number of cytological and architectural abnormalities have been reported in the sessile serrated polyp, especially those that have been associated with carcinoma (33,36,37,39). The abnormal proliferation/dysmaturation features include persisting nuclear atypia with large nuclei and nucleoli high (upper third) in the crypts, high (upper third of the crypt) mitoses figures and irregular distribution of dystrophic goblet cells. Architectural abnormalities include basal crypt dilatation, horizontally oriented crypts, crypt branching, an increased epithelial:stromal ratio (>50%), inverted crypts, prominent serration, increased surface villosity/papillations and the lack of a surface basement membrane thickening typical of convention hyperplastic polyps. Some authors suggest that a diagnosis of sessile serrated polyp requires the presence of at least four of the architectural and abnormal proliferation features mentioned above (43). Immunohistochemical differences between hyperplastic polyp and sessile serrated polyp have been described using Beta-catenin, CDX2 and MUC6 (48,49) but it is uncertain whether this will be useful clinically.

Once recognized, the sessile serrated polyp creates a patient management dilemma. Calling them “sessile serrated adenomas” may not be an appropriate default diagnosis because it can be confused by the clinician for serrated adenoma. It is unknown whether colonic resection which is typically done for incompletely excised adenomas should be recommended for sessile serrated polyps which are incompletely excised at endoscopy. Furthermore, endoscopic follow-up for serrated adenoma would typically be at three-years (if the clinician considers serrated adenoma or sessile serrated adenoma a variant villous adenoma) or in five-years. In a cohort of 91 patients with sessile serrated polyps preceding MSI-H carcinomas, 19 predated the carcinomas by less than three years (36). Sessile serrated polyps should be treated by complete excision if possible. Until more is known, a shorter surveillance interval (e.g., 1-2 years) seems prudent for these types of polyps that are incompletely excised or associated with additional similar endoscopically appearing polyps that have remained unsampled (34-37).
REFERENCES


