COLORECTAL CANCER MOLECULAR PATHWAYS AND THE SERRATED POLYP

Charles J. Kahi, MD, MS
Indiana University School of Medicine
Richard L. Roudebush VA Medical Center
Indianapolis, Indiana

The First CRC Screening & Quality in Colonoscopy Symposium
April 28th, 2018
Beirut, Lebanon
Older Paradigm: Fearon-Vogelstein model

- Almost all colorectal cancers (CRC) develop along the adenoma-carcinoma sequence
- One precursor lesion-conventional adenoma
- Proximal/distal colon dichotomy

CRC is a heterogeneous disorder!

Several pathways with significant overlap contribute to CRC

Fundamentally characterized by genomic and epigenomic instability or alterations, the combination of which defines different pathways to CRC

Despite multitude of molecular signatures, 3 major pathways can be defined, based on 2 precursor lesions

Molecular events and neoplasia pathways have important clinical implications.
Loss of genomic stability facilitates the acquisition of multiple mutations that drive the development of CRC.

Genomic instability can take a number of forms, including chromosomal instability (CIN), microsatellite instability (MSI), aberrant DNA methylation, and DNA repair defects.

Genome-wide analysis of mutations in CRC has identified acquired somatic mutations in 100s of genes, and an average of 80 mutations in any single CRC!
<table>
<thead>
<tr>
<th>Gene or group of genes</th>
<th>Description</th>
<th>Mechanism for mutation increasing CRC risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APC</strong></td>
<td>Tumor suppressor gene</td>
<td>Inactivating mutation causes loss of regulation of spindle microtubules during mitosis</td>
</tr>
<tr>
<td><strong>TP53</strong></td>
<td>Tumor suppressor gene</td>
<td>Inactivating mutation causes loss of regulation of cell-cycle arrest and cell death</td>
</tr>
<tr>
<td><strong>RAS</strong></td>
<td>Oncogene</td>
<td>Activating mutations drive cell growth through MAPK pathway</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>Oncogene</td>
<td>Activating mutations drive cell growth through MAPK pathway</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>Oncogene</td>
<td>Activating mutation upregulates PI3 K pathway, enhancing prostaglandin E2 synthesis and inhibiting apoptosis</td>
</tr>
<tr>
<td><strong>MLH1, MSH2, MSH6, PMS2</strong></td>
<td>Mutation Mismatch Repair genes</td>
<td>Inactivating mutation impairs ability to repair strand slippage within nucleotide repeats</td>
</tr>
<tr>
<td><strong>EPCAM</strong></td>
<td>Codes for transmembrane glycoprotein epithelial cell adhesion molecule</td>
<td>Deletion of 3’ end of EPCAM leads to epigenetic silencing of MSH2</td>
</tr>
<tr>
<td><strong>MYH</strong></td>
<td>Base excision repair gene</td>
<td>Germline inactivating mutation of MYH leads to somatic mutation of APC</td>
</tr>
</tbody>
</table>

Chromosomal Instability (CIN) Pathway

• Precursor lesion: Conventional adenoma

• CIN refers to structural changes or numerical gain or loss in the karyotype of cells (aneuploidy), and subsequent loss of heterozygosity (LOH) of genes

• **Early molecular events:**

1. *APC* tumor suppressor gene mutation (Wnt pathway)
   - Accumulation of β-catenin protein
   - Stimulation of TCF-1 and LEF-1 transcription factors
   - Proliferation of colorectal cells

2. Gain-of function mutation of β-catenin gene (*CTNNB1*)

3. *KRAS* proto-oncogene mutation (MAPK cascade)
   - Resistance to inhibition of cell surface receptors
   - Colorectal cells evade apoptosis.
Wingless-type (Wnt) canonical pathway

Fumi Takahashi-Yanaga, and Michael Kahn
Clin Cancer Res 2010;16:3153-3162
Chromosomal Instability (CIN) Pathway

• **Later molecular events:**
  1. **Mutation of p53 tumor suppressor gene**
     - *p53* (Chr 17p) a.k.a. “guardian of the genome”
     - Mutation in *p53* prevalence increases from adenoma, to HGD, to CRC
     - Unclear if absolutely required

  2. **LOH (Chr 18q)**
     - Occurs in 70% of CRC
     - Often synchronous with *p53* loss
     - Affects Deleted in Colorectal Carcinoma (*DCC*), *SMAD4*, and *SMAD2* which are involved in regulation of cell proliferation and apoptosis

  3. **Other events**
     - PI3K pathway mutations—accelerated cell growth
     - microRNA upregulation or downregulation: Effectively act like oncogenes and tumor suppressor genes.
Microsatellite Instability (MSI) Pathway

- Precursor lesion: Conventional adenoma

- 15% of CRC have MSI
  - 20% have hereditary cause-Lynch syndrome
  - Remaining associated with hypermethylation of MLH1 (serrated pathway)

- Microsatellites are mononucleotide or dinucleotide repeats found throughout the genome, vulnerable to transcription errors during replication

- Mutation Mismatch Repair (MMR) system: Identifies and corrects errors

- Germline mutations leading to loss of function have been identified in four genes involved in MMR: MLH1, MSH2, MSH6 and PMS2

- Germline deletion mutations in the EPCAM gene leads to epigenetic silencing of the neighboring MSH2 MMR gene.
Microsatellite Instability (MSI) Pathway

- Most labs assess MSI using a panel of 5 mononucleotide markers
  - BAT-25, BAT-26, NR-21, NR-24, MONO-27

- At least 2 of 5 (40%): MSI or MSI-High
  - MSI-Low versus MSS distinction is controversial

- Additional molecular alterations in hereditary MSI pathway:
  - Wnt pathway activation and β-catenin gene mutation
  - K-RAS mutations are common
  - TGF-β gene mutation (inhibitor of epithelial cell growth)
  - BAX gene mutation leads to evasion from apoptosis (similar to p53).
The Serrated Pathway(s)

• Precursor lesion: Sessile serrated polyp (SSP)

• Key feature: Hypermethylation of CpG islands on the promoter regions of tumor suppressor genes, leading to epigenetic silencing

• CIMP phenotype

• \textit{BRAF} oncogene mutation

• Associated with MSI in about 50\% of cases (\textit{BRAF} mutation distinguishes from Lynch-syndrome related MSI).
**Chromosomal Instability Pathway**
- APC mutation (β-catenin)
- KRAS
- SMAD4
- p53

**Microsatellite Instability Pathway**
- MMR Germline mutations
  - MLH1, MSH2, MSH6, PMS2, (EPCAM)

**Serrated Pathway (Main)**
- BRAF mutation
- MLH1 hypermethylation (epigenetic silencing)

**Other Serrated Pathways**
- SSP-based MGMT or p16 hypermethylation
- TSA-based KRAS mutation

**CRC signature**
- CIMP – MSS (Sporadic CRC, FAP)
- CIMP + MSI-H
- Lynch Syndrome

**Molecular and genetic events**
- CIMP + MSI-H

**Percentage**
- Chromosomal Instability Pathway: 60% - 70%
- Microsatellite Instability Pathway: ~3%
- Serrated Pathway (Main): 25% - 30%
Clinical Applications and Implications

- **Fecal FIT-DNA test for average-risk CRC screening**
  - Detection of abnormal DNA shed in stool by CRC
  - *KRAS*, aberrantly methylated *BMP3* and *NDRG4* promoter areas, beta-actin
  - Sensitivity for CRC 92% (FIT 74%)
  - Highest sensitivity for advanced polyps among all non-invasive tests (40%)
  - Sensitivity for large SSP about 42%


- **Chemoprevention with aspirin/NSAIDs** (degradation of β-catenin)

- **Genetic testing for familial CRC and polyposis syndromes**
  - *APC* for FAP and its variants
  - *SMAD4* for juvenile polyposis…
Am J Gastroenterol 2014; 109:1159–1179
Clinical Applications and Implications

**Prognostic**
- MSI CRC (both Lynch and serrated pathways) has better prognosis than MSS CRC, even with adjustment for stage
- *BRAF* mutation in MSS CRC marker of worse prognosis
- 18q LOH associated with worse prognosis in stage II and III CRC

**Predictive**
- *KRAS* and *BRAF* mutant CRC do not respond to anti-EGFR therapy (cetuximab)
- MSI CRC require oxalaplatin addition to 5-FU for response
- *MLH1* hypermethylated CRC: Poor response to 5-FU, but respond to irinotecan
- Stage IV MSI CRC (but not MSS) respond to immunotherapy (pembrolizumab).

Dichotomy concept is obsolete: The frequencies of CIMP-high, MSI-high, and \textit{BRAF} mutation in CRC increase gradually from rectum to ascending colon.
Serrated Pathway and Post Colonoscopy
CRC: Overlap of Molecular Signatures

• Compared to sporadic CRC, PCCRC is more likely to:

  - Be located in the proximal colon
  - Demonstrate MSI
  - Be associated with CIMP

Sawhney et al. Gastroenterology 2006; 131: 1700-5
Arain et al. Am J Gastroenterol 2010; 105: 1189-95
Serrated polyps: Old-New lesions

- Prior to 1990: Adenoma or hyperplastic (benign)

- Polyps with “mixed” features
  

- 1996: “sessile serrated adenoma” coined to distinguish the polyps of “giant hyperplastic polyposis” from true hyperplastic polyps,
  

- Histologic features further refined in 2003
  
Classification of Serrated Lesions of the Colorectum

• Hyperplastic Polyp
  - Microvesicular HP (MVHP)
  - Goblet-cell rich HP (GCHP)
  - Mucin-poor HP (MPHP)

• Sessile Serrated Adenoma/Polyp (SSA/P)
  - SSA/P without cytological dysplasia
  - SSA/P with cytological dysplasia

• Traditional Serrated Adenoma (TSA)

The Serrated Pathway: How Long?

- Pathological review of 2319 SSP:
  - Median age of patients (years):
    - SSP without dysplasia: 61
    - SSP with LGD: 66
    - SSP with HGD: 72
    - SSP with CA: 76


- Other studies have shown more aggressive behavior, related in part to patient age (inactivation of MLH1 associated with advancing age)

  Goldstein NS. Am J Clin Pathol 2006; 125: 132-45

- Serrated lesion-carcinoma sequence duration is variable
- May take 10-15 years, but key event is MLH1 methylation/silencing, which accelerates progression to CRC.
Pathology Overview-HP vs SSP

- Hallmark: Crypt elongation
- Crypts straight, narrow bases
- Proliferation in lower third of crypt → serration in the luminal aspect

- Distorted architecture due to displaced proliferative zone
- Crypts are dilated and branched
- Crypts may be filled with mucin (endoscopic mucus cap).
Pathology Considerations

- Often difficult to distinguish HP from SSP, criteria not validated
- Considerable inter-observer variation, even among expert pathologists
  
  Khalid O et al. World J Gastroenterol 2009;15:3767-70

  - Contributes to high variability in serrated polyp endoscopic detection rates
  

  - Pathologic diagnoses of SSP are increasing over time
  

  - Right-sided location and larger size predictive of reclassification of HP to SSP
  
  Singh et al. Gastrointest Endosc 2012; 76: 1003-8

Practical Bottom lines:

- Communicate with your pathologist
- Treat right-sided HP ≥ 1 cm as SSP

Endoscopic Features-HP vs. SSP

- Usually sessile and 1-5 mm
- Typically distal (rectosigmoid)
- Multiple, pale
- May disappear with insufflation

- Mucus cap (64%)
- Rim of debris or bubbles (52%)
- Alteration of the contour of a fold (37%)
- Interruption of underlying mucosal vascular pattern (32%)

Endoscopic Prediction SSP vs. HP

Indistinct borders
(OR ~ 2)

Cloud-like appearance
(OR ~ 5)

Dark spots inside the crypts
(OR ~ 2)

Screening Relevance of SSP

• High prevalence in colonoscopy series
  - SSP overall 8%-9%
  - SSP-CD 0.4%-0.6% (5% to 8% of SSP)

• Association with synchronous advanced neoplasia
  - Meta-analysis of 9 studies with 34,000 patients
  - OR of AN in individuals with serrated polyps: 2.0
  - Higher if proximal and/or large SP
**CRC Risk: SSP are Adenoma-Equivalent**

- Population-based Danish study 1977-2009
  - Case-control study within individuals who had received colonoscopy (n=272,342)
  - 2045 CRC cases, 8105 cancer-free controls, age and sex-matched
  - Hyperplastic polyps reviewed by expert pathologists using 2010 WHO criteria

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>10-year risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSP with CD</td>
<td>4.8 (2.6-8.7)</td>
<td>4.4%</td>
</tr>
<tr>
<td>SSP without CD</td>
<td>2.75 (2.0-3.8)</td>
<td>2.6%</td>
</tr>
<tr>
<td>SSP with synchronous conventional adenomas</td>
<td>2.7 (1.7-4.2)</td>
<td>2.5%</td>
</tr>
<tr>
<td>SSP without synchronous conventional adenomas</td>
<td>3.4 (2.4-4.9)</td>
<td>3.2%</td>
</tr>
<tr>
<td>Conventional adenomas without SSP</td>
<td>2.5 (2.2-2.8)</td>
<td>2.3%</td>
</tr>
<tr>
<td>TSA</td>
<td>4.8 (2.4-9.9)</td>
<td>4.5%</td>
</tr>
<tr>
<td>Hyperplastic polyps only</td>
<td>1.3 (0.9-1.8)</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Serrated polyposis syndrome (SPS)

I. At least 5 serrated polyps proximal to the sigmoid, of which ≥ 2 are ≥ 10 mm in size
II. At least one serrated polyp proximal to sigmoid, in first-degree relative of patient with SPS
III. > 20 serrated polyps throughout the colon.

SPS: Most Common Polyposis Syndrome

- Prevalence estimates higher than previously thought
  - FIT/FOBT screening cohorts: 1:111 to 1:127 colonoscopies
  - Screening colonoscopy cohorts: 1:238 colonoscopies
    
    Rivero-Sanchez et al. Endoscopy 2017; 49: 44-53

- Unusual syndrome—Genetic basis unclear/uncertain
  - Criterion II alone uncommon
  - Average age of diagnosis > 50 years
    
    Carballal et al. Gut 2016; 65: 1829-37

  - About 50% have a family hx of CRC
    
SPS: Important Clinical Points

• Recognition is key!

• CRC risk is highest at time of diagnosis

• Excluding baseline surgery for CRC or high polyp burden, endoscopic control feasible for most patients (~90%) and requires average of 2 colonoscopies

• Endoscopic clearance and effective surveillance associated with decreased CRC risk

→ Importance of high-quality baseline examination
→ Referral to endoscopist/center with expertise.
Optimizing Detection of Serrated Polyps

- Strong correlation between ADR and proximal SP detection
  
  *Kahi et al. Gastrointest Endosc 2012; 75: 515-20*
  

- Longer WT associated with better proximal SP detection
  
  *De Wijkerslooth et al. Gastrointest Endosc 2013; 77: 617-23*

- SP detection increases with each minute of WT above 6 minutes, with maximum benefit at 9 minutes
  

- Risk factors overlap with those of conventional adenomas:
  - Smoking, red meat
  - NSAIDs protective
  - Obesity, fiber intake, alcohol, calcium: Not associated
  
Optimizing Resection of Serrated Polyps

- Identification of lesion margin is key to complete resection
  - Submucosal injection with contrast agent
  - Electronic chromoendoscopy
### Optimizing Resection of Serrated Polyps

- **Australian Multicenter study (ACE):**
  246 patients with 323 SSP ≥ 20 mm

<table>
<thead>
<tr>
<th></th>
<th>SSP</th>
<th>Adenomas</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eradication rate</strong></td>
<td>99.1%</td>
<td>94.5%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Intraprocedure bleeding</strong></td>
<td>6.9%</td>
<td>16.7%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Delayed bleeding</strong></td>
<td>5.7%</td>
<td>6.3%</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Perforation</strong></td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>2-year cumulative recurrence rate</strong></td>
<td>7%</td>
<td>28.4%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Serrated Surveillance Guidelines
(High-quality baseline colonoscopy)

<table>
<thead>
<tr>
<th></th>
<th>USMSTF (1)</th>
<th>ESGE (2)</th>
<th>BSG (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP &lt; 10 mm</td>
<td>10</td>
<td>10</td>
<td>No clear indication for surveillance unless meeting SPS criteria</td>
</tr>
<tr>
<td>(rectosigmoid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSP &lt; 10 mm</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>SSP ≥ 10 mm</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SSP-CD TSA</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Serrated polyposis syndrome</td>
<td>1</td>
<td>1-2</td>
<td></td>
</tr>
</tbody>
</table>

(1) Lieberman et al. Gastroenterology 2012; 143: 844-57
(2) Hassan et al. Endoscopy 2013; 45: 842-51