Genetics of Familial Colorectal Cancer Syndromes

Wade S. Samowitz, M.D.
Professor, Dept. of Pathology
University of Utah
Familial Colorectal Cancer

- Lynch syndrome
- Familial adenomatous polyposis (FAP)
- MYH-associated polyposis (MAP)
- Hamartomatous polyposis
Lynch syndrome (HNPPCC)

- Early onset colon cancer
- Right-sided
- Extra-colonic cancers: endometrium, ovary, renal pelvis, ureter, small intestine, stomach, hepatobiliary tract
- Muir-Torre: Lynch + sebaceous neoplasms
- Turcot’s: Lynch + brain tumor (GBM) (Hamilton, NEJM, 1995)
Germline mutations in mismatch repair genes: \textit{MLH1}, \textit{MSH2}, \textit{MSH6} or \textit{PMS2}

- Autosomal dominant
- Phenotype not so obvious (unlike FAP, for example)
- Family history not always obvious or available
- Fortunately, we can use the molecular features of the tumor (Microsatellite instability) to help in work-up
Microsatellite repeats

- Type of repetitive DNA in which repeat unit is short (1-6 nucleotides, aka STR)
  - Mononucleotide: AAAAAAAAAAAAA
  - Dinucleotide: CACACACACACACA

- Most in non-coding regions
  - Some exceptions: Mononucleotide repeats such as in TGFBRII

- Often slippage during DNA replication of these repeats
  - Leads to changes in number of repeats

- Usually fixed by mismatch repair apparatus
Microsatellite instability

- Expansion or contraction of microsatellite repeats
  - For example, 10 CA’s to 14 CA’s
- Requires a mistake in replication plus deficiency in mismatch repair
Chromosome 5  Maternal

Chromosome 5  Paternal

10 CA

12 CA
PCR of Normal

120
Maternal

124
Paternal
PCR of MSI

118 120 122 124 126

Maternal  Paternal
Lynch/HNPCC
  • Germline mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6, or PMS2)

10-15% Sporadic colorectal cancer
  • Acquired hypermethylation of MLH1 promoter
Bethesda Consensus Panel

- Two mononucleotide repeats, three dinucleotide repeats
- MSI high: Instability in two or more repeats
- Microsatellite stable (MSS): No instability
- MSI low: Instability in one repeat
  - Controversial
  - Lynch-associated cancers show MSI high, not low
Mononucleotide repeats are probably more sensitive and specific for MMR deficiency.

New panel(s) of 5 mononucleotide repeats:
- MSI high: two or more unstable, although typically all (or almost all) repeats are unstable.
- Since instability in even one mononucleotide repeat may indicate MMR deficiency, instability in one repeat is termed “indeterminate” rather than MSI low.
Normal

Tumor
MMR proteins are expressed in proliferating cells (like cancer)
Lack of MMR expression of one or more proteins is a good surrogate test for MSI
IHC can also guide subsequent germline MMR evaluation
MLH1 complexes with PMS2
MSH2 complexes with MSH6
The stability of PMS2 and MSH6 depends upon these complexes
Therefore, if MLH1 is negative, PMS2 is usually negative; if MSH2 is negative, MHS6 is negative.
Corollary usually not true (MLH1 and MSH2 bind to other proteins as well)
<table>
<thead>
<tr>
<th>IHC Result</th>
<th>Likely Defective Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of MLH1, PMS2</td>
<td>MLH1</td>
</tr>
<tr>
<td>Loss of MSH2, MSH6</td>
<td>MSH2</td>
</tr>
<tr>
<td>Isolated Loss of MSH6</td>
<td>MSH6</td>
</tr>
<tr>
<td>Isolated Loss of PMS2</td>
<td>PMS2</td>
</tr>
</tbody>
</table>
Complementary

- MSH6 negative tumors can be stable by PCR
  - Mononucleotide repeat panels may help identify these
- Missense mutations may be normal by IHC
- IHC can be hard to interpret
- IHC can guide subsequent mismatch repair gene testing
10-15% of all colon cancer
Acquired hypermethylation of $MLH1$ promoter
More common than Lynch/HNPCC
Leads to IHC profile: $MLH1$/$PMS2$ negative
Lynch due to $MLH1$ germline mutation can have the same IHC profile
Unstable, MLH1/PMS2 negative: sporadic or Lynch?

- BRAF V600E mutation in about 50% of sporadic unstable colorectal cancers, only rarely occurs in Lynch/HNPCC (so far, minority of those with PMS2 germline mutation; Senter, Gastroenterology, 2008)
- **BRAF V600E not useful for extra-colonic tumors like endometrium
- MLH1 methylation in most sporadic unstable tumors, only rarely in Lynch/HNPCC
- Rare reports of germline MLH1 methylation
Requirements for MSI (PCR and IHC), BRAF and methylation

- All work on formalin-fixed, paraffin-embedded tissue
- MSI by PCR requires both normal and tumor
- All require only one 5 micron slice (maybe more if scant tissue, e.g. biopsy)
- Cancers better than adenomas, resections better than biopsies (exception: rectal cancer obliterated by preop chemorads)
Strategies for Lynch work-up

- Revised Bethesda criteria
- General screening
Table 2. The Revised Bethesda Guidelines for testing colorectal tumors for microsatellite instability (MSI)

<table>
<thead>
<tr>
<th>Tumors from individuals should be tested for MSI in the following situations:</th>
</tr>
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<tbody>
<tr>
<td>1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.</td>
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<tr>
<td>2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors, regardless of age.</td>
</tr>
<tr>
<td>3. Colorectal cancer with the MSI-H histology (TILs, Crohn’s-like lymphoid reaction, mucinous/signet ring, medullary) diagnosed in a patient who is less than 60 years of age.</td>
</tr>
<tr>
<td>4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years</td>
</tr>
<tr>
<td>5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.</td>
</tr>
</tbody>
</table>

Umar, JNCI, 2004
General Screening

- Individuals can present with Lynch syndrome at older ages (Hampel, NEJM, 2005)
- Family history not always available
- Histologic features are not 100% predictive for MSI
- IHC is good, fairly cheap screening test
Lynch Syndrome Test Algorithm

Consider Lynch syndrome

MSI by:
Immunohistochemical stains or PCR

Negative for MSI

Probably not Lynch syndrome

Abnormal MLH1 staining

Test for BRAF V600E mutation, MLH1 methylation

Alteration absent (wild-type)

Seq & Del/Dup MLH1

Positive for MSI

Normal MLH1 staining and abnormal for MSH2, MSH6, or PMS2

Seq & Del/Dup MSH2, MSH6, PMS2

Alteration present

Probable sporadic colorectal cancer
Sequencing
Southern blots or multiplex ligation-dependent probe amplification (MLPA) for deletions of an exon or more, especially important for MSH2 and PMS2
PMS2 Pseudogenes

LR 1: 9,964 bp
LR 2: 9,440 bp
LR 3: 8,812 bp
LR 4: 9,804 bp

Clendenning, et al. 2006
Vaughn, et al. 2010
PMS2: Detection of 3’ deletions

New MLPA kit
Sequence both the gene and pseudogene
(Vaughn et al, 2011)
Constitutional mismatch repair deficiency syndrome

- Bi-allelic mutations in an MMR gene
- Often in setting of consanguinity
- Childhood cancers
  - Hematologic malignancies
  - Brain tumors
  - Colorectal cancers
- NF 1 manifestations (café au lait spots)
Germline methylation

- **MLH1 methylation**
  - Rare, usually not inherited

- **MSH2 methylation**
  - Deletion of 3’ end of EPCAM (aka TACSTD1)
  - Transcription read through into MSH2 leads to promoter hypermethylation
  - Cancer risk mostly limited to colorectal cancer
Colon with hundreds of polyps
Adenomatous polyp
Familial Adenomatous Polyposis

- Risk of colorectal cancer is basically 100%, average age onset 39 years
- Extra-colonic intestinal manifestations: duodenal (mostly peri-ampullary) and jejunal adenomas and carcinomas, gastric antral adenomas (?cancer), fundic gland polyps (some with superficial adenomatous change)
Variable number of adenomas, usually less than 100, lower risk and later onset of colon cancer than classic FAP

Mutations, although knockout type like in classic FAP, are at the extreme five prime and three prime ends of the gene, probably leading to a partially functional APC protein

Upper GI tract manifestations are variable, may not be attenuated
Named FAP syndromes

- Gardner’s: FAP plus various extra-intestinal manifestations, including desmoids, osteomas, dental abnormalities, and epidermal cysts (also APC mutations, probably more extreme end of spectrum)
- Turcot’s (recently renamed Crail’s): FAP plus brain tumors; if due to APC gene usually medulloblastoma
- Other extra-intestinal manifestations: congenital hypertrophy of the retinal epithelium (CHRPE), hepatoblastoma (in children) and thyroid cancer
Acquired APC gene mutations are thought to be the initiating event in adenoma formation.

Adenomas are the probable precursor of most cases of sporadic colorectal cancer.

APC is a tumor suppressor gene: “two hits” are required to inactivate the gene.

In FAP the first mutation is inherited; in sporadic colorectal cancer, both mutations are acquired.
MYH-associated polyposis (MAP)

- Colonic phenotype usually more like attenuated FAP
- Extra-colonic manifestations: duodenal adenomas/carcinomas, cancer of ovary, bladder, skin, sebaceous gland reported, no desmoids or other APC related conditions
- Most mutations are homozygous or compound heterozygotes of Y179C and G396D (formerly known as Y165C and G382D)
- Sometimes referred to as FAP (or AFAP) caused by MYH mutations
Oxidative damage to DNA causes oxidation of guanine leading to 8-oxo-7,8-dihydroxyguanine. This mispairs with adenine, leading to G:C to T:A transversion. MYH performs base excision repair during replication, removing the adenine.
Hamartomatous polyposis

- All probably have increased risk of colorectal cancer
- Cowden’s syndrome: PTEN gene mutations in 80% (also thyroid, breast, endometrium)
- Peutz-Jegher: STK11/LKB1 mutations in 50-90% (also breast, pancreas, ovarian, lung)
- Juvenile polyposis: BMPR1A mutations in 20%, SMAD4 mutations in 20%
- See Genetests for descriptions
Next generation sequencing

- Biggest impact on Lynch syndrome
- May decrease the need for many tissue tests as costs come down for sequencing
- PMS2 still likely to be problematic
- Will facilitate genetic definitions of syndromes, especially hamartomatous polyposes