Microsatellite Instability: The Basics for Pathologists

James P. Grenert, MD, PhD
UCSF Molecular Pathology Laboratory

Overview

- Terminology
- HNPCC/Lynch syndrome
- Clinical criteria
- Problems in pt identification
- MSI histology
- HNPCC testing options, pros/cons
- Testing results
- Pitfalls
- MSI testing algorithm

Terminology

- **Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome**: Autosomal dominant inherited cancer syndrome, discovered by Dr. Henry Lynch. →*What we are trying to identify.*
- **Mismatch repair (MMR) genes**: The affected genes in HNPCC. Their protein products can be analyzed by immunohistochemistry.
- **Microsatellite instability (MSI)**: Genomic changes caused by defective MMR. These can be detected by a PCR test.

Terminology

- **Microsatellites**: Short, repetitive sequences of DNA (~3% of the genome). Repeating element may be from one to six bases long.
  - Dinucleotide repeat (repeat of two bases) e.g., ATATAT or CTCTCTCT
  - Mononucleotide repeat (repeat of one base) e.g., GGGG or AAAA
Hereditary nonpolyposis colorectal cancer
• **Autosomal dominant** cancer syndrome

HNPCC/Lynch syndrome
• Individuals with HNPCC are at greatly increased risk of colorectal cancer
  – ~85% lifetime risk (approaches 100% in males)
• NOT a polyposis syndrome.
• Also increased risk of non-colorectal cancers, including gynecologic, gastric and genitourinary.

HNPCC/Lynch syndrome
• Individuals with HNPCC have defective DNA mismatch repair (MMR) proteins.
• MMR proteins correct errors in DNA replication.
• MMR protein dysfunction causes accumulation of genetic errors.
  → **CANCER**
HNPCC/Lynch syndrome

• Errors also occur in replication of microsatellites.
• If errors are not corrected, microsatellite length will change from added or deleted bases.
• This size change is seen as “instability” of a microsatellite.
• So: Individuals with HNPCC have microsatellite instability.

Sporadic MSI

• ~15-20% of unselected colorectal cancers show microsatellite instability/defective mismatch repair.
• HNPCC accounts for ~2% of colorectal carcinomas.
• The majority of these (~85%) are NOT HNPCC. They are sporadic.
• Sporadic MSI tumors are caused by silencing of one of the MMR proteins (MLH1) by hypermethylation of the gene.

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Bethesda criteria

• 1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
• 2. Presence of multiple primary (synchronous or metachronous) colorectal or other HNPCC-associated tumors, regardless of age.
Bethesda criteria
HNPCC-associated tumors include:
- Colorectal
- Endometrial
- Stomach
- Ovarian
- Ureter and renal pelvis
- Biliary tract
- Pancreas
- Small bowel
- Brain (usually glioblastoma as seen in Turcot syndrome) tumors
- Sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome

Bethesda criteria
- 3. Colorectal cancer with the **MSI-H histology** diagnosed in a patient who is less than 60 years of age. Note that inclusion of the age criteria is controversial.
- 4. Colorectal cancer in a patient with one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years.
- 5. Colorectal cancer in a patient with two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

Clinical criteria limitations
- Criteria are not perfect.
  - Maximum ~80% sensitivity
  - Lower sensitivity in less-specialized settings
- History not always available.

Clinical criteria limitations
- Pathology to the rescue!
- Histologic features up to 60-90% sensitive, including patients that do not meet Bethesda criteria.
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MSI histology

• Tumor-infiltrating lymphocytes
• Crohn’s-like lymphocytic reaction
• Mucinous or signet ring cell differentiation
• Medullary carcinoma

Tumor-infiltrating lymphocytes

• Lymphocytes present within tumor epithelium
• Approximately 4 lymphocytes per 10 high-power fields
• Assessed by H&E
**Crohn’s-like lymphocytic response**

- Nodular lymphocytic aggregates
- Sometimes with germinal centers
- Occurs beyond advancing tumor front
- Often subserosal or in pericolonic fat
- Resembles transmural inflammation of Crohn’s disease

**Mucinous differentiation - Extracellular mucin**

**Signet ring cell adenocarcinoma**
Medullary carcinoma

- Poorly-differentiated/Undifferentiated carcinoma
- Solid, syncytial growth
- Vesicular nuclei, prominent nucleoli
- Often prominent TILs
- Rare, but very closely associated with MSI

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MMR protein immunohistochemistry

- Commercially-available antibodies against four MMR proteins:
  - MLH1
  - PMS2
  - MSH2
  - MSH6
- Look for loss of one or more of these proteins in the tumor.

Microsatellite instability testing by PCR

- A PCR test to look for a change in microsatellite size in the tumor compared to the patient’s non-tumor tissue.
- Microsatellite repeats amplified from tumor and normal.
- Amplified DNA separated by size using electrophoresis.

Normal tissue. Allele size is 134 b.p.

MMR protein IHC

**PROS**
- Antibodies available
- No special techniques
- >90% sensitive
- Can "rule in" HNPCC
- Directs subsequent gene sequencing
- Test any slide with tumor

**CONS**
- Patchy staining
- Defective MMR may still be antigenic
- Untested MMR proteins
- No further testing for MLH1+PMS2 loss

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MSI testing by PCR

**PROS**
- Commercial kit available (Promega)
- >90% sensitive
- Detection independent of affected MMR protein
- Opportunity to evaluate MLH1 loss with BRAF testing

**CONS**
- Specialized laboratory required
- Does not indicate affected MMR protein
- Requires both tumor and normal samples
- May be less sensitive to MSH6 loss

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MMR protein IHC

- Mismatch repair proteins include
  - MLH1 (most commonly affected)
  - PMS2
  - MSH2
  - MSH6
- They are located in the nucleus.
- It is NORMAL for these proteins to be present, therefore:
- Loss of staining is abnormal.

Normal MMR protein stain-

Normal MMR protein stain-

normal tissue

tumor tissue
**Loss of MMR protein staining**

**MMR protein dimerization**

- MMR proteins function as dimers (pairs):
  - MLH1 and PMS2
  - MSH6 and MSH2
  - MSH2 and others not tested
  - MLH1 and others not tested
- Loss of one protein results in loss of its partner
  e.g., MLH1 loss causes PMS2 loss

**MMR protein loss patterns**

- **MLH1 and PMS2**: Most common pattern of loss. Both sporadic and hereditary tumors may show this pattern, but most are sporadic.
- **MSH2 and MSH6**: Only lost in HNPCC. Most common exclusively hereditary pattern.
- **MSH6 only**: Rare, HNPCC only.
- **PMS2 only**: Rare, HNPCC only.
MMR IHC pitfalls

- Patchy staining
  - Insufficient tumor (false negative)
  - MSH6 especially patchy
- Cytoplasmic staining

MSI PCR results

- A panel of microsatellite markers tested.
- Characterized as:
  - Microsatellite stable (MSS): All markers stable.
  - MSI-Low (MSI-L): Instability in <40% of tested markers.
  - MSI-High (MSI-H): Instability in ≥40% of tested markers

MSI PCR results

- MSS is **NOT** associated with HNPCC.
- MSI-L in most cases does not indicate HNPCC.
- MSI-H **MAY** indicate a patient with HNPCC. Further evaluation is necessary:
  - Genetic counseling
  - Correlation with IHC results
  - Further testing (sequencing, BRAF mutation, methylation analysis)

BRAF V600E mutation

- BRAF is a tyrosine kinase in the RAS pathway.
- Commonly activated by Val→Glu mutation at codon 600 (“V600E”).
**BRAF V600E mutation**

- Hypermethylation of MLH1 in sporadic tumors is the most common cause of microsatellite instability.
- Closely associated with BRAF V600E mutation (32-83% of cases) in colon cancer (not others).
- BRAF V600E mutation is extremely rare in HNPCC colon cancers.
- So: **BRAF V600E mutation in colon cancer almost always excludes HNPCC.**

**MSI pitfalls**

- Insufficient material (biopsies)
- Lack of separate tumor and normal tissue

**Tumor and normal**

- Five 10-micron unstained sections used

**Ideal case**
Biopsies- Mixed tumor & normal

Biopsy – Separate tumor area

Little tumor present

Avoiding pitfalls

- Use resection specimen.
- “Tumor” areas should contain 50% tumor cells.
- “Normal” tissue can be any non-tumor tissue:
  - Negative resection margin
  - Negative lymph node
  - Prior hernia sac or inflammatory skin biopsy
HNPCC testing algorithm

MSI histology

Bethesda criteria met

Clinician request

Microsatellite instability testing by PCR and/or Mismatch repair protein immunohistochemistry

Tests disagree

Both abnormal

Which protein is lost?

MLH1 (usually with PMS2)

BRAF V600E mutation testing

Mutation negative

Mutation positive

HNPCC possible

Unlikely HNPCC

MLH1 (usually with PMS2)

BRAF V600E mutation testing

Mutation negative

Mutation positive

HNPCC possible

Unlikely HNPCC

Consider technical error, specimen limitation (e.g. too few tumor cells)

Which protein is lost?

• MSH2 and/or MSH6
• PMS2 without MLH1 loss

Likely HNPCC

Unlikely HNPCC

Likely HNPCC

BRAF V600E mutation testing

Mutation negative

Mutation positive

HNPCC possible

Unlikely HNPCC

Confirm with sequencing
HNPCC testing algorithm

Which protein is lost?

- MSH2 and/or MSH6
- PMS2 without MLH1 loss

MLH1 (usually with PMS2)

- Likely HNPCC
- BRAF V600E mutation testing

Mutation negative
- HNPCC possible

Mutation positive
- HNPCC possible

Gene sequencing is an alternative

HNPCC testing algorithm

Which protein is lost?

- MSH2 and/or MSH6
- PMS2 without MLH1 loss

MLH1 (usually with PMS2)

- Likely HNPCC
- BRAF V600E mutation testing

Mutation negative
- Evaluate with MLH1 sequencing
- HNPCC possible

Mutation positive
- Unlikely HNPCC

Get genetic counseling involved!