The Molecular Pathology of Colon Cancer for the Community Pathologist

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Objectives

• Convey an understanding of current common molecular diagnostic testing relevant to colorectal cancer:
  – Mis-Match Repair deficiency
    » Microsatellite Instability
    » IHC
  – BRAF
  – KRAS
  – CIMP
Colorectal Cancer - molecular pathways

- Hypermutability Pathway
- Chromosomal Instability Pathway
Frequent chromosomal loss in CRC
Figure 2. Genetic Alterations during Colorectal-Tumor Progression.
Genetic changes during colorectal tumor development

Vogelstein, et al., NEJM 319:525-532, 1988
Molecular pathways in colon cancer

**Chromosomal Instability Pathway**

- Normal Colorectal Epithelium
- Early Adenoma
- Intermediate Adenoma
- Advanced Adenoma
- Colorectal Carcinoma (aneuploid)
- Invasive Carcinoma
- Metastatic Carcinoma

- APC
- K-ras
- Unknown Locus on 18q
- p53
- ?
- ?

**Hypermutability Pathway**

- Normal Colorectal Epithelium
- Unknown Tumorigenic Stages
- Colorectal Carcinoma (diploid)
- Invasive Carcinoma
- Metastatic Carcinoma

- hMLH1
- Other DNA Mismatch-Repair Genes
- hMSH2
- TGFB, pR1
- IGF, hMSH3
- IGF, hMSH6
- BAX
- ?

- Tumor Suppressor Gene
- Proto-Oncogene
- Unknown Status
Hypermutability Pathway
Chromosomal Instability Pathway
FAP
HNPCC
Hypermutable Pathway

• 15 – 20% of all CRC

• Defective DNA Mis-Match Repair
  – Inherited predisposition – 3% (Lynch Syndrome)
  – Sporadic – 12%

• Significance
  – Genetic
  – Prognostic
  – Therapeutic
What is DNA mismatch repair?
What are Microsatellite DNA’s?

- Short, repeated sequences are called microsatellite DNA.

- The number of repeats at any locus is constant, and is dependent on DNA Mis-Match Repair.

- Defective MMR leads to microsatellite instability (MSI).
DNA MMR and Microsatellite Instability

Diagram showing the process of replication error, normal mismatch repair, and abnormal mismatch repair leading to DNA sequences with 4 and 5 repeats.
Microsatellite DNA instability - MSI
Date Specimen Collected: 9/24/2002
Date Specimen Received: 5/26/2004
Date & Time Reported: 5/27/2004 16:52

Test(s) Submitted:
DNA Microsatellite Instability

Results and Interpretation:

<table>
<thead>
<tr>
<th>Marker</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT25</td>
<td>Unstable</td>
</tr>
<tr>
<td>BAT26</td>
<td>Unstable</td>
</tr>
<tr>
<td>D2S123</td>
<td>Unstable</td>
</tr>
<tr>
<td>D5S346</td>
<td>Unstable</td>
</tr>
<tr>
<td>D17S250</td>
<td>Unstable</td>
</tr>
</tbody>
</table>

Microsatellite instability is seen at two or more microsatellite loci. This tumor shows high-frequency microsatellite instability (MSI-H). Further evaluation of this patient is recommended to determine if the patient carries a germine mutation in genes affecting DNA Mismatch Repair.
What value is there in recognizing MSI colorectal tumors?

1. Prognosis
2. Response to chemotherapy
3. Screen for Lynch Syndrome (HNPCC)
Lynch Syndrome
(HNPCC – Hereditary Non Polyposis Colon Cancer)

• Aldred Warthin – 1913

• Henry Lynch
  – Lynch syndrome I - colon only
  – Lynch syndrome II - colon and extracolonic tumors
Lynch Syndrome (HNPCC)

- Accounts for 3-4% of all colon cancers
- Accounts for 15-20% of MSI tumors
- Inherited *predisposition* to many different cancers, including colon cancer
Lynch Syndrome (aka HNPCC) - cardinal features

- autosomal dominant inheritance
- gene penetrance for CRC of 85-90%
- develop CRC at an early age - 45 yrs
- most CRC (70%) proximal to splenic flexure
- multiple CRC’s common - synchronous and metachronous
- prognosis better than sporadic CRC
- associated pathologic features
- increased risk for other malignancies
<table>
<thead>
<tr>
<th>Tumor Site</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>endometrium</td>
<td>second most common</td>
</tr>
<tr>
<td>stomach</td>
<td>older generations</td>
</tr>
<tr>
<td>small bowel</td>
<td>risk 25X in HNPCC</td>
</tr>
<tr>
<td>hepatobiliary tract</td>
<td>5% risk</td>
</tr>
<tr>
<td>ureter and pelvis</td>
<td>14 - 20% risk</td>
</tr>
<tr>
<td>skin</td>
<td>Muir - Torre</td>
</tr>
<tr>
<td>pancreas</td>
<td>trend for increase</td>
</tr>
<tr>
<td>brain</td>
<td>GBM in some HNPCC (Turcot)</td>
</tr>
<tr>
<td>hematologic</td>
<td>case reports</td>
</tr>
<tr>
<td>soft tissue</td>
<td>case reports</td>
</tr>
<tr>
<td>larynx</td>
<td>case report</td>
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</tbody>
</table>
### Lynch Syndrome - cumulative cancer risk in LS carriers by age 70

<table>
<thead>
<tr>
<th>Site of tumor</th>
<th>Finnish population (%)</th>
<th>HNPCC families (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>colon/rectum</td>
<td>1.6</td>
<td>82</td>
</tr>
<tr>
<td>endometrium</td>
<td>1.3</td>
<td>60</td>
</tr>
<tr>
<td>stomach</td>
<td>0.8</td>
<td>13</td>
</tr>
<tr>
<td>ovary</td>
<td>1.3</td>
<td>12</td>
</tr>
<tr>
<td>bladder, ureter, urethra</td>
<td>0.7</td>
<td>4.0</td>
</tr>
<tr>
<td>brain</td>
<td>0.9</td>
<td>3.7</td>
</tr>
<tr>
<td>kidney</td>
<td>0.8</td>
<td>3.3</td>
</tr>
<tr>
<td>biliary tract, gallbladder</td>
<td>0.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Lynch Syndrome - cumulative cancer risk by age 70

Cancer incidence in women HNPCC carriers:
- endometrial cancer 60%
- colon cancer 54%

Lynch Syndrome CRC - pathological features

- poor differentiation
- increased signet cells
- medullary features
- peritumoral lymphocyte infiltration
- Crohn’s like reaction
- tumor infiltrating lymphocytes (TIL’s)
## Comparison of CRC subgroups

<table>
<thead>
<tr>
<th></th>
<th>All CRC</th>
<th>Right colon</th>
<th>HNPCC</th>
<th>sporadic MSI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>65-70</td>
<td>70</td>
<td>40-50</td>
<td>60</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>M&gt;F</td>
<td>F&gt;M</td>
<td>M=F</td>
<td>M=F</td>
</tr>
<tr>
<td><strong>Proximal Location</strong></td>
<td>30%</td>
<td>-</td>
<td>70%</td>
<td>94%</td>
</tr>
<tr>
<td><strong>MSI</strong></td>
<td>10-15%</td>
<td>29%</td>
<td>86-92%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Poorly differentiated</strong></td>
<td>10%</td>
<td>10%</td>
<td>39%</td>
<td>53%</td>
</tr>
<tr>
<td><strong>Mucinous</strong></td>
<td>10-20%</td>
<td>20-30%</td>
<td>15-45%</td>
<td>35%</td>
</tr>
<tr>
<td><strong>Crohn’s-like reaction</strong></td>
<td>20-28%</td>
<td>10-36%</td>
<td>42%</td>
<td>47%</td>
</tr>
</tbody>
</table>
5 Year Experience with MSI Testing (2004-2008)
ACL Laboratories

Distribution of Cases by Result

<table>
<thead>
<tr>
<th>Result</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS</td>
<td>170</td>
<td>70%</td>
</tr>
<tr>
<td>MSI-L</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>MSI-H</td>
<td>71</td>
<td>29</td>
</tr>
<tr>
<td>TOTAL</td>
<td>243</td>
<td>100</td>
</tr>
</tbody>
</table>
Anatomic Distribution of MSI-H and MSS Tumors

MSI-H
- 1 (2%)
- 2 (1%)
- 11 (17%)
- 24 (15%)
- 36 (56%)
- 31 (20%)
- 7 (11%)
- 16 (10%)

MSS
- 5 (3%)
- 7 (11%)
- 17 (11%)
- 2 (3%)
- 77 (50%)
- 24 (15%)
- 11 (17%)
- 36 (56%)
- 2 (1%)
- 1 (2%)
Age Distribution

MSS Age/Sex Distribution

MSI-H Age/Sex Distribution
HNPCC – The search for associated genes - 1994

- Exclude FAP and known CRC associated genes
- Studied genetic markers linked to HNPCC
- Two observations made:
  1. Predisposition genes located on chromosomes 2p and 3p
  2. Microsatellite markers show unusual changes (microsatellite instability - MSI or replication error phenotype - RER)
HNPCC predisposition genes

- Sequence homology to known bacterial and yeast genes that:
  1. Are required for a specific type of DNA repair
  2. Defects result in increased mutation rates

\[ \text{mut}S \quad \text{human } \text{mut}S \text{ homologue} \quad \text{hMSH2} \]

\[ \text{mut}L \quad \text{human } \text{mut}L \text{ homologue} \quad \text{hMLH1} \]
<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>E.coli homologue</th>
<th>Freq. In HNPCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2</td>
<td>2p15-2p16</td>
<td>mutS</td>
<td>~35%</td>
</tr>
<tr>
<td>MLH1</td>
<td>3p21</td>
<td>mutL</td>
<td>~35%</td>
</tr>
<tr>
<td>PMS1</td>
<td>2q31</td>
<td>mutL</td>
<td>rare</td>
</tr>
<tr>
<td>PMS2</td>
<td>7p22</td>
<td>mutL</td>
<td>~15%</td>
</tr>
<tr>
<td>GTBP/MSH6</td>
<td>2p15-2p16</td>
<td>mutS</td>
<td>~15%</td>
</tr>
<tr>
<td>other</td>
<td>??</td>
<td>??</td>
<td>rare</td>
</tr>
</tbody>
</table>
When to suspect Lynch Syndrome

- Amsterdam Criteria
  - Clinical guidelines for when to suspect HNPCC
- Bethesda Guidelines
  - Guidelines for when to do MSI (or MMR) testing
- Screen all new colon cancers?
  - EGAPP Working Group Report
HNPCC - Amsterdam criteria II - 1998

- Patient with HNPCC associated cancer (CRC, endometrium, small bowel, ureter, renal pelvis)
- FAP must be excluded
- 3 affected relatives with HNPCC associated cancer
- at least one must be a first degree relative of the other two
- two successive generations affected
- one of the affected <50 yr
- histologically verified tumors

Vasen, et al., Gastroenterology 116: 1453-6, 1999
Revised Bethesda Guidelines for testing colorectal tumors for MSI - 2004

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer in a patient less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC associated tumors, regardless of age.
3. Colorectal cancer with the MSI-H histology diagnosed in a patient less than 60 yr.
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 yr.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

“…found sufficient evidence to recommend offering genetic testing for Lynch syndrome to individuals with newly diagnosed colorectal cancer to reduce morbidity and mortality in relatives.”

= universal screening of all new CRCs

What value is there in recognizing MSI colorectal tumors?

1. Prognosis
2. Response to chemotherapy
3. Screen for Lynch Syndrome (HNPCC)
Prognostic significance of MSI in sporadic CRC

Gryfe, R et al, NEJM 2000; 342:69-77
MSI and response to chemotherapy?

Tumor Microsatellite-Instability Status as a Predictor of Benefit from Fluorouracil-Based Adjuvant Chemotherapy for Colon Cancer

## HNPCC - mismatch repair gene mutations

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<th>Location</th>
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<th>Freq. In HNPCC</th>
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<tbody>
<tr>
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<td>mutS</td>
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</tr>
<tr>
<td>other</td>
<td>??</td>
<td>??</td>
<td>rare</td>
</tr>
</tbody>
</table>
IHC for MMR Protein Expression

MLH1

MSH2

MSH6

PMS2
Can I use IHC in lieu of MSI?

- Must look for all 4 proteins with well validated assays
- Sensitivity of ISH for LS mutations is ~ 95%
- IHC can direct mutation testing
- MSH2 IHC highly correlated with MSH2 mutation
  - Is this a “genetic” test?
- Optimal detection = MSI + IHC
Summary

• Two biologically distinct pathways for CRC
• Recognizing the MSI pathway is significant for prognosis, therapy, and LS screening
• Sufficient evidence exists for universal CRC screening for Lynch Syndrome
• Pathologists have responsibility for recognizing features suggestive of Lynch Syndrome
• MSI and IHC are both good, but have limits: optimal screening would need both
KRAS 30%

BRAF 11%
Receptor tyrosine kinase signaling in cancer
KRAS Mutations and anti-EGFR Monoclonal Therapy

- Cetuximab
- Panitumumab

- Ineffective if KRAS has codon 12 or 13 mutation
Predictive and prognostic value of KRAS mutations in metastatic colorectal cancer patients treated with cetuximab: A meta-analysis of 22 studies


<table>
<thead>
<tr>
<th></th>
<th>KRAS Mutated</th>
<th>KRAS Wild Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Response Rate</td>
<td>14%</td>
<td>39%</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>6.9 mo</td>
<td>13.5 mo</td>
</tr>
</tbody>
</table>
- KRAS codon 12 and 13 mutation testing is required for use of monoclonal antibody inhibitors of EGFR for metastatic CRC

- Evidence is good that BRAF mutation V600E is also predictive of non-response
Selection of blocks is important!
What is the basis of sporadic MSI-H?

- Hypermutability Pathway
- Chromosomal Instability Pathway
- FAP
- HNPCC

Image: Pie chart showing the distribution of the basis of sporadic MSI-H.
Allelic MLH1 gene inactivation

Normal allele

Mutated allele (genetic inactivation)

Methylated allele (epigenetic inactivation)

MLH1 protein

Mutant MLH1 protein

No MLH1 expression
What is the basis of sporadic MSI-H?

- Hypermutability Pathway
- Chromosomal Instability Pathway
- FAP
- HNPCC

Mostly MLH1 promoter hypermethylation
CpG islands

- Most CpG sites are methylated (i.e. inactive)
- Frequently CpG islands within promoters are protected from methylation
- These are targets for abnormalities in cancer cells
- CpG Island Methylation may represent an overlying pathway for CRC development
CpG Island Methylator Phenotype - CIMP

- Significant for both Chromosomal Instability and Hypermutability pathways

- Possible relationship to “serrated” histology

CpG Island Methylator Phenotype - CIMP

- Methylation status of 5 genes:
  - hMLH1, p16, MINT1, MINT2, MINT31

- Identified 3 classes:
  - CIMP-High (4-5 methylated loci)
  - CIMP-Low (1-3 methylated loci)
  - CIMP-No (no methylated loci)
# CIMP Classification of CRC

<table>
<thead>
<tr>
<th></th>
<th>No-CIMP</th>
<th>CIMP-Low</th>
<th>CIMP-High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>49%</td>
<td>34%</td>
<td>17%</td>
</tr>
<tr>
<td><strong>MSI-H</strong></td>
<td>3.2</td>
<td>6.6</td>
<td>61.5</td>
</tr>
<tr>
<td><strong>BRAF mut</strong></td>
<td>2.5</td>
<td>7.1</td>
<td>57.9</td>
</tr>
<tr>
<td><strong>KRAS mut</strong></td>
<td>32.5</td>
<td>45.1</td>
<td>18.1</td>
</tr>
</tbody>
</table>
# MSI-H and CIMP

<table>
<thead>
<tr>
<th></th>
<th>No-CIMP</th>
<th>CIMP-Low</th>
<th>CIMP-High</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF mut</td>
<td>33.3</td>
<td>7.7</td>
<td>81.0</td>
</tr>
<tr>
<td>KRAS mut</td>
<td>33.3</td>
<td>23.1</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>No-CIMP</td>
<td>CIMP-Low</td>
<td>CIMP-High</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>BRAF mut</td>
<td>1.4</td>
<td>7.1</td>
<td>78.4</td>
</tr>
<tr>
<td>KRAS mut</td>
<td>32.5</td>
<td>46.7</td>
<td>43.2</td>
</tr>
</tbody>
</table>
Relative survival according to methylation status in MSS group

Figure 1. Relative survival according to methylation status in MSS group ($P < 0.001$). No-CIMP, ---; CIMP-Low, - - -; CIMP-High, ---.
BRAF - summary

• Mutations activate MAPK signaling pathways

• Mutated in ~90% of sporadic MSI-H tumors

• Never mutated in Lynch Syndrome tumors

• Most (90%) mutations: c.1799T>A (p.Val600Glu) aka BRAF V600E
Applications for BRAF mutation testing

- In MSI-H (MLH1) tumors, BRAF mutation correlates with hypermethylation, not Lynch Syndrome
  - No testing for Lynch needed

- Selection of therapy
  - BRAF mutations indicate no response to anti-EGFR inhibitors

- Prognosis
  - BRAF mutation correlated with poor outcome in MSS tumors
 Colon cancer tissue specimen

Do KRAS mutation test.

If KRAS is mutated, tumor is unlikely to respond to EGFR inhibitors.

If KRAS is not mutated, do BRAF mutation test, and if BRAF is mutated then patient is unlikely to respond to EGFR inhibitors.

If BRAF is not mutated, continue workup for CpG island methylator phenotype, which suggests the tumor is sporadic, or refer patient for genetic counseling and offer sequencing of mismatch repair genes.

If microsatellite instability test reveals MSI-H or if immunohistochemistry reveals loss of expression of MLH1, MSH2, PMS2, or MSH6, continue workup for possible Lynch syndrome.

If immunohistochemistry shows lost MLH1 expression, do BRAF mutation test.

If BRAF is mutated, cancer is probably sporadic.
Important Concepts

- Polyp – carcinoma sequence
- Vogelstein Model
- MSI vs MSS tumors
- Lynch Syndrome
- KRAS and MAPK pathway (EGFR Inhibitors)
- BRAF and MAPK pathway (EGFR Inhibitors)
- Epigenetic control of cancer and sporadic MSI
- BRAF and sporadic MSI
- CIMP classification of CRC