Clinical Colorectal Cancer Genetics for the General Surgeon

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Cancer is a Genetic Disease

- Cancer is in essence a genetic disease
- Cancer is not an event, it is a multistep process
- Genetic mutations contribute to, rather than cause cancer
- Alterations in cancer cell DNA
  - ↑ cell proliferation
  - ↓ cell death (apoptosis)
  - Local invasiveness
  - Metastatic spread
- Most cancers result from mutations in somatic cells
  - Sporadic colorectal cancer
- Some cancers result from mutations in germline cells
  - Inherited colorectal cancer (Lynch, FAP, MAP, JPS, PJS)
Colorectal Cancer Genetics

• Pubmed: colorectal AND cancer AND genetics = >10,000 articles in the past 5 years
  Enormous topic

Today’s talk:

• Selected, clinically relevant aspects of colorectal cancer molecular genetics:
  - Cancer treatment - medical & surgical issues
  - Cancer prognosis
  - Response to therapy

• Genetic emphasis:
  - Microsatellite instability & DNA mismatch repair
  - EGFR & VEGF signaling pathways
The Adenoma to Carcinoma Sequence: Multiple Genetic Alterations

- APC mutation & 5q loss
- MMR-deficiency
- K-Ras or BRAF mutation
- Global Hypomethylation
- COX-2 overexpression
- Loss of 18q SMAD2,4,DCC?
- p53 mutation & 17p loss

Mutator predisposition pathways → multiple genetic alterations
Microsatellite Instability (MSI)
DNA Mismatch Repair (MMR)

Lynch Syndrome &
Sporadic Colorectal Cancer
The MSI Mutator Pathway

Mismatch repair deficiency
- Loss of function of one MMR gene: MLH1, MSH2, MSH6, PMS2
- → MSI
- 15% of sporadic colorectal cancer
- Lynch syndrome (2-4%)
- ↑↑ specific mutations (i.e. BRAF, TGFBRII, CTNNB1)

<table>
<thead>
<tr>
<th></th>
<th>MSI</th>
<th>MSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal to SF</td>
<td>80%</td>
<td>42%</td>
</tr>
<tr>
<td>AJCC I/II</td>
<td>77%</td>
<td>52%</td>
</tr>
<tr>
<td>Poor grade</td>
<td>32%</td>
<td>6%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>30%</td>
<td>10%</td>
</tr>
<tr>
<td>Signet ring</td>
<td>26%</td>
<td>8%</td>
</tr>
</tbody>
</table>

all p<0.0001

Yamuachi Gut 2012
1,443 colorectal cancers
**MMR Immunohistochemistry**

**DNA Mismatch Repair**

- **MLH1**
- **MSH2**
- **PMS2**
- **MSH6**

**MLH1**-deficient
15% of sporadic CRC
45% of Lynch syndrome

**PMS2**-deficient
<5% of Lynch syndrome

**MSH2**-deficient
45% of Lynch syndrome

**PMS2**-deficient
<5% of Lynch syndrome

**MSH6**-deficient
<10% of Lynch syndrome

**MMR IHC can help guide genetic testing & clinical management**
What is Sporadic MSI Colorectal Cancer?

**Normal**
- promoter unmethylated
- gene transcribed & translated

**Sporadic MSI CRC**
- promoter hypermethylated
- transcription blocked
- ~15% of sporadic CRC

**CpG Island Methylator Phenotype**
- Epigenetic CIMP pathway
- 20-30% of colorectal cancers
- often older, female, right-sided
- often BRAF mutations

**Lynch MSI CRC**
- MLH1 mutated
- ~45% of Lynch (MSH2, MSH6, PMS2)
Colorectal Cancer Mutator Pathways

- **Chromosomal Instability Pathway (CIN)**
  - CIN/MSS: 80% < 1%
  - CIN/CIMP: 5% not reported

- **CpG Island Methylator Pathway (CIMP)**
  - MSI/CIMP: 15% rare?

- **Microsatellite Instability Pathway (MSI)**
  - MSI: rare 2-4%

**Sporadic Inherited**
- CIN/MSS: 80% < 1%
- CIN/CIMP: 5% not reported
- MSI/CIMP: 15% rare?
- MSI: rare 2-4% Lynch
Clinical Implications of MSI Beyond Lynch Syndrome
**MSI & Colorectal Cancer Survival**

Cox Proportional Hazards $MSI \text{ vs } MSS = 0.45 \ (0.30-0.68), \ p<0.001$

*MSI is prognostic of independent, multivariate improved survival*

Gryfe NEJM 2000
**MSI: Colorectal Cancer Prognosis**

Guastadisegni EJC 2010

- meta-analysis
- 20 studies
- 9,243 patients

**Hazard Ratio**

**MSI-H vs MSS = 0.60 (0.53-0.69)**

- association maintained across cancer stages
- no evidence of:
  - publication bias
  - study heterogeneity

**MSI is associated with an improved prognosis in colorectal cancer**
Colorectal Cancer Molecular Genetics and Therapy
Why Do We Need Predictive Biomarkers?

<table>
<thead>
<tr>
<th>Stage II/III</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery alone</td>
<td>55%</td>
<td>64%</td>
</tr>
<tr>
<td>5yr benefit FULV</td>
<td>+12%</td>
<td>+7% Gill JCO 2004</td>
</tr>
<tr>
<td>additional 5yr benefit FOLFOX</td>
<td>+6%</td>
<td>+0-5% Andre JCO 2009</td>
</tr>
<tr>
<td></td>
<td>+18%</td>
<td>+7-17%</td>
</tr>
</tbody>
</table>

82-93% do **not** benefit from adjuvant chemotherapy

- 55-64% cured by surgery alone & will **never** benefit
- Toxicity (grade III/IV): FULV >20%, FOLFOX >40%
- ↑ complications & cost with FOLFOX
Prognostic & Predictive Biomarkers

**Prognostic** - marker status is associated with a difference in clinical outcome
  • cancer characteristic

**Predictive** - marker status is associated with a difference in response to treatment
  • more complex cancer-treatment characteristic

**Predictive Studies:**

- Both treated & untreated patients are necessary
- Surgery only arm required to determine which patients benefit from FULV (7-12%)
- Ethical dilemma in an era where FOLFOX is the standard of care (but benefits 7-18% of patients)
MSI & Predicting 5-FU Response

**MSS, but not MSI, is predictive of improved survival with adjuvant 5-FU compared to surgery alone.**
# MSI: Predicting 5-FU Response

<table>
<thead>
<tr>
<th>Study</th>
<th>Journal</th>
<th>Patients</th>
<th>MSI (%)</th>
<th>Good Prognosis</th>
<th>Predicts 5-FU Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinicrope, 2011</td>
<td>JNCI</td>
<td>2,141</td>
<td>16</td>
<td>MSI</td>
<td>MSS/LS MSI**</td>
</tr>
<tr>
<td>Hutchins, 2011</td>
<td>JCO</td>
<td>1,913</td>
<td>11</td>
<td>MSI</td>
<td>No</td>
</tr>
<tr>
<td>Ohrling, 2010</td>
<td>Acta Oncol</td>
<td>1,006</td>
<td>16</td>
<td>No</td>
<td>No**</td>
</tr>
<tr>
<td>Ribic, 2004</td>
<td>NEJM</td>
<td>570</td>
<td>17</td>
<td>MSI</td>
<td>MSS</td>
</tr>
<tr>
<td>Kim, 2007</td>
<td>JCO</td>
<td>542</td>
<td>18</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Halling, 1999</td>
<td>JNCI</td>
<td>508</td>
<td>15</td>
<td>MSI</td>
<td>No</td>
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<tr>
<td>Sargent, 2010</td>
<td>JCO</td>
<td>457</td>
<td>15</td>
<td>MSI</td>
<td>MSS</td>
</tr>
<tr>
<td>Barratt, 2002</td>
<td>Lancet</td>
<td>368</td>
<td>24</td>
<td>No</td>
<td>MSS***</td>
</tr>
<tr>
<td>Storojeva, 2005</td>
<td>Onc Rep</td>
<td>160</td>
<td>NA</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

* MSS & LS MSI benefit, not sporadic MSI  
** Negative 5-FU RCT  
*** trend

MSS predicts 5-FU benefit: 2-4 of 9 RCTs
MSI benefit from 5-FU: 0 of 9 RCTs

Adjuvant FOLFOX not recommended for MSI AJCC II
MSI & Adjuvant Irinotecan?

**CALGB, 723 stage III**

<table>
<thead>
<tr>
<th>MSI-Status</th>
<th>Rx</th>
<th>5-yr DFS</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI</td>
<td>FULV</td>
<td>57%</td>
<td>0.52 (0.25-1.07), p=0.07</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>MSS</td>
<td>FULV</td>
<td>61%</td>
<td>1.01 (0.79-1.29)</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>59%</td>
<td></td>
</tr>
</tbody>
</table>

- Suggests that MSI is predictive of improved survival with irinotecan
- Not validated by PETACC-3 RCT of FU vs IFL in 1,254 stage II/III

Tejpar, JCO 2009
Molecular Genetics-based Therapeutics

anti-EGFR therapy
anti-VEGF therapy
EGFR Targeted Colorectal Cancer Therapy: Cetuximab (Erbitux) & Panitumumab (Vectibix)

Cetuximab & Panitumumab:
- Anti-EGFR monoclonal antibodies

K-Ras & BRAF:
- Oncogenes
- Downstream of EGFR
- Circumvent anti-EGFR therapy
- Activating mutations:
  - KRAS 40% of colorectal cancers
  - BRAF 15% of colorectal cancers
KRAS<sup>WT</sup>: Predicting Anti-EGFR Response

Amado JCO 2008
- 427 patients, stage IV
- 43% KRAS mutation
- panitumumab

Karapetis, NEJM 2008
- 394 patients, stage IV
- 42% KRAS mutation
- cetuximab

KRAS<sup>WT</sup> is predictive of improved survival with anti-EGFR palliative therapy
What about BRAF mutation & anti-EGFR therapy?
Complicated interrelationships: MSI, CIMP & EGFR

CIMP (20%):
- 67% MSI (sporadic)
- 33% MSS

BRAF (15%):
- 70% CIMP
- 55% MSI (70% of sporadic)

KRAS (35%):
- 90% CIMP-negative
- 95% MSS
- 5% MSI (35% of LS)
- BRAF & KRAS mutually exclusive

Based on Yamauchi Gut 2012
1,443 colorectal cancers

Significant associations of mutator pathways & somatic mutations
# BRAF Mutation and Prognosis

<table>
<thead>
<tr>
<th>Study</th>
<th>Journal</th>
<th>RCT</th>
<th>Patients</th>
<th>BRAF (%)</th>
<th>Poor Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hutchins, 2011</td>
<td>JCO</td>
<td>Adjuvant</td>
<td>1,584</td>
<td>8</td>
<td>MSS/BRAF</td>
</tr>
<tr>
<td>Roth, 2009</td>
<td>JCO</td>
<td>Adjuvant</td>
<td>1,307</td>
<td>8</td>
<td>MSS/BRAF</td>
</tr>
<tr>
<td>Ogino, 2011</td>
<td>Clin Cancer Res</td>
<td>Adjuvant</td>
<td>506</td>
<td>15</td>
<td>MSS/BRAF</td>
</tr>
<tr>
<td>Maughan, 2011</td>
<td>Lancet</td>
<td>Palliative*</td>
<td>1,269</td>
<td>8</td>
<td>BRAF</td>
</tr>
<tr>
<td>Van Cutsem 2011</td>
<td>JCO</td>
<td>Palliative*</td>
<td>999</td>
<td>6</td>
<td>BRAF</td>
</tr>
<tr>
<td>Richman, 2009</td>
<td>JCO</td>
<td>Palliative</td>
<td>711</td>
<td>8</td>
<td>BRAF</td>
</tr>
<tr>
<td>Tol, 2010</td>
<td>EJC</td>
<td>Palliative*</td>
<td>559</td>
<td>9</td>
<td>BRAF</td>
</tr>
<tr>
<td>Tveit, 2012</td>
<td>JCO</td>
<td>Palliative*</td>
<td>498</td>
<td>12</td>
<td>BRAF</td>
</tr>
<tr>
<td>Price, 2011</td>
<td>JCO</td>
<td>Palliative</td>
<td>315</td>
<td>11</td>
<td>BRAF</td>
</tr>
</tbody>
</table>

* anti-EGFR RCT

**Oncogenic BRAF mutation is associated with poor prognosis**
- Poor survival prognosis MSS/BRAF specific
- MSI/BRAF (sporadic MSI) not associated with poor prognosis
- BRAF not predictive of response to therapy, including anti-EGFR
Anti-VEGF, Angiogenesis-inhibition therapy
Bevacizumab in Stage IV CRC

Bevacizumab (Avastin)
- Monoclonal antibody VEGF inhibitor
- Inhibits angiogenesis
- Potentially complicates wound healing

Galfrascoli Dig Liver Dis 2011
- Systemic review 6 RCTs, 3,385 stage IV CRC pt’s
  - OS = 0.80 (0.71-0.91)
  - PFS = 0.62 (0.52-0.74)
Bevacizumab and Surgery

Galfrascoli Dig Liver Dis 2011
- HTN (Gr III/IV) = 2.98 (2.32-3.84)
- Bleeding (Gr III/IV) = 2.07 (1.19-3.62)
- GI perforation = 5.04 (1.72-14.79)
- GI perforation = 1-4% in CRC
  = 3-11% in ovary
  = 1% in others

Manufacturer Warning:
- Half life = 11-50 days
- Do not give Avastin within 28d of surgery
- Hold Avastin at least 28d for elective surgery
- Discontinue Avastin in patient with wound dehiscence or wound healing complications

Grade III / IV wound healing or bleeding complications within 60d postop
- 528/1,132 in phase II/III had surgery

<table>
<thead>
<tr>
<th>Time of Surgery</th>
<th>Chemo</th>
<th>Chemo + Avastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>194</td>
<td>230</td>
</tr>
<tr>
<td>Complications</td>
<td>(1) 0.5%</td>
<td>(3) 1.3%</td>
</tr>
<tr>
<td>During study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>29</td>
<td>75</td>
</tr>
<tr>
<td>Complications</td>
<td>(1) 3.4%</td>
<td>(10) 13.3%</td>
</tr>
</tbody>
</table>

Scappaticci J Surg Onc 2005

Anti-VEGF molecular therapy ↑ cost & associated with ↑ toxicity & ↑ surgical complication rates
Summary

MMR-deficiency → MSI
• Lynch syndrome & 15% sporadic colorectal cancer
• ↑ prognosis
• MSS, not MSI likely predictive of ↑ 5-FU response
• Sporadic MSI associated with CIMP & BRAF mutation

EGFR signaling
• KRAS mutation in 40% of colorectal cancer circumvents cetuximab / panitumumab anti-EGFR therapy
• MSS/BRAF mutation associated with ↓ prognosis (not MSI/BRAF)

Anti-VEGF angiogenesis inhibitors
• Bevacizumab ↑ palliative prognosis, but associated with spontaneous GI perforations, hemorrhage & ↑ surgical complications