Genetic Modifiers of Chemotherapy for Colorectal Cancer

September 27, 2011

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Colorectal Cancer

- 1,233,700 cases/year Worldwide
- ~146,000 cases/year in the United States (2011)

Colorectal Cancer

• Carcinomas generally begin as adenomas
  – therefore, removal of adenomas should reduce cancer incidence

• Screening
  – Tests that detect cancers
    • FOBT, FIT, Stool DNA tests
  – Tests that detect adenomas and cancers
    • DCBE, Flexible sigmoidoscopy, Colonoscopy, CT Colonography
Polypectomy
Colon Cancer - Endoscopic View
## Staging for Colorectal Cancer

<table>
<thead>
<tr>
<th>Stage</th>
<th>AJCC TNM Stage</th>
<th>Astler-Coller-Dukes Stage</th>
<th>Five-Year Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1-2, N0, M0</td>
<td>A, B1</td>
<td>85-95</td>
</tr>
<tr>
<td>II</td>
<td>T3-4, N0, M0</td>
<td>B2, B3</td>
<td>60-80</td>
</tr>
<tr>
<td>III</td>
<td>Any T, N1-3, M0</td>
<td>C</td>
<td>30-50</td>
</tr>
<tr>
<td>IV</td>
<td>Any T, any N, M1</td>
<td>D</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>
Stage II Prediction of Behavior

Stage II Prediction of Behavior

Personalized Approach for Patients with Stage II CRC

- Genetic markers may predict which stage II patients might behave like stage III patients, separating those who may behave like stage I patients.
- May predict who could benefit from chemotherapy – micrometastasis.
# Drugs for Advanced Colorectal Cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Stage for Treatment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-fluorouracil (5-FU)</td>
<td>antimetabolite</td>
<td>III, IV</td>
<td>Used with leukovorin</td>
</tr>
<tr>
<td>Irinotecan (Camptosar)</td>
<td>Topo-isomerase I inhibitor</td>
<td>III, IV</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin (Eloxitin)</td>
<td>platinates DNA</td>
<td>III, IV</td>
<td></td>
</tr>
<tr>
<td>Avastin (bevacizimab)</td>
<td>VEGF</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>Erbitux (cetuximab)</td>
<td>EGFR/HER1/c-ERB1</td>
<td>IV</td>
<td>WT KRAS</td>
</tr>
<tr>
<td>Vectibix (panitumab)</td>
<td>EGFR</td>
<td>IV</td>
<td>WT KRAS</td>
</tr>
</tbody>
</table>
RAS Signaling in Colon Cancer

EGFR: overexpressed
RAS/RAF: mutational activation

RAS: ~50% CRC
RAF: in MMR-deficient sporadic tumors

Facilitates size growth
EGFR inhibitors ineffective with mutant RAS

DUAL PATHWAYS FOR COLON CANCER DEVELOPMENT

Normal → Adenoma

Genomic instability

Wnt:APC/β-catenin Pathway

Chromosomal Instability

LOH, Aneuploidy

RAS, p53 mutations

B-RAF mutation

Hypermutable Phenotype

ACVR2, TGFBR2, BAX, MMR genes, IGF2R, E2F4 frameshifts

FAP (germline APC mut)
-Lynch (germline MMR mut)
-Sporadic (LOH APC or hMLH1 hypermethylation)

p53 LOH

Cancer

Cancer
# MSI vs. MSS Colorectal Tumors

<table>
<thead>
<tr>
<th>MSI</th>
<th>MSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsatellite instability</td>
<td>Loss of heterozygosity (LOH)</td>
</tr>
<tr>
<td>Diploid</td>
<td>Aneuploid</td>
</tr>
<tr>
<td>Frequently mucinous</td>
<td>Few mucinous tumors</td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>Well differentiation</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>Fewer proximal tumors</td>
</tr>
<tr>
<td>Young (germline) / Old (hypermethylated hMLH1) patients</td>
<td>Few young patients</td>
</tr>
<tr>
<td>Few p53 mutation/LOH</td>
<td>p53 mutation/LOH</td>
</tr>
<tr>
<td>Lymphoid Crohn’s-like histology</td>
<td></td>
</tr>
</tbody>
</table>
DNA Mismatch Repair

- Requires all components for full function
  - “major”: hMSH2, hMLH1
  - “minor”: hMSH6, hMSH3, hPMS2
- Proteins interact as heterodimer:
  - hMSH2-hMSH6, hMSH2-hMSH3
  - hMLH1-hPMS2
- Mutations cause Lynch syndrome
- Second copy is inactivated in tumors (tumor suppressor gene)
- Perturbed function causes phenotype of microsatellite instability and mutations in target genes
DNA Mismatch Repair

Grady and Carethers. Gastroenterology 2008
O\textsuperscript{6} meG Mispairing

Thymine

\[
\begin{array}{c}
\text{CH}_3 \\
\text{C} \\
\text{H} \\
\text{C} \\
\text{N} \\
\text{C} \\
\text{O}
\end{array}
\begin{array}{c}
6 \\
3 \\
1 \\
2 \\
4 \\
5 \\
4
\end{array}
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{C} \\
\text{C} \\
\text{N} \\
\text{C} \\
\text{H}
\end{array}
\begin{array}{c}
\text{CH}_3 \\
\text{O} \\
\text{N} \\
\text{H} \\
\text{O} \\
\text{H} \\
\text{H}
\end{array}
\]

O\textsuperscript{6} Methylguanine

\[
\begin{array}{c}
\text{C} \\
\text{C} \\
\text{N} \\
\text{C} \\
\text{O} \\
\text{C} \\
\text{N}
\end{array}
\begin{array}{c}
6 \\
5 \\
1 \\
2 \\
3 \\
4 \\
9
\end{array}
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{C} \\
\text{C} \\
\text{N} \\
\text{C} \\
\text{H}
\end{array}
\begin{array}{c}
\text{CH}_3 \\
\text{O} \\
\text{N} \\
\text{H} \\
\text{O} \\
\text{H} \\
\text{H}
\end{array}
\]

Deoxyribose

Deoxyribose
**G₂/M Arrest Mechanism**

First S Phase (DNA Synthesis)

- Repair attempted (recognizes mispair)
- G₂/M Arrest or Cell Death
- Fidelity of DNA maintained

S Phase Delay (within 24 hours)

- MMR-proficient
  - Repair attempted (recognizes mispair)
  - G₂/M Arrest or Cell Death
  - Fidelity of DNA maintained

- MMR-deficient
  - No repair attempted
  - Mitosis completed
  - New point mutations introduced

5-Fluorouracil (5-FU) and Treatment of Colon Cancer

- Principle treatment for stage III colorectal cancer
- Cellular toxicity of this fluoropyrimidine due to:
  - Incorporation into all forms RNA
  - Blockage of thymidylate synthetase (TS)
    - Prohibits conversion of dUTP to dTTP
- DNA incorporation reported in breast cancer cells
  - Effect on toxicity thought to be minor mechanism
Figure 2. Intracellular metabolism of 5-FU enzymes are as follows: 1, uridine phosphorylase; 2, uridine kinase; 3, orotate phosphoribosyltransferase; 4 and 9, pyrimidine monophosphate kinase; 5 and 10, pyrimidine diphosphate kinase; 6, RNA polymerase; 7, thymidine phosphorylase; 8, thymidine kinase; 11, DNA polymerase, 12, ribonucleotide reductase; 13, deoxyuridine triphosphate pyrophosphatase, 14, uracil-DNA-glycosylase; TS = thymidylate synthase.
5-FU Hydrogen Bonding
5FU Sensitivity and DNA MMR

5-FU Sensitivity With Re-expression of \textit{hMLH1}

- SW48 human colorectal cancer cells
  - Hypermethylated \textit{hMLH1}
  - Resistance to 5-FU
- Demethylated with 5-Aza-2’ deoxycytidine
  - Re-expression of \textit{hMLH1} by mRNA, protein expression
  - Sensitive to 5-FU

Arnold, \textit{et. al}. Int J Cancer 2003;106:66-73
Univariate Analysis for Survival: MSI and 5-FU

<table>
<thead>
<tr>
<th>Analysis</th>
<th>No. of Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on MSI Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSI-H overall</td>
<td>36</td>
<td>0.99</td>
</tr>
<tr>
<td>non-MSI-H overall</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>MSI-H with 5-FU</td>
<td>10</td>
<td>0.74</td>
</tr>
<tr>
<td>Non-MSI-H with 5-FU</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>MSI-H without 5-FU</td>
<td>26</td>
<td>0.998</td>
</tr>
<tr>
<td>non-MSI-H without 5-FU</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Based on 5-FU Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No 5-FU overall</td>
<td>138</td>
<td>0.04</td>
</tr>
<tr>
<td>5-FU overall</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>No 5-FU with MSI-H</td>
<td>26</td>
<td>0.52</td>
</tr>
<tr>
<td>5-FU with MSI-H</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>No 5-FU with non-MSI-H</td>
<td>112</td>
<td>0.04</td>
</tr>
<tr>
<td>5-FU with non-MSI-H</td>
<td>56</td>
<td></td>
</tr>
</tbody>
</table>

Kaplan-Meier: Survival and 5FU

All Patients

MMR-deficient (MSI)

MMR-proficient (non-MSI)

Studies of 5-FU Treatment, Survival and MSI Status

Table 3. Chemotherapy in Colorectal Cancer with Microsatellite Instability

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Study design</th>
<th>Adjuvant chemotherapy regimen</th>
<th>No. of patients (MSI/MSS)</th>
<th>Benefit of chemotherapy in patients with MSI</th>
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</thead>
<tbody>
<tr>
<td>Elsaleh</td>
<td>2000</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>63/669</td>
<td>Yes</td>
</tr>
<tr>
<td>Ribic</td>
<td>2003</td>
<td>Randomized controlled study</td>
<td>5-FU</td>
<td>95/475</td>
<td>No</td>
</tr>
<tr>
<td>Carethers</td>
<td>2004</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>36/168</td>
<td>No</td>
</tr>
<tr>
<td>de Vos tot Nederveen Cappel</td>
<td>2004</td>
<td>Lynch syndrome patients</td>
<td>5-FU</td>
<td>28/0</td>
<td>No</td>
</tr>
<tr>
<td>Storojeva</td>
<td>2005</td>
<td>Randomized controlled study</td>
<td>5-FU/mitomycin</td>
<td>21/139</td>
<td>No</td>
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<tr>
<td>Benatti</td>
<td>2005</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>256/1007</td>
<td>No</td>
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<tr>
<td>Popat</td>
<td>2005</td>
<td>Pooled data from multiple studies</td>
<td>5-FU</td>
<td>1277/6365</td>
<td>No</td>
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<tr>
<td>Lanza</td>
<td>2006</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>75/288</td>
<td>No</td>
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<tr>
<td>Jover</td>
<td>2006</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>66/688</td>
<td>No</td>
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<tr>
<td>Kim</td>
<td>2007</td>
<td>Prospective study</td>
<td>5-FU/leucovorin</td>
<td>98/444</td>
<td>No</td>
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<tr>
<td>Des Guetz</td>
<td>2009</td>
<td>Meta-analysis</td>
<td>—</td>
<td>454/2871</td>
<td>No</td>
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<tr>
<td>Bertagnolli</td>
<td>2009</td>
<td>Randomized controlled study</td>
<td>5-FU/irinotecan/leucovorin</td>
<td>106/677</td>
<td>No</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; MSS, microsatellite stable.

5FU may shorten survival in some MMR-deficient patients.

Summary

• There is improved survival in patients with non-MSI tumors after treatment with 5-FU.

• There is *no* improvement in survival in patients with MSI-H tumors after treatment with 5-FU.

• Stratification of patients by their tumor’s genomic instability can predict survival by treatment with 5-FU.
DNA Mismatch Repair

Grady and Carethers. Gastroenterology 2008
EMSA of hMutSα and 5-FU Oligos

Tajima, et. al. Gastroenterology 127:1678-1684, 2004
Biosensor (SPR) Analysis of hMutSα and 5-FU Binding and Release by ATP

Tajima, et. al. Gastroenterology 127:1678-1684, 2004
EMSA of hMutSβ and 5-FU Oligos

<table>
<thead>
<tr>
<th>Matched DNA</th>
<th>AA Loop</th>
<th>5FU Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMutSβ</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ATP</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Tajima, et. al. (submitted)
Comparison Binding of 5-FU with hMutSα and hMutSβ

Tajima, et. al. (submitted)
Cell growth with 5FU (5 μM) is affected by MMR background

MTT metabolic growth rate (% of control)

* (p < 0.05)

DLD1 (hMutSβ alone)

HCT116+chr3 (hMutSα alone)

SW480 (hMutSα and hMutSβ)

Tajima, et. al. (submitted)
Mismatch Repair / 5-FU Mechanism

5-FU

Thymidylate synthetase
↑ dUTP and ↑ FdUTP
↓ dTTP

DNA Synthesis:
dCTP, dATP, dGTP
dUTP or FdUTP (no dTTP)

CCA (5-FdU)CT ATC

-hMutSα
-hMutSβ

 Increase Cyclin E
 Decrease Histone H3

Chemosensitivity
Cell Toxicity and Death

mRNA, tRNA, etc.

G1 Arrest

Attempt Repair?

Plasmid to Isolate DNA Effects of 5-FU

Pure MMR Execution of 5-FU Cytotoxicity

Pure MMR Execution of 5-FU Cytotoxicity

Summary

• hMutSβ recognizes and binds 5FU-modified oligonucleotides, but to a lesser extent than hMutSα

• Reduced growth and increased apoptosis with 5FU:
  \[ h\text{MutS}_\alpha + h\text{MutS}_\beta > h\text{MutS}_\alpha > h\text{MutS}_\beta \]
Personalized Approach for Patients with MSI Cancers

- Immunohistochemistry (and MSI testing) of sample screens for possibility of MMR-deficient tumor
  - Lynch syndrome
  - Sporadic \textit{hMLH1}-deficient tumor

- Oncologist can use information for treatment decision making
  - Avoid stage II 5FU treatment if MMR-deficient
  - Stage III patients still get treated with 5FU regimens, but likely should not
Summary: DNA MMR and 5-FU

- Differences in response to 5-FU chemotherapy
  - *In vitro* cell death with intact DNA MMR
    - Re-expressed DNA MMR turns resistant cell line into sensitive
  - Improved patient survival with intact DNA MMR
  - DNA MMR proteins (both complexes) bind and recognize 5-FU in DNA

- Loss of DNA MMR (i.e. tumor MSI) abrogates 5-FU toxicity

- Mechanism for MMR triggering cellular death with 5-FU incompletely understood
  - Triggers G1 cell cycle proteins for G1 arrest (ie cyclin E)
  - Decreases Histone H3 at mRNA and protein levels
Acknowledgements

• Carethers’ Laboratory
  – Akihiro Tajima
  – Moriya Iwaizumi
  – Heekyung Chung
  – Deena Ream-Robinson
  – Stephanie Tseng-Rogenski
  – Maike Wilk

• Collaborators
  – C. Richard Boland
  – Richard Kolodner
  – Josef Jiricny
  – Robert Sandler

• Support
  – NIH