Aberrant DNA Methylation in Colorectal Cancer

April 20, 2010
Rebecca Fausel
THANK YOU

- Dr. Bill Grady and the Grady Lab:
  - Slavomir Dzieciatkowski
  - Andrew Kaz
  - Shelli Morris
  - Jam Kanngurn
  - Patty Trobridge
  - Michael Luo
Pathogenesis of Colon Cancer

- Multi-step process, polyp --> cancer sequence
- Accumulation of genetic and epigenetic alterations
- Three colorectal cancer molecular subgroups:
  - Microsatellite Unstable (MSI)
  - Chromosome Unstable (CIN)
  - CpG Island Methylator Phenotype (CIMP)
Epigenetic Alterations

- Epigenetic alterations include **aberrant methylation** of 5’ promoter regions of genes with CpG islands.
- Methylation causes transcriptional silencing and can **inactivate tumor suppressor genes**.
- Can be found in normal colon of people at increased risk for CRC – "field effect"
RET gene

• Rearranged during Transfection, Chromosome 10
• Using methylation microarrays, it was discovered that RET can be aberrantly methylated in colon cancer
  ▪ Receptor tyrosine kinase
  ▪ Activates major intracellular pathways
  ▪ Involved in cell cycle, proliferation, differentiation, motility, survival
**RET**

- **Dependence receptor:**
  - Presence of ligand: mediates a proliferative/anti-apoptotic signal
  - Absence of ligand: caspase-mediated cleavage of receptors, promotion of apoptosis
- Medullary Thyroid Cancer, MEN2A, MEN2B
- Hirschsprung’s disease
- RET expression in human embryonic kidney cells leads to marked increase in caspase activation and cell death (inhibited by GDNF)
Figure 1. Scheme of principal targets and potential therapeutic options.
GDNF (Glial-Derived Neurotrophic Factor)

- Ligand for RET receptor
- **Strong anti-apoptotic effects** on colonic epithelial cells, induces phosphorylation of p42 MAPK and Akt
- Essential for survival of enteric neurons
- **No GDNF immunoreactivity** could be found in normal human colonic epithelium (Gastroenterology 2003)
Previous Research in Grady Lab

- Transfection with RET increases cell death and caspase activity in colorectal cancer cell lines
  - Addition of GDNF inhibits apoptosis and caspase activity (concentration dependent)
  - MethyLight assays have shown there is significant difference in RET methylation between colon cancer and normal colon tissue
Question/Hypothesis

- Does methylation of RET lead to adenoma formation and/or adenoma progression to colorectal adenocarcinoma?
- To study this question, I assessed the frequencies of RET methylation in normal colon mucosa versus adenomas (early/advanced) versus colorectal cancers
Methylation Specific PCR

- **Bisulfite Treatment:** Converts unmethylated (but NOT methylated) cytosines to uracil
- **Primers designed to include CpG sites in CpG island**
- **Two different primers:** One is specific for methylated *RET* and one is specific for unmethylated *RET*
- **Polymerase Chain Reaction amplifies methylated vs. unmethylated DNA**
Methylation-specific primers

---AUUUUGG---TUUAUGC---

m

---ATTTATGG---TTTATCGTT---
---TAAATGC---AAATGCAA---

PCR

No product

Gel electrophoresis

MSP

Real-time detection

MethyLight

ConLight-MSP

Heat/melting curve

Mc-MSP

Methylation Specific PCR
RET, Original Primers
Adenomas and CRC
12/20/09
Methylation Specific PCR
RET, Original Primers
Adenomas
10/15/09
<table>
<thead>
<tr>
<th>Samples</th>
<th>Methylated</th>
<th>Unmethylated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Colon Mucosa*</td>
<td>2 (14%)*</td>
<td>12 (86%)*</td>
</tr>
<tr>
<td>Early Adenomas</td>
<td>0 (0%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Advanced Adenomas</td>
<td>3 (30%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Total Adenomas</td>
<td>3 (17%)</td>
<td>15 (83%)</td>
</tr>
<tr>
<td>Stage I-II CRC</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Stage III CRC</td>
<td>7 (70%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Stage IV CRC</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total CRC</td>
<td>11 (69%)</td>
<td>5 (31%)</td>
</tr>
</tbody>
</table>

P value (CRC vs. adenoma) = 0.0045
Conclusion

- Statistically significant difference in methylation of \textit{RET} in adenomas compared with colon cancers -> \textit{RET} is more frequently aberrantly methylated in colon cancer than in adenomas

- This result suggests that inactivation of \textit{RET} may be involved in the progression of colon cancer
Further Research Opportunities

- Recognition of other genes that are aberrantly methylated in the initiation and progression of colon cancer
- Development of biomarkers for prevention, detection, and management of colorectal cancer
- Epigenetic changes are potentially reversible (5-azacitidine can reverse methylation)
Sources