Sporadic vs. Colitis-Associated Colon Cancer: Vive la difference!

Steven H. Itzkowitz, M.D.
The Dr. Burrill B. Crohn Professor of Medicine
Mount Sinai School of Medicine
New York City, N.Y.
Conclusion

There **must** be a difference between SCC and CAC.

Otherwise, many of us would not get research funding!
Risk of Developing Colorectal Cancer

- General pop’n: 5%
- Personal hx of adenoma/CRC: 15%–20%
- IBD: 15%–40%
- HNPCC: 70%–80%
- FAP: >95%

Lifetime risk (%)
Dysplasia-Carcinoma Sequence

- Normal
- Adenoma
- Cancer
- Colitis
- Dysplasia
- Cancer
Dysplasia in IBD

Flat Dysplasia (not macroscopically visible)

DALM (within colitis)

Adenomatous Polyps (proximal to colitis)

Adenomalike DALM (within colitis)

Photos courtesy of David T. Rubin, MD.
## Clinicopathological Features of CRC

<table>
<thead>
<tr>
<th></th>
<th>Sporadic</th>
<th>IBD</th>
<th>HNPCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dysplasia:</strong></td>
<td>polyp</td>
<td>flat/polyp</td>
<td>polyp</td>
</tr>
<tr>
<td><strong>Age @ cancer:</strong></td>
<td>60’s</td>
<td>30’s</td>
<td>30’s</td>
</tr>
<tr>
<td><strong>Multiple cancers:</strong></td>
<td>2-3%</td>
<td>10-15%</td>
<td>10-15%</td>
</tr>
<tr>
<td><strong>CRC location:</strong></td>
<td>distal</td>
<td>prox&gt;distal</td>
<td>prox&gt;distal</td>
</tr>
<tr>
<td><strong>Histologic type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mucinous:</td>
<td>rare</td>
<td>common</td>
<td>common</td>
</tr>
<tr>
<td>poorly diff’d:</td>
<td>rare</td>
<td>common</td>
<td>common</td>
</tr>
<tr>
<td>low-grade TGA</td>
<td>very rare</td>
<td>11%</td>
<td>?</td>
</tr>
<tr>
<td><strong>Surveillance interval</strong></td>
<td>5-10 yr</td>
<td>1-2 yr</td>
<td>1-3 yr</td>
</tr>
<tr>
<td><strong>Genetic v. Environ:</strong></td>
<td>Env&gt;Gen.</td>
<td>Env&gt;Gen</td>
<td>Gen&gt;Env.</td>
</tr>
</tbody>
</table>
Low-Grade Tubulo-Glandular Adenocarcinoma (LGTGA)

- Arises directly from LGD.
- Accounts for 11% of IBD-associated CRC.
- Well-differentiated adenocarcinoma with distinct histological features:
  - rounded, oval or tubular glands
  - minimal desmoplastic reaction
  - minimal intraluminal necrosis
  - low-grade nuclear cytology

Does Inflammation Cause CRC in IBD?

Evidence “For”

CRC risk is increased with:

- longer duration of colitis (>7 yrs)
- greater extent of colitis
  - pancolitis > left-sided > proctitis
- primary sclerosing cholangitis
- active inflammation (histologic, endoscopic)

CRC risk is decreased with:

- anti-inflammatory drugs
  - 5-ASA
  - steroids (oral or topical)
### Severity of Inflammation as a Risk Factor for CRC in UC

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR of CRC (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonoscopic inflammation score*</td>
<td>2.54 (1.45-4.44)</td>
<td>.001</td>
</tr>
<tr>
<td>Histologic inflammation score*</td>
<td>5.13 (2.36-11.14)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Mean (standard deviation); odds ratio (OR) is for a 1-unit increase in score. CI=confidence interval.

Velayos et al. Am J Gastro 100:1345, 2005

Cancer:
O.R. = 0.51 (0.37-0.69)

Dysplasia:
O.R. = 1.18 (0.41-3.43)

CRC or Dysplasia:
O.R. = 0.51 (0.38-0.69)
### 6MP is Not Chemopreventive in UC

Proportional Hazards Analysis

<table>
<thead>
<tr>
<th>Any Neoplasia HR (CI)</th>
<th>6MP Exposure</th>
<th>Avg Daily Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Exposure</td>
<td>&gt;25mg/d</td>
</tr>
<tr>
<td>Any Neoplasia</td>
<td>1.06 (0.59-1.93)</td>
<td>1.00 (0.54-1.87)</td>
</tr>
<tr>
<td>Advanced Neoplasia</td>
<td>1.30 (0.45-3.75)</td>
<td>1.49 (0.52-4.32)</td>
</tr>
</tbody>
</table>

Does Inflammation Cause CRC in IBD?

Evidence “Against”

Why don’t patients with proctitis have an increased risk of rectal cancer?

Why does it take 7-8 years of colitis before neoplasia occurs?

Why do patients with quiescent inflammation also have a high risk of CRC?

Why do 6-MP and Azathioprine appear to have no chemopreventive role?

Why don’t all studies indicate that mesalamine use prevents CRC?
PATHWAYS OF COLON CARCINOGENESIS

Chromosomal Instability (e.g. FAP)
- Aneuploidy
- LOH
- Tumor suppressor gene mutations

K-ras  DCC/18q genes  p53

70-85%

APC

Normal mucosa  Early adenoma  Intermediate adenoma  Late adenoma  Carcinoma

15%

Microsatellite Instability (e.g. HNPCC)
- Hypermethylation/mutation of DNA MMR genes
- Target gene alterations (TGFβRII; BAX; others)

25-30%

CpG Island Methylation (CIMP)
- Suppression of gene expression by promoter hypermethylation
- Target genes: hMLH1, MGMT, others
Although the biology of UC-associated dysplasia (p53 lesions early, APC late) and sporadic adenomas differ, the biology of UC-associated cancer and sporadic cancer are remarkably similar.
### p53 Mutations Correlate with Dysplasia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Mutated*</th>
<th>Aneuploid</th>
<th>LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>6</td>
<td>83%</td>
<td>83%</td>
<td>83%</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>56</td>
<td>48%</td>
<td>46%</td>
<td>44%</td>
</tr>
<tr>
<td>Indefinite</td>
<td>96</td>
<td>3%</td>
<td>15%</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>57</td>
<td>29%</td>
<td>5%</td>
<td>0</td>
</tr>
</tbody>
</table>

*based on colectomy material from 2 patients with mutations of p53 codon 248 (exon 7); PCR --> Msp1 endonuclease digest

**p53 mutation -> aneuploidy -> p53 LOH**

## APC Mutations in UC

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients</th>
<th>APC mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC CA</td>
<td>33</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Sporadic CA</td>
<td>23</td>
<td>17 (74%)</td>
</tr>
</tbody>
</table>

*APC* mutations are uncommon and late in UC neoplasms

# K-Ras Mutations in UC

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Redston</th>
<th>Meltzer</th>
<th>Burmer</th>
<th>Chen</th>
<th>Bell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>3/7 (43%)</td>
<td>1/4 (25%)</td>
<td>1/12 (8%)</td>
<td>3/5 (60%)</td>
<td>8/33 (24%)</td>
</tr>
<tr>
<td>HGD</td>
<td>4/8 (50%)</td>
<td>2/6 (33%)</td>
<td>0/12</td>
<td>1/3 (33%)</td>
<td>--</td>
</tr>
<tr>
<td>LGD</td>
<td>1/7 (14%)</td>
<td>0/6</td>
<td>0/1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Indefinite</td>
<td>5/14 (36%)</td>
<td>0/5</td>
<td>0/3</td>
<td>0/8</td>
<td>--</td>
</tr>
<tr>
<td>Negative</td>
<td>0/17</td>
<td>--</td>
<td>--</td>
<td>0/2</td>
<td>--</td>
</tr>
</tbody>
</table>

*K-ras mutations occur late in UC neoplasms*

## MSI in Non-Neoplastic UC Mucosa

Loci: D2S119, D2S123, D2S136, D3S1067, D5S346, D6S87, D8S255, D13S175, D17S87, D17S261, D18S34, D18S35

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>MSI &gt;1 locus</th>
<th>MSI &gt;2 loci</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long Term UC:</strong> (mean 20 yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative dyspl.</td>
<td>6/10 (60%)</td>
<td>5/10 (50%)</td>
</tr>
<tr>
<td>HGD</td>
<td>11/13 (85%)</td>
<td>6/13 (46%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>2/5 (40%)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td><strong>Short Term UC:</strong> (mean 2.2 yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative dyspl.</td>
<td>5/6 (83%)</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>Ischemic colitis:</td>
<td>0/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

MSI is an early event in UC carcinogenesis

**p16^{INK4a} Promoter Methylation in UC Neoplasia**

<table>
<thead>
<tr>
<th>Tissue Status*</th>
<th>p16 methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No dysplasia</td>
<td>7/21 (33%)</td>
</tr>
<tr>
<td>Dysplasia/cancer</td>
<td>18/24 (75%)</td>
</tr>
</tbody>
</table>

*tissue taken from 3 colectomy specimens*

Methylation is an early event in UC carcinogenesis

**hMLH1 Methylation in UC Neoplasia**

<table>
<thead>
<tr>
<th>Tissue Status</th>
<th>hMLH1 Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI-H</td>
<td>6/13 (46%)</td>
</tr>
<tr>
<td>MSI-L</td>
<td>1/16 (6%)</td>
</tr>
<tr>
<td>MSS</td>
<td>4/27 (15%)</td>
</tr>
</tbody>
</table>

*only dysplasia or cancer tissues used

Methylation occurs in MSI-H tumors, but also MSS

*Fleisher et al. Cancer Res. 60:4864, 2000*
# Age-Related Gene Methylation in UC

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>(Age)</th>
<th>ER</th>
<th>MYOD</th>
<th>p16</th>
<th>CSPG2</th>
<th>(% gene methylation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>(53.4)</td>
<td>7.4</td>
<td>3.0</td>
<td>2.4</td>
<td>30.6</td>
<td></td>
</tr>
<tr>
<td>UC-ND</td>
<td>(42.0)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.3</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>UC-HGD/CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEG</td>
<td>(53.2)</td>
<td>20.1</td>
<td>18.4</td>
<td>7.9</td>
<td>35.2</td>
<td></td>
</tr>
<tr>
<td>IND</td>
<td>(40.0)</td>
<td>20.0</td>
<td>27.7</td>
<td>13.0</td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>HGD/CA</td>
<td>(54.0)</td>
<td>40.0</td>
<td>44.0</td>
<td>9.4</td>
<td>57.5</td>
<td></td>
</tr>
</tbody>
</table>

Age-related methylation is enhanced in UC neoplasia

*Issa et al. *Cancer Res.* 61:3573, 2001*
Normal epithelium

Environmental trigger
• Bacterial/viral infection
• NSAIDs
• Other toxins

Inflamed epithelium (colitis)

Inflammatory cells

Oxidative stress
• Reactive oxygen species (ROS)
• NO; H2O2; others

Damaged epithelium

Genetic/epigenetic changes:
• p53 mutation/activation
• damage/mutation of DNA MMR system
• methylation of regulatory genes
• telomere shortening

Additional genetic changes

Increased epithelial cell turnover
• proliferation
• apoptosis

Dysplasia

Cancer

Itzkowitz and Yio, Am J Physiol. 287:G7-17, 2004
p53 Mutations in Non-Neoplastic UC Tissues

- 50% of bx’es from inflamed UC tissues exhibited:
  - G-to-A transition at codon 248 (CGG->CAG)
  - C-to-T transition at codon 247 (AAC->AAT)
- Only found in UC tissues, not normal controls
- In UC tissues, only in “lesional” biopsies
- Correlated with NOS2 activity

Chronic inflammation can induce p53 mutations

Hussain et al. Cancer Res. 60:3333-7, 2000
DNA Mismatch Repair

H₂O₂

base-base mismatch

1 base insertion/deletion loop

2-8 base insertion/deletion loop

MSH2

MSH6

MLH1

ummer

PMS2

MLH1

MLH3

MLH1

MLH3

MLH1

PMS1

MLH1

PMS2

MLH1

PMS2

H₂O₂
Inflammation Predisposes to Colon Cancer: 
*In Vivo* Evidence from Animal Models

Three main approaches:

1. **Normal mice:**
   Induce colitis with noxious agent and monitor for development of neoplasia.

2. **Cancer prone mice:**
   – Induce colitis and monitor for higher rates of neoplasia
   -or-
   – Reduce colitis and monitor for lower rates of neoplasia

3. **IBD-prone mice:**
   Determine whether IBD-prone mice develop neoplasia

*Itzkowitz and Yio, Am J Physiol.* 287:G7-17, 2004
Inflammation--->Cancer Normal Mice:

• Dysplasia and cancer can be induced after repeated cycles of dextran sulfate sodium (DSS)
• Longer disease duration --> increased rate of neoplasia, even in the setting clinical remission
• Neoplasia associated with more severe degrees of inflammation, especially in the distal colon.
• Treatment with antioxidant (N-acetylcysteine) reduced both inflammation and tumor incidence

Seril et al.  *Carcinogenesis* 23:993, 2002
## Cancer-Prone Mice:

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC^{min/+}</td>
<td>intestinal adenomas</td>
</tr>
<tr>
<td>APC^{min/+} + DSS</td>
<td>intestinal adenomas &amp; cancers</td>
</tr>
<tr>
<td>MSH2^{-/-}</td>
<td>small bowel cancer</td>
</tr>
<tr>
<td>MSH2^{-/-} + DSS</td>
<td>colonic dysplasia and cancer</td>
</tr>
<tr>
<td>p53^{-/-}</td>
<td>no intestinal cancer</td>
</tr>
<tr>
<td>p53^{-/-} + DSS</td>
<td>colon cancer (100%) &amp; dysplasia</td>
</tr>
<tr>
<td>APC^{min/+}</td>
<td>colonic adenomas</td>
</tr>
<tr>
<td>APC^{min/+} + C. <em>rodentium</em></td>
<td>more colonic adenomas</td>
</tr>
</tbody>
</table>
Cancer-Prone Mice: 
\[ \downarrow \text{inflammation} \Rightarrow \downarrow \text{tumors} : \]

- \( \text{APC}^{\text{min}+/+} \times \text{COX-2}^{-/-} \)
- \( \text{APC}^{\text{min}+/+} + \text{COX-2 inhibitor} \)
- \( \text{APC}^{\text{min}+/+} \times \text{iNOS}^{-/-} \)
- \( \text{APC}^{\text{min}+/+} + \text{iNOS inhibitor} \)
- \( \text{AOM} \rightarrow \text{DSS} \)
  - IKKB\(^{-} \) in epithelial cells
    - \( \uparrow \text{epith. cell apoptosis} \)
  - IKKB\(^{-} \) in myeloid cells
    - \( \downarrow \text{pro-inflamm. cytokines} \)

\[ \Rightarrow 75\% \text{ fewer tumors} \]
\[ \Rightarrow 50\% \text{ fewer, smaller tumors} \]

# Colitis-Prone Mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Histology</th>
<th>Neoplasia</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2/-</td>
<td>colitis (UC)</td>
<td>dysplasia</td>
<td></td>
</tr>
<tr>
<td>IL2/β2M DKO</td>
<td>milder colitis</td>
<td>dysplasia; CRC</td>
<td>APC; p53; MSI</td>
</tr>
<tr>
<td>IL10/-</td>
<td>enterocolitis</td>
<td>CRC (25-60%)</td>
<td>No APC, p53, kras, Msh2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-10 rx: ↓ colitis &amp; CRC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Req. flora; <em>E. faecalis</em></td>
</tr>
<tr>
<td>Rag2/-</td>
<td>colitis</td>
<td>dysplasia; CRC</td>
<td>Requires <em>H. hepaticus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-10 rx: ↓ colitis &amp; CRC</td>
</tr>
<tr>
<td>Rag2/TGFβ1 DKO</td>
<td></td>
<td>dysplasia; CRC</td>
<td>Requires <em>H. hepaticus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CRC independ. of colitis</td>
</tr>
<tr>
<td>TCRβ/p53 DKO</td>
<td>colitis (UC)</td>
<td>dysplasia; CRC</td>
<td>Requires flora</td>
</tr>
<tr>
<td>Gpx1/Gpx2 DKO</td>
<td>ileocolitis</td>
<td>dysplasia; CRC</td>
<td>Requires flora</td>
</tr>
</tbody>
</table>

Lessons From Animal Models

- Colonic neoplasia develops in genetically susceptible hosts
- Requires prolonged periods of chronic inflammation
- Requires fecal flora (*Helicobacter* sp; others?)
Culture of mucosa-associated bacteria in IBD and colon cancer

Martin et al, Gastroenterology 2004 127(1):80-93
Increased haemagglutinating activity by mucosa-associated *E. coli* in Crohn's disease and colon cancer correlates with their ability to adhere to and invade intestinal epithelial cells.

*Martin et al* Gastroenterology 2004;127:80-93
Mucosal bacteria

Inflammation
- Unesterified arachidonic acid
  (Cao et al, PNAS 2000;97:11280-5)
- Mucosal apoptosis

NFkappaB Activation

Cox2 activation

Prostaglandin E2-induced nuclear localisation of Beta catenin

Mutagenesis

Carcinogens

Cancer

Common mechanisms for IBD-associated and sporadic colon cancer

Adapted from Rhodes & Campbell Trends Molecular Med 2002
Summary

- There are more clinicopathologic and molecular differences between SCC and CAC than there are similarities.
- This appears to be due to chronic inflammation.
- Whether bacteria play a role in human colitis-associated neoplasia (or perhaps even sporadic CRC) requires further investigation.
Summary-Human Observations

• IBD patients are at increased risk for CRC.
• The risk of CRC rises with increased duration, extent, and severity of inflammation, and with associated PSC.
• Use of certain anti-inflammatory medications (5-ASA, steroids) may reduce the development of CRC in IBD.
• Oxidative stress causes genomic instability leading to the development of dysplasia.
• Molecular markers may help to identify patients at increased risk of CRC.