Overview

• Inflammation and cancer

• Infection and cancer

• The future
#1
What is the relationship between inflammation and cancer?
Inflammation and GI Cancer

• Every chronically inflamed GI mucosa is at increased risk for cancer (IBD, chronic gastritis, Barrett’s esophagus, etc.)
• Inflammation and oxidative stress are directly mutagenic
• Chronic inflammation actually leads to “relaxation” of DNA repair systems
• Inflammation leads to mucosal repair, which is mitogenic
• This is a “perfect storm” for carcinogenesis
What are the molecular pathways to colorectal cancer?
Mutational Signatures

**CIN** – chromosomal instability (LOH)
  » genes deleted by allelic imbalance

**MSI** – microsatellite instability
  » mutations, especially at repetitive DNA sequences

**CIMP** – CpG island methylator phenotype
  » methylated gene promoters
Genesis of Colorectal Neoplasia

CIN
- Genes deleted (LOH)
- Lynch syndrome
- MLH1 silenced (methylation)
- Genes silenced by promoter methylation

CIMP

MSI
- Mutated genes

CRC with CIN
CRC with MSI
CRC with CIMP
By what mechanisms does chronic colorectal inflammation lead to IBD-associated CRC?
Mechanisms of Carcinogenesis in IBD

• **CIN**: often aneuploid, mechanism unknown
  – Telomere shortening + anaphase bridges (O’Sullivan, Nat Genet 2002)

• **MSI**: present in many UC-associated neoplasms
  – also found in the *premalignant* colon

• **CIMP**: frequently present in UC-associated CRCs
  – methylation of MLH1 (Fleisher, Cancer Res 2000)
  – methylation of multiple other genes in UC CRCs (Schulman, Gastro 2005)
  – methylation is found in normal colons, and in colitic colons
Response of the DNA MMR system to oxidative stress/inflammation
Model of Oxidative Stress

• In vitro model, HEL cells
• Non-lethal dose of $\text{H}_2\text{O}_2$ added
• DNA MMR activity directly measured
  • used Kunkel assay of DNA repair
• Recombinant DNA MMR proteins made in baculovirus system
  – system reconstituted with MSH2+MSH6 and MLH1+PMS2

DNA MMR activity in HEL cells is ‘relaxed’ by H$_2$O$_2$. 

![Bar chart showing DNA MMR activity in HEL cells with increased H$_2$O$_2$ concentrations. The activity decreases with increasing concentrations.]
DNA MMR Activity is Restored with Recombinant DNA MMR Proteins

$S_\alpha = MSH2 + MSH6$

$L_\alpha = MLH1 + PMS2$

Figure 4
Chang et al.
DNA MMR Protein Expression is Reduced in Response to Oxidative Stress

Figure 3
Chang et al.

<table>
<thead>
<tr>
<th>H$_2$O$_2$ (mM)</th>
<th>0</th>
<th>0.1</th>
<th>1</th>
<th>0</th>
<th>0.1</th>
<th>1</th>
<th>0</th>
<th>0.1</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-treatment</td>
<td>0 h</td>
<td>3 h</td>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Summary:
Oxidative Stress and DNA MMR

- Chronically inflamed colonic mucosa shows microsatellite instability (MSI-L)
- Many of the CRCs in IBD show MSI
- DNA MMR activity falls when \( \text{H}_2\text{O}_2 \) is added
  - confirmed with other models of oxidative stress
- DNA MMR proteins are reduced after exposure to \( \text{H}_2\text{O}_2 \)
- DNA MMR activity is restored by adding the MMR proteins (in vitro)
Infection and GI Cancer

- Hp is associated with gastric cancer
  - presumably carcinogenesis is a consequence of chronic inflammation
  - this is mechanistically complex
  - involves both bacterial and host factors

- Might there be other mechanisms?
  - viruses bring additional potential mechanisms (insertional mutagenesis; viral oncogenes)
JC Virus and CRC

• JC virus is a ubiquitous human polyomavirus
  – a tiny, circular DNA virus
  – one of two human “SV40-like” viruses
  – encodes a T-antigen (‘transforming’)
  – will induce aneuploid cancers when injected into the CNS of a rodent or monkey

• JCV DNA can be found in most CRCs
  – 89% (Laghi et al., PNAS 1999)
  – 81% (Enam et al., Cancer Res 2002)
JC Virus (Mad1, complete genome)

- ~5 KB DNA virus
- Closed circular genome
- Supercoiled
- Encodes 5 genes
  - T Antigen
  - 3 viral capsids
    - VP1, VP2, VP3
  - Agnoprotein
JC Virus T Antigen

688 amino acids, alternatively spliced

JCV is in most GI tracts

Upper GI Tract (71%)
- esophagus: 10/15 (67%)
- gastric corpus: 9/17 (53%)
- gastric antrum: 8/17 (47%)
- duodenum: 10/17 (59%)

Lower GI Tract (81%)
- cecum: 10/15 (67%)
- transverse colon: 11/16 (69%)
- sigmoid colon: 8/16 (50%)
- rectum: 10/16 (63%)

Ricciardiello et al, Gastroenterology 119:1228 2000
How Many Copies of JCV are in Human Colorectal Neoplasms?

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>N</th>
<th>MEAN (SD) (copies/µg DNA)</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>49</td>
<td>14.3 (+4.2) X10³</td>
<td>9-20 X 10³</td>
</tr>
<tr>
<td>Adenomas</td>
<td>15</td>
<td>14.0 (+3.1) x 10³</td>
<td>9-20 X 10³</td>
</tr>
<tr>
<td>Adjacent</td>
<td>35</td>
<td>242 (+127)</td>
<td>50-450</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
<td>147 (+50)</td>
<td>100-250</td>
</tr>
</tbody>
</table>

Thoedoropoulos et al, *Dis Colon & Rectum* 2005
INTRODUCTION OF JCV T Ag INTO HCT116 CELLS

• HCT116 cells
  – diploid, MSI, no CIN
  – does not support JCV infection
• T Ag/GFP construct inserted into plasmid
• Plasmid transfected into HCT116
JC Virus T-Antigen Transfection Causes CIN in HCT116 Cells

- **Dicentrics:**
  - A, C, F, G

- **Breaks:**
  - A, B, E

- **Fused:**
  - D, F, I

- **Rings:**
  - H
Transfection of the JCV genome into diploid CRC cells

• Model: RKO cells
  – diploid, microsatellite instability (MSI)
    • hypermethylated hMLH1
  – wild type p53, APC, β-catenin
  – a CIMP model

• JCV cloned into pBR322 plasmid
  – full length, Mad-1 inserted into plasmid
  – transfected into RKO
Results: RKO transfected with JCV

• JCV integrates into RKO
• T antigen expressed within 7 days
  – nuclear localization of T Ag protein
• VP1 expressed (late gene; viral capsid)
  – low level expression
    • suggests viral replication
• T Ag and β-catenin interaction
• p53 stabilized
• CIN induced

JCV INDUCES CIN
Stable Transfection of “normal” colonic cells with T-Ag

Shin and Goel, 2007 (unpublished)
Transformation with T-Ag

vector

T-Ag

Shin and Goel, 2007 (unpublished)
Intranuclear Interactions with JCV T-Antigen

- DNA: causes CIN
- p53: leads to inactivation
- pRB: leads to degradation

β-catenin: stabilization, nuclear localization, initiation of the neoplastic phenotype (prior to mutation of APC)
Model: JCV T antigen stabilizes β-catenin and initiates the neoplastic phenotype
Nuclear stabilization of \(\beta\)-catenin precedes loss of APC
JCV and DNA Methylation

• 100 sporadic CRCs evaluated for MSI, CIN, promoter methylation at 9 genes (CIMP), IHC for T-Ag

• JCV DNA found in 77% of CRCs, and 56% of those expressed T-Ag
  – Significantly associated with CIN (p=.017)
  – Significantly associated with CIMP (p=.01)

Goel et al, Gastroenterology 130:1950, 2006
Summary: JC Virus and CRC

1. JCV has oncogenic potential (T-Ag)
2. JCV is in most colonic epithelia, and in most CRCs
3. JCV can induce both CIN and CIMP
4. JCV is present in esophageal, gastric and pancreatic cancers
5. Is this virus a key to carcinogenesis throughout the digestive tract?
Luigi Laghi, M.D.
Milan, Italy

First report of JCV in CRC from our lab
PNAS, 1999
KEY RESEARCH COLLABORATORS

Ajay Goel, PhD: methylation pathways, classification of CRCs, etc.

Luigi Ricciardiello, MD: JCV in gut, induction of CIN in diploid cells, promoter rearrangements, etc.

La Jolla, 2000
#3
Where will these insights take us in the future?
Genesis of Colorectal Neoplasia

- **CIN**
  - Genes deleted (LOH)
  - JC Virus
  - Lynch syndrome
  - MLH1 silenced (methylation)
- **CIMP**
  - Genes silenced by promoter methylation
- **MSI**
  - Mutated genes
- **CRC with CIN**
- **CRC with MSI**
- **CRC with CIMP**
NFκB Signaling Networks

Nutripreventive Signal Blockade
Mechanisms of Nutriceuticals

- Curcumin: inhibitor of NFκB, Cox-2, EGRr, angiogenesis, cyclin D1, etc.
- Resveratrol: inh NFκB, p450s, IGF, etc; prolongs lifespan in mice, etc.
- Annurca apple extract: demethylating agent
- Olive oil extract: p53 mediated apoptosis
- Vitamin D: non-calcium anticancer effects
- Etc.
Conclusions: the future

• CRC is a complex disease that can evolve through several pathways
• The most unifying feature of all CRCs is the presence of JC virus
• Prevention may require a multifaceted approach that inhibits several independent pathways
• Will we someday immunize vs. JCV?