Cancer originating from bone marrow-derived stem cells

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Inflammation and Cancer

- 1863 - Rudolf Virchow noted leukocytes in neoplastic tissues, made the connection between inflammation & cancer.
- 1986 - HF Dvorak (NEJM 315:1650) described cancers as “wounds that do not heal.”

<table>
<thead>
<tr>
<th>Condition</th>
<th>Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory bowel disease</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>H. pylori infection</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td>Chronic viral hepatitis</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Liver fluke infection</td>
<td>Cholangiocarcinoma</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>Bladder cancer</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>Lymphoma/papillary cancer of the thyroid</td>
</tr>
<tr>
<td>Human papilloma virus</td>
<td>Cervical cancer</td>
</tr>
<tr>
<td>Pelvic inflammatory disease</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>Esophageal adenocarcinoma</td>
</tr>
<tr>
<td>Asbestosis</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>Bronchial lung cancer</td>
</tr>
</tbody>
</table>

* Chronic pancreatitis  Pancreatic Cancer
Classical model for inflammation and cancer

Inflammation

- Macrophages
- NF-κB
- CD4(+) - T cells

Cytokines
- Chemokines
- Prostaglandins
- Growth factors

Tumor Promotion

- Oxidative stress
- Tissue destruction/Regeneration
- Increased proliferation

Cancer

Mitotic error
Mutagenesis

Tissue/Stem cells
Properties of stem cells:  

- Longevity  
- Self-renewal  
- Multipotentiality  

But behavior of stem cells is largely governed by the stem cell niche.
Cancer Stem Cells

Criteria for Cancer Stem Cells (or Cancer Initiating Cells)
- Enriched for tumorigenic ability
- Enable serial propagation in vivo
- Asymmetric (or symmetric) self-renewal ability
Cancer Stem Cells

AML

Breast CA

CRC

Pancreatic CA

Brain CA

CD133(+) CD133(-)


CD44+CD24+/low

CD44+CD24+ESA+
## Markers of Normal and Malignant Adult Tissue Stem Cells

<table>
<thead>
<tr>
<th>Organ</th>
<th>Normal Tissue</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic</td>
<td>CD34⁺CD38⁻Thy1⁺ c-kit⁺IL-3Ra⁻</td>
<td>CD34⁺CD38⁻Thy1⁻ c-kit⁻IL-3Ra⁺</td>
</tr>
<tr>
<td>Breast</td>
<td>CD24⁺medCD49fhigh or CD29hiCD24⁺</td>
<td>CD44⁺CD24⁻/low</td>
</tr>
<tr>
<td>Brain</td>
<td>CD133⁺</td>
<td>CD133⁺</td>
</tr>
<tr>
<td>Lung</td>
<td>Sca-1⁺CD34⁺</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>CD44⁺/a2b₁hi/CD133⁺</td>
<td>CD44⁺/CD24⁺</td>
</tr>
<tr>
<td>Colon</td>
<td>CD133⁺ or CD44⁺</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>CD133⁺cMet⁺ (?)</td>
<td>CD44⁺CD24⁺ESA⁺</td>
</tr>
</tbody>
</table>
Origins of Cancer Stem Cells

- Resident tissue stem cell is the most likely candidate but -
- Markers of CSCs are often not present consistently on tissue stem cells
- Difficulties in accounting for the “field effect” and early epigenetic changes seen in precancerous lesions
- Need to account for the role of inflammation
Helicobacter infection induces gastric cancer in humans and mice.

Correa Model of Human Gastric Cancer
**H. Pylori** activates host immunity

- Early infiltration by F4/80 (+) and NF-kB (+) macrophages
- **H. pylori** activates monocytes & macrophages through TLR2
- LPS from **H. pylori** can weakly activate TLR4 on gastric epithelial cells

**Infect. Immun. 2004;72:6446-54**
**H. felis** infection to NF-κB-GFP mice

<table>
<thead>
<tr>
<th>GFP</th>
<th>anti-GFP stain</th>
<th>Merged</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. felis</strong> infection (2m); x200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No felis infection; x200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• No atrophy and increased colonization seen in immunodeficient mice.
• *H. felis* leads to atrophy in Th1-polarized mice (*C57BL/6*).
• Adoptively transferred CD4+ lymphocytes to H. felis-infected SCID mice enhances gastritis.
• Deletion of Th1 cytokines (*IFNγ/-*) protects against atrophy, while deletion of Th2 cytokines (*IL10/-*) worsens atrophic gastritis.
• Co-infection with parasite (*H. polygyrus*) results in Th2 polarization and protection from H. felis-induced dysplasia in B6 mice (Nat Med 2000).
• *H. pylori*-dependent gastric cancer is associated with high-expressing IL-1β and TNF-α genotypes (El-Omar, Nature 2000).
Chronic *Helicobacter* infection leads to TFF2-positive metaplasia (SPEM)

SPEM in *H. felis*-infected C57BL/6 mouse stomach

SPEM expresses unusual transcripts such as Xist

Intestinal metaplasia arises from SPEM
Inflammation and injury leads to mobilization of bone marrow-derived progenitor cells (BMDCs)

Hematopoietic stem cells (HSCs)

Endothelial Progenitor cells (EPCs)

Key role for cytokines, chemokines and hypoxia in this recruitment process

Rafii S, Lyden D, 2003

Korbling M et al, 2002
Methods to study cellular origins in mice

Tracking bone marrow progeny

- ROSA 26 (mouse transgenic for bacterial β-galactosidase)
- Lethal irradiation
  - Detection of β-galactosidase (Histochemistry)
- GFP (mouse transgenic for jellyfish green fluorescent protein)
  - Detection of GFP (UV fluorescence)
- Bone Marrow Transplant
  - Detection of Y-chromosome (In situ hybridisation)

Male (XY) into female recipient (XX)

Nick Wright
What is the role of bone marrow-derived cells (BMDC) in acute and chronic gastric injury?

C57BL/6 mice lethally (900 rads) irradiated and reconstituted with Rosa26 (C57BL/6JGtrosa 26) bone marrow (Houghton et al, Science 2004).

- No injury → No engraftment
- Acute injury → No engraftment (cryoinjury or acetic acid)
- Parietal Cell Loss → No engraftment
- Acute *H. felis* infection → No engraftment
- Chronic *H. felis* infection → BMDCs engraft
Gastric metaplasia arises from bone marrow-derived cells

30 wks *H. felis* infection, X-gal

30 wks *H. felis*, X-gal + TFF2

30 wks *H. felis*, X-gal + Alcian blue/PAS

*Science 2004; 306:1568-1571*
Gastric dysplasia and cancer arise from bone marrow-derived cells

Science 2004; 306:1568-1571
IHC shows beta-galactosidase expression by dysplastic gastric glands
Colocalization of β-GAL with cytokeratin

DAPI = blue
Beta-Gal = red
Cytokeratin = green
Merge = yellow

DAPI = blue
Beta-Gal = red
CD45 = green
Merge = yellow

Science 2004; 306:1568-1571
Evidence supporting BMDC origin of metaplasia/dysplasia

- X-Gal staining
- -Gal IHC
- LCM/PCR
- GFP IF/IHC
- Y-FISH

GFP+CD45- X and Y donor

WT donor

Rosa donor

WT donor

B

C

GFP+CD45- X and Y donor

WT donor

Rosa donor
Key factors in BMDC model

- **Mobilization** into circulation of progenitor cells
- **Recruitment** to sites of inflammation
- Increase in **adhesion** molecules to retain BMDCs
- **Tissue stem cell failure** to maintain BMDCs in niche
- **Altered stem cell niche** to release BMDCs from growth inhibition

Increased IL-1, IL-6, G-CSF levels

<table>
<thead>
<tr>
<th>control</th>
<th>12 mo H. felis</th>
<th>16 mo H. felis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDF-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Increased P-selectin, VCAM-2, MAdCAM-1

Apoptosis in gastric progenitor zone

Increases in myofibroblasts and COX-2, VEGF, and MMP-9, etc
Carcinogen-dependent apoptosis initiates cancer

**Normal stomach**
- Apoptosis regulated
- Proliferation regulated
- Homeostasis
- Normal cell turnover

**H. pylori gastritis**
- Fas/FasL, IL-1β, TNF-α
- ↑ Apoptosis
- ↑ Proliferation
- Homeostasis
- Increased cell turnover

*Apoptosis (TUNEL) 4-6 wks*

Uninfected

*H. felis* -infected

Apoptosis in stem cell zone = empty niche
Helicobacter infection leads to increases in gastric myofibroblasts

McCaig et al, Gastroenterology 2006
Body snatcher model of cancer

CSC’s are really pod people…
New Model of Epithelial Cancer

Gastroenterology 2004
Models for Inflammation & Cancer
Balkwill et al, 2006

**Initiation by Inflammation**
- Epithelial cells (green-initiated or transformed cells)
  - Proinflammatory cytokines and chemokines
  - Growth factors

**Promotion by Stroma**
- Matrix metallo-proteases
- ROS free radicals
- COX enzymes
- Angiogenic factors

**Promotion by Initiated Cells**
- Initiated tissue stem cells are subject to tumor promotion by chronic inflammatory mediators
  - e.g., colon cancer
  - Liver cancer

Bone marrow stem cells repopulate the epithelium and become initiated or
Tissue stem cells are mutated by free radicals generated by chronic inflammation
  - e.g., stomach cancer

Initiated stem cells produce inflammatory mediators that attract tumor-promoting inflammatory cells and set up cytokine networks
  - e.g., skin cancer
  - Liver cancer
  - Ovarian cancer

Stromal cells
Gastritis and colitis both predispose to CA

Similarities with respect to inflammation as a major risk factor include

• Importance of duration of inflammation ( > 10 years)
• Extent and severity of inflammation - pan-colitis or pan-gastritis are greatest risk
• Field effect - multiple dysplasias - entire colon at risk
• Early p53 mutations (? AID effect)
• Early epigenetic and genetic alterations before histologic evidence of dysplasia
• Potential benefit of eradication or other anti-inflammatory therapies.
BMDCs could contribute to tissue repair in multiple ways in IBD

• BMDCs have been shown to form
  – Activated myofibroblasts (Brittan et al, 2005)
  – Endothelial cells or neovasculogenesis (Brittan et al, 2005; Khalil et al, 2007 in press)
  – Epithelial cells (Komori et al, 2005, Matsumoto et al, 2005)

• Reports of bone marrow transplant for therapy of Crohn’s disease.

• Trial of infusion of MSCs for Crohn’s.
Transplanted BMDCs contribute to myofibroblasts in mouse colon

- Alpha-smooth muscle actin (αSMA) - red/pink
- Y chromosome representing donor BMDCs - brown nuclear dot
- BMDCs contribute to 50% of myofibroblasts in colon

Brittan M et al., *Gut* 2002
A Regenerative Role for Bone Marrow Following Experimental Colitis: Contribution to Neovasculogenesis and Myofibroblasts

MAIRI BRITAN,* VICTORIA CHANCE,† GEORGE ELIA,* RICHARD POULSOM,* MALCOLM R. ALISON,*.§ THOMAS T. MACDONALD,† and NICHOLAS A. WRIGHT*.§


Mouse Colon. H&E, 6 days post-TNBS (10x)
Venule formed *entirely* from transplanted bone marrow?

Brittan and Wright
Do BMDCs contribute to dysplasia/cancer in colitis?

AOM/DSS model in C57BL/6 mice transplanted with ROSA26 marrow

Colonic polyp, H&E
Polypoid mass lacks goblet cells, AlcBlue/PAS
Polypoid mass lacks β-gal+ epithelium. IHC.

Preliminary results negative but experiment not ideal.
Studies by others have shown BMDC recruitment in DSS colitis
But is this the appropriate model for IBD-associated cancer?
Bone marrow-derived cells fuse with normal and transformed intestinal stem cells

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