Inflammatory Bowel Disease
Neoplasia

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Neoplastic Progression in Chronic Inflammatory GI Dz

Inflammation

Dysplasia

Carcinoma
Chronic Inflammatory GI Disease & Cancer

- Barrett’s Esoph
  - Esoph CA
- HP Gastritis
  - Gastric CA
- Hepatitis B & C
  - HCC
- Ch Pancreatitis
  - Panc CA
- UC and Crohn’s
  - Intestinal CA
Ulcerative Colitis: A Paradigm
Managing Cancer Risk in UC

• Ignore it

• “Prophylactic” colectomy

• Colonoscopic surveillance for dysplasia / early carcinoma
Optimal Colonic Biomarker

• Pancolonic distribution
• Predate incurable cancer
• Objective
• Sensitive, Specific, ↑PPV, ↑NPV
Gold Standard Biomarker: Dysplasia
Dysplasia: Problems

• **Sampling**

• Distinction from reactive change

• Observer variation

• Natural history incompletely understood
### Adequate Bx Sampling

<table>
<thead>
<tr>
<th></th>
<th>Dysplasia</th>
<th>Cancer</th>
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<tbody>
<tr>
<td>90% confidence</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>95% confidence</td>
<td>56</td>
<td>64</td>
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UC Surveillance Protocol

10 cm

5 cm
Rectosigmoid Predominance of Ulcerative Colitis Cancer

Location of Colorectal Carcinoma

<table>
<thead>
<tr>
<th>RS</th>
<th>D</th>
<th>T</th>
<th>A/C</th>
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<tbody>
<tr>
<td>52%</td>
<td>12%</td>
<td>21%</td>
<td>15%</td>
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Choi PM. *Gastroenterology* 1993;104:666  Summary of 5 Studies
Dysplasia: Problems

- Sampling
- Distinction from reactive change
- Observer variation
- Natural history incompletely understood
Dysplasia: Problems

- Sampling
- Distinction from reactive change
- Observer variation
- Natural history incompletely understood
Outcome of 40 UC LGD Patients

- 78% no progression, avg f/u 5y (1-13 y)
- 22% HGD, avg f/u 1.5 y (1-3 y)
- ≥3 LGD biopsies: 9x↑ progression risk
- 2 non-compliant patients developed Dukes’ A cancer

Dysplastic Field: Limited
Better Biomarkers of Cancer Risk Greatly Needed!
Chromosomal Instability?

• FCM Aneuploidy - Detects gross chromosomal instability

• CGH - Detects clonal gains and losses of chromosomal regions

• FISH - Detects clonal and non-clonal chromosomal abnormalities
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<th>Cancer</th>
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<tbody>
<tr>
<td>No. Bx for</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>90% confidence</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>No. Bx for</td>
<td>95% confidence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>14</td>
</tr>
</tbody>
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Morphologic + DNA Ploidy
Neoplastic Field: Larger
Metaphase Comparative Genomic Hybridization in UC

39% (15/38) of diploid bx’s near dysplasia or cancer showed CGH detectable alterations

Performed in collaboration with F. Waldman, UCSF
Array-based Comparative Genomic Hybridization (CGH)

- Chromosomes replaced by ordered array of targets
- Karyotyping of metaphase spreads not necessary
- Greatly increased resolution
Array CGH in UC

- 100% (9/9) UC-progressors extensive chromosomal gains and losses
- FISH and PCR targets identified

Bronner MP, *Mod Pathol* 2010;23:1624-33
Ulcerative Colitis A-CGH

PROGRESSORS

NON-PROGRESSORS

BAC CGH Whole Genome Log2-Ratio Plots of All Chromosomes

Normal Non-UC Control

UC Non-progression

UC Progressor

UC Progressor

Morphology
+ DNA Ploidy
+ CGH

Neoplastic Field:
Larger Still
Non-Clonal Change in UC: Wider Field?

- DNA Flow & CGH detect clonally expanded abnormalities only

- Larger fields of non-clonal instability? Detectable in negative biopsies, even from rectum?

- Assessed by Fluorescence In Situ Hybridization (FISH)?
Hypothesis:

UC progresses differ from UC non-progressors using non-clonal genomic instability biomarkers on single negative rectal biopsies.
FISH

• Interphase nuclear suspensions placed on glass slide

• Locus specific probes (Chrom 8, 11, 17, 18) & centromeres (green and red)

• Red and green FISH spots counted per 100 nuclei
Control Normal Colon FISH
Chrom11 Probe Set

% of Nuclei

Red and Green Signal Counts

0r2g 1r1g 1r2g 1r3g 2r1g 2r2g 2r3g 2r4g 3r2g 3r3g
Diploid Neg Rectal Bx UC Progressor Chrom11 Probe Set

Red and Green Signal Counts

% of Nuclei

0r2g: 4
1r1g: 2
1r2g: 8
1r3g: 1
2r1g: 1
2r2g: 74
2r3g: 11
2r4g: 0
3r2g: 0
3r3g: 0
FISH in Ulcerative Colitis

- Arm loss
- Arm gain
- Centromere loss
- Centromere gain

% cells with FISH abnormalities

- Non-UC controls N=10
- UC non-progressors N=18
- UC progressors N=12

- p ≤ 0.001
- p = 0.001

ROC Analysis of FISH Biomarkers

8q: c-myc

11q: CyclinD1

18q: DCC

17p: p53

ROC Analysis of FISH Biomarkers

All 4 chromosomes combined

Consequences of Shortened Telomeres

• Sticky chromosomal ends
• Bridge-breakage-fusion cycles
• Chromosomal arm losses/gains and dicentrics

Studied by peptide nucleic acid (PNA) probe ISH or RT PCR
Telomere Shortening in UC

Epithelial: Stromal Telomere Ratio

Non-UC control

Non-progressors

Progressors

p=0.001

p=0.08

p=0.02

NGS miRNA bioclassifier of UC patients at increased risk of colon cancer

• Why miRNAs?
  – Small size (~21nt) more stable, less ribonuclease degradation
  – Readily detectable in FFPE and stained slides
  – Important roles in immune regulation
miRNAs misregulation in UC-P, UC-NP

- Linear discriminant analysis to predict UC-P vs. UC-NP
- Robust candidate panel selected for RT-PCR & additional cohort validation

UC-NP vs. nl (26 miRNAs)

UC-P vs. nl (29 miRNAs)

11

15

18

miRNA Panel

UC-NP

9/10

UC-P

10/10

Normalized Read count
Histology
+ DNA Ploidy
++ CGH
++ FISH
++ Telomeres
++ Ana Bridges
++ miRNA
Neoplastic Field:
Entire Colon
UC Polypoid Dysplasia

You’re dalmed if you do, and dalmed if you don’t

Teri Brentnall, MD
Dysplasia in UC vs Adenoma

• No clinical features
• No endoscopic features
• No pathologic features
• No molecular tests
HOWEVER

• If the lesion can be demonstrably completely removed endoscopically

• Has only Low-Grade Dysplasia

• There is no other dysplasia on adequate sampling

• Then, careful follow-up may be considered
UC Dysplasia Management

Continue Surveillance with adequate sampling:

– Single site LGD while in surveillance

– Indefinite of negative for dysplasia
UC Dysplasia Management

Consider Colectomy:

– Multiple LGD sites
– LGD on more than one endoscopy
– LGD at initial colonoscopy
– Excessive inflammatory polyps
Inflammatory Polyps
UC Dysplasia Management

Colectomy Indicated:

– HGD

– Endoscopically unresectable dysplastic lesion
Conclusions

• Molecular alterations are widespread in UC, CD, CP, HP, HCV

• Single non-dysplastic bx alterations show promise for reducing sampling error

• Paradigm for cancer in chronic inflammatory disease
Further Work:

- Reproducibility
- Longitudinal analyses
- Prospective validation
- High throughput
- Reduced numbers of markers
- Mechanism: *why progressors?*
Thanks To My Colleagues:

Bonnie Shadrach
Teri Brentnall
Peter Rabinovitch
Ru Chen
David Crispin
Rosana Risques
Jacintha O’Sullivan
Noah Welker
Keith Lai
Danielle Elsberry
Ryan O’Connell
June Round
John Valentine