Carcinogenesis in IBD

Dr Simon Leedham, Oxford, UK
Carcinogenesis in Inflammatory Bowel Disease

Dr Simon Leedham
Cancer Research UK Clinician Scientist
Honorary Consultant Gastroenterologist
University of Oxford
Pathogenesis of cancer in IBD

- Cell of origin
- What's driving cancer forwards
- Genetic mutations
- Epigenetic change
- Mutation spread and field cancerisation
- Challenges and opportunities
Cell of origin
Cell of origin in cancer

Apc KO in Lgr5-ve cells

Apc KO in Lgr5+ve stem-cells

Tight morphogen regulation of stem cells

Scoville D et al, Gastroenterology, 2008: 134(3), 849-64
How does inflammation affect this balance?

Cell of origin in inflammation driven cancer

- Polarised morphogen expression disrupted by lamina propria inflammatory signalling
- De-differentiation and stem-cell plasticity

Disrupted morphogen signalling
Carcinogen (e.g. AOM)

Inflammation (e.g. DSS)

Inflammation + Carcinogen

Carcinogen but treat inflammation

Kirchberger S, et al. JEM, 2013

Cooper H et. al, Acta Pharm, 2007
What drives IBD associated cancer

Hanahan and Weinburg, Cell, 2000
APC and Wnt signalling
APC mutation frequency

Sporadic

87

Colitis-associated

17
Sporadic adenomas

Colitis associated dysplasia

β-catenin staining

Leedham et al, Gastroenterology, 2009
What is driving colitis associated carcinogenesis?

- Reactive oxygen and nitrogen species
- Telomere shortening and chromosomal end fusion
- NF-KB activation
Chromosomal instability

- Inflammation and repair provides the proliferative drive
- Chromosomal instability seen early - even in non-dysplastic tissue
- More seen in ‘cancer progressors’
Genetic mutations
So what genes are important in colitis neoplasia?
Genetic dependency analysis

Figure 2. Topographical clonal map from patient 2

A. H&E

B. Laser capture


D. | Morphology | Low-grade dysplasia (LGD) | Hyperplasia | Dysplasia (LGD) | Hyperplasia | Chronic inflammation |
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<thead>
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<td>β-catenin stain</td>
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Colitis associated gate-keeping mutations

- p53, 46%
- K-Ras, 18%
- APC, 9%
- None found, 27%
p53 as the commonest initiating mutation

- p53 – the guardian of the genome
- Tumours driven by chromosomal instability need to inactivate p53 to progress
The genetic road to cancer
Epigenetic changes
Methylation

- **Normal**
  - Exon CpG methylated
  - Promoter CpG islands unmethylated

- **Age associated** – widespread global change

- **Cancer associated** – tumour suppressor genes
  - Promoter CpG hypermethylation
  - Global hypomethylation

[Diagram showing DNA structure with methylation and transcription processes]
Methylation in sporadic cancer

- **Sporadic serrated neoplasia pathway**
  - BRAF/KRAS mutation initiates
  - Aberrant methylation detectable leading to CpG Island Methylator Phenotype (CIMP)
  - Eventual methylation of TSG’s leads to rapid progression when dysplasia sets in
  - Connection between genetic event (BRAF mutation and aberrant methylation unknown)
Methylation in colitis associated cancer

- Less CIMP panel positive lesions seen in CAC
- Inflammatory context alters the mediators of DNA methylation
- Methylation occurs as a response to inflammatory environment rather than a genetic insult
- Acceleration of age-related global methylation changes
- Colitis causes premature epigenetic aging of cells
Mesenchymal-epithelial interaction

- Methylation in stromal cells
  - IL-6 stabilises DNA methyltransferase 1
  - DNMT1 expression higher in CAC than sporadic CRC samples
  - Increased DNMT1 expression seen in both tumour and peritumoural stroma
  - Altered DNA methylation in the mesenchyme affecting the malignant transformation of the epithelium

Foran E et. Al, 2010, Molecular Cancer Res
Lesion spread
Lesion spread - Crypt fission in UC

- 60% of crypts in fission in active UC (Brittan, 2005)
- Main mechanism of epithelial restitution to heal ulcers
- FISH identifies abnormal chromosome 17 (p53) in the 2 halves of daughter crypts

Chen et al, Carcinogenesis, 2005
Field Cancerisation - microscopically

Figure 2. Topographical clonal map from patient 2

C. Dysplastic
Hyperplastic
Dysplastic
Hyperplastic
Chronic Inflammation

D.

<table>
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<tr>
<th>Morphology</th>
<th>Low-grade dysplasia (LGD)</th>
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Widespread field cancerisation

<table>
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<tr>
<th>Year</th>
<th>Tissue sites and genotypes</th>
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<td><img src="image12" alt="Diagram" /></td>
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Genotypes:
- **TP53 c.731G>A**
- **TP53 c.775G>T**
- **TP53 c.742C>T**
What can we learn from human tissue sampling?

- Single crypt gene expression analysis
- Single crypt mutation burden
- Multi-region biopsy and cancer heterogeneity
- Organoid formation, clonogenic assays
Identifying the progressor from the non-progressor

- Histology is an incomplete gold standard when field cancerisation present
  - Improved endoscopic targeting (dye spray, NBI, ?confocal)
  - Molecular phenotyping, genetic risk stratification
  - Fluorescent biomarkers (cf Barrett's esophagus\(^1\))

Intestinal inflammation and cancer

- Define the molecular pathogenesis
  - Determining the cell of origin. Stem-cell plasticity in the human?
  - Cancer progression in the biological therapy era – impact of mucosal healing as a therapy goal

Personalising therapy

- Impact of sequencing technology
  - Targeting the right pathway(s)
  - The cancer heterogeneity problem

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