Colon Cancer Genetics and Development

Progression of neoplasia of the Colon and rectum
Inherited Predisposition to Colorectal Cancer

Polyposis and Colon Cancer Predisposition Syndromes

- Familial Adenomatous Polyposis, Hereditary Nonpolyposis Syndrome, Juvenile Polyposis
  - Genetic analysis of these syndromes has identified their genetic causes and led to understanding of sporadic colorectal cancer
Familial Adenomatous Polyposis

- Autosomal dominant
- Multiple benign adenomas of the colon and rectum
- Increased risk of colon cancer
- Patients develop adenomas in 2nd and 3rd decade
- Inherited deletion of chromosome 5q

Adenomatous Polyposis Coli
APC Mutations in Colon Cancers

Mechanism of Action of APC

- Identify protein-protein interactions
- Beta-catenin

Wnt signaling pathway

- Drosophila genetics
- Mammalian cells
- Xenopus development
Growth-related genes (e.g. C-myc)

Regulation by
• protein-protein interactions
• phosphorylation events
• ubiquitin-mediated proteolysis

Inherited Predisposition to Colorectal Cancer

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial Adenomatous Polyposis</td>
<td>40%</td>
</tr>
<tr>
<td>Hereditary Nonpolyposis Colon Cancer</td>
<td>20%</td>
</tr>
<tr>
<td>Undefined Inherited Colorectal Cancer</td>
<td>10%</td>
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<tr>
<td>Sporadic Colorectal</td>
<td>30%</td>
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</tbody>
</table>
Hereditary Nonpolyposis Colon Cancer Syndrome

- At least three affected family members in two generations
- Autosomal Dominant
- One member diagnosed under age 50
- Right sided colon cancer most common
- Also associated with endometrial carcinoma

HNPCC Pedigrees
Microsatellite DNA

- Repeats of simple single, double, triple or tetra nucleotides
- Commonly (A)$_n$ or (CA)$_n$/(GT)$_n$ repeats
- $>10^4$-$10^5$ found throughout genome

Linkage analysis in HNPCC Families

- Log of the Odds (LOD) ratio of greater than 5 seen in HNPCC pedigrees
- Linkage to 2p16 and 3p24
  - Inheritance of phenotype was linked with specific allele
Microsatellite Instability and HNPCC

- Simple repeated sequences such as CA_n and A_n are mutated in tumors from HNPCC patients
- Mutations typically delete or insert repeat sequence
- Point mutations occur at high frequency

HNPCC Tumors Have Mutations in Microsatellite DNA
Mismatch repair and Microsatellite Instability

• Resembles mutator phenotype seen in e. coli and s. cerevesiae that are deficient in mismatch repair
• Cells with microsatellite instability also have increased rate of point mutations
• Cells with microsatellite instability have no functional mismatch repair

E. Coli Mismatch Repair

• E coli: MutS, MutL and MutH
• MutS binds mismatch
• MutL binds MutS and DNA repair machinery
• MutH recognizes daughter strand
• Mismatches can be repaired with purified factors in a cell free system
Mismatch Repair

Eukaryotic Mismatch Repair

- MSH2 and (MSH3 or MSH6) bind DNA
- MLH1 binds MSH complex and recruits DNA repair machinery
- Mutation of one subunit inactivates repair
- Purified factors able to repair mismatches
Mismatch Repair Defects in Yeast Cause Microsatellite Mutations

TABLE 1  Rates of alteration in lengths of poly(GT) tracts in yeast strains with mutations affecting DNA mismatch repair

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Tract location</th>
<th>Rate of tract alterations</th>
<th>Rate relative to wild type</th>
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<tbody>
<tr>
<td>MS85</td>
<td>Wild type</td>
<td>pSH31</td>
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<td>pSH31</td>
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<td>MS90</td>
<td>mih1</td>
<td>pSH31</td>
<td>1.7 x 10^-3</td>
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<tr>
<td>MS94</td>
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<td>pSH31</td>
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<td>MS97</td>
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<tr>
<td>MS86</td>
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<td>pSH31</td>
<td>2.2 x 10^-6</td>
<td>0.7</td>
</tr>
</tbody>
</table>

HNPCC linked to 2p16 and MSH2

HNPCC KINDREDS

CHROMOSOME 2

GENETIC MAP IN CM

hMSH2

MEROTIC RECOMBINANTS

MEROTIC RECOMBINANTS
Mutations of hMSH2 occur in HNPCC

The critical subunits are hMLH1 and hMSH2
Inherited Predisposition to Colorectal Cancer

- Familial Adenomatous Polyposis
- Hereditary Nonpolyposis Colon Cancer
- Undefined Inherited Colorectal Cancer
- Sporadic Colorectal

Juvenile Polyposis Syndrome

- Onset of hamartomatous polyps in first decade
- Increased risk of colon cancer
- Autosomal dominant
- Associated with germline mutations of SMAD4 and BMP 1A receptor
TGF-β Pathway

- TGF-β
- ↓
- Receptor types I and II
  (TGFβ and BMP receptors)
- ↓
- SMADs
  (SMAD4)
- ↓
  Growth inhibition

Genetics of Sporadic Colorectal Cancer
Progression of neoplasia of the Colon and rectum

Nowell’s Hypothesis of Tumor Development: Multiple Waves of Clonal Expansion
Predictions of the Nowell’s Hypothesis

- Mutations of oncogenes are rare events
- Solid tumors require multiple genetic alterations within a single clone
- Multiple waves of mutation require increased mutation rate

Microsatellite Mutations in Sporadic colon Cancer

- Instability due to lack of hMLH1 expression associated with promoter methylation.
- Inactivation is somatic.
Epidermal Growth Factor Receptor/Mitogen Activated Protein Kinase Pathway—an oncogenic pathway

Ligand (EGF, TGF-α) → EGFR → K-RAS → RAF → MAPK → Mitogenesis

K-RAS Mutations are selected after adenoma development

K-RAS → NORMAL EPITHELlIUM → ADENOMA → ADENOMA → CARCINOMA → METASTASIS
K-RAS Mutations

- Mutated in 50% of sporadic colorectal cancer
- Mutated in 50% of sporadic adenomas
- Mutations are somatic
- Mutations affect only one allele
- Mutations are dominant and oncogenic
- Mutations are not seen in small adenomas or aberrant crypts
- Mutations inactivate GTPase activity of K-RAS

RAS Signaling
Epidermal Growth Factor Receptor/Mitogen Activated Protein Kinase Pathway—an oncogenic pathway

Ligand (EGF, TGF-α)

↓

EGFR

↓

K-RAS

↓

B-RAF Mutated in 20%

of colorectal cancer

MAPK

↓

Mitogenesis

EGFR Pathway mutations in colon cancer

K-RAS (50%)

B-RAF(20%)

NORMAL EPITHELIIUM → ADENOMA → ADENOMA → CARCINOMA → METASTASIS
Loss of Heterozygosity in Colorectal Cancer

P53 is a Tumor Suppressor

Normal Mucosa

Normal Mucosa

p53 mutation

Chromosome 17

Chromosome 17

A B

A B

Alleles
p53 Mutations

- Mutations inactivate both alleles.
- Mutations inactivate DNA-binding domain.
- Mutations occur in 50-80% of Carcinomas
- No mutations occur in Adenomas
- Mutations are somatic

p53 Domains
The p53 Tumor Suppressor Pathway

- Radiation
- ATM/ATR
- Chk1/chk2
- MDM2
- p53

Cell Cycle Arrest
Apoptosis

p53 Mutations Occur During Carcinoma Development

- K-RAS/B-RAF
- p53

NORMAL EPITHELIUM → ADENOMA → ADENOMA → CARCINOMA → METASTASIS
Growth-related genes (e.g. C-myc)

Wnt/APC/β-catenin Pathway

Regulation by
- protein-protein interactions
- phosphorylation events
- ubiquitin-mediated proteolysis

APC and β-catenin Mutations Occur During Sporadic Carcinoma Development: It’s the WNT pathway!

NORMAL EPITHELIUM → ADENOMA → ADENOMA → CARCINOMA → METASTASIS
TGF-β Pathway

TGF-β
↓
Receptor types I and II
(TGFβ and BMP receptors)
↓
SMADs
(SMAD4)
↓
Growth inhibition

TGF-β Pathway altered in sporadic Cancer

NORMAL
EPITHELIUM

APC
β-catenin
K-RAS
B-RAF
SMAD4
TGFβRII
p53

ADENOMA
ADENOMA
CARCINOMA
METASTASIS
Genomic Instability in Colon Cancer

- APC inactivation causes a mitotic checkpoint defect
- p53 mutations cause G1 and G2 checkpoint defect
- MMR defects associated with diploid karyotype and 1000 fold increase in mutation rate
- Cause of aneuploidy remains poorly understood but likely to involve mitotic checkpoint pathways

Checkpoint inactivation can lead different forms of genetic instability
Aneuploidy occurs in most colorectal cancer

Aneuploidy is associated with chromosomal instability
Comparison of MMR deficient and Aneuploid Tumors: The TGF-β pathway

- 100% inactivation of TGFβ type II receptor in MMR-deficient cases.
- Typically, frame shift mutation in A_{10} tract in open reading frame
- Not mutated in aneuploid tumors.
- Chromosome 18q lost in 80% of aneuploid cases.
- Both alleles altered for SMAD4 and SMAD2 in substantial proportion of cases.

Comparison of WNT pathway Alterations (MMR + v. MMR -)

- β-catenin mutations more common than APC mutations in MMR-deficient tumors
- APC mutation occur in MMR proficient tumors
- Mutations are mutually exclusive
- WNT pathway is targeted in all colorectal cancers
Therefore:

- Different genetic instabilities reveal distinct “hot-spots” within different genes on a pathway.
- The pathways are the target
- Multiple pathways are activated in an invasive carcinoma

Accumulation of Genetic Changes During Tumor Progression
Pathways altered in sporadic Colorectal Cancer

WNT (APC/Beta-Catenin) → K-RAS → SMAD4/TGFβRII → p53

Increased Genetic Instability:
MMR pathway (hMLH1 and hMSH2)