Progression of neoplasia of the Colon and rectum

Inherited Predisposition to Colorectal Cancer
- Familial Adenomatous Polyposis
- Hereditary Nonpolyposis Colon Cancer
- Undefined Inherited Colorectal Cancer
- Sporadic 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
**APC Mutations in Colon Cancers**

**Mechanism of Action of APC**

- Identify protein-protein interactions
- Beta-catenin
- Wnt signaling pathway

**Wnt/APC/β-catenin Pathway**

Regulation by:
- Protein-protein interactions
- Phosphorylation events
- Ubiquitin-mediated proteolysis

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**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. cerevisiae 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

HNPCC linked to 2p16 and MSH2

Mutations of hMSH2 occur in HNPCC

Human Mismatch Repair Germline Mutations in HNPCC

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

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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 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

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

EGFR Pathway mutations in colon cancer

K-RAS (50%)
B-RAF (20%)

Normal Epithelium → 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

Mutations inactivate both alleles.
Mutations inactivate DNA-binding domain.
Mutations occur in 50-80% of Carcinomas
No mutations occur in Adenomas
Mutations are somatic
The p53 Tumor Suppressor Pathway

RADIATION
ATM/ATR
MDM2
Chk1/chk2
p53
Cell Cycle Arrest Apoptosis

p53 Mutations Occur During Carcinoma Development

K-RAS/B-RAF
p53
NORMAL EPITHELIUM
ADENOMA
ADENOMA
CARCINOMA
METASTASIS

Wnt/APC/β-catenin Pathway

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

Growth-related genes (e.g. C-myc)

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

APC β-catenin
K-RAS
B-RAF
p53
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

APC β-catenin
K-RAS
B-RAF
SMAD4
TGFBRII
p53
NORMAL EPITHELIUM
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 A10 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**

Increased Genetic Instability:
MMR pathway (hMLH1 and hMSH2)