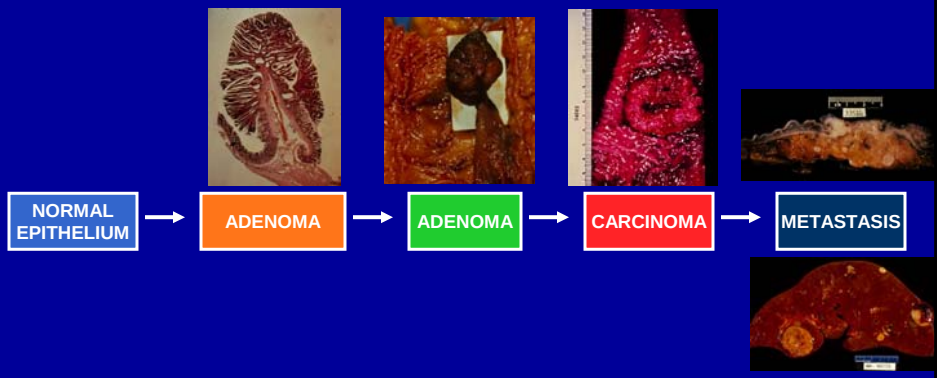
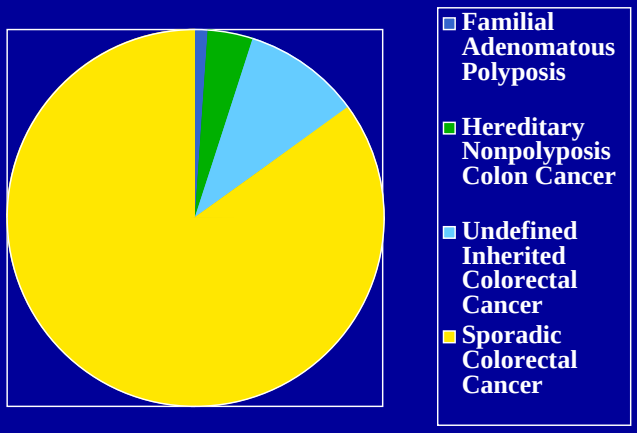
Colon Cancer Genetics and Development

Progression of neoplasia of the Colon and rectum



Inherited Predisposition to Colorectal Cancer



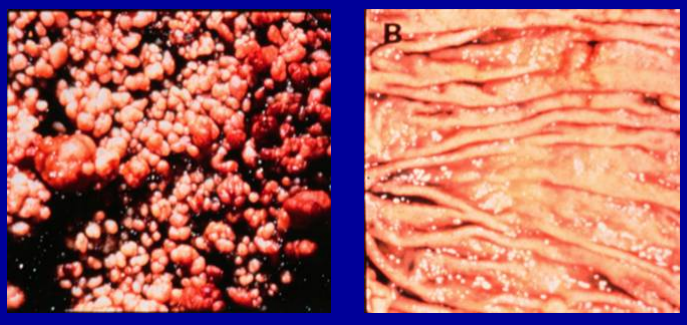
Polyposis and Colon Cancer Predisposition Syndromes

- 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

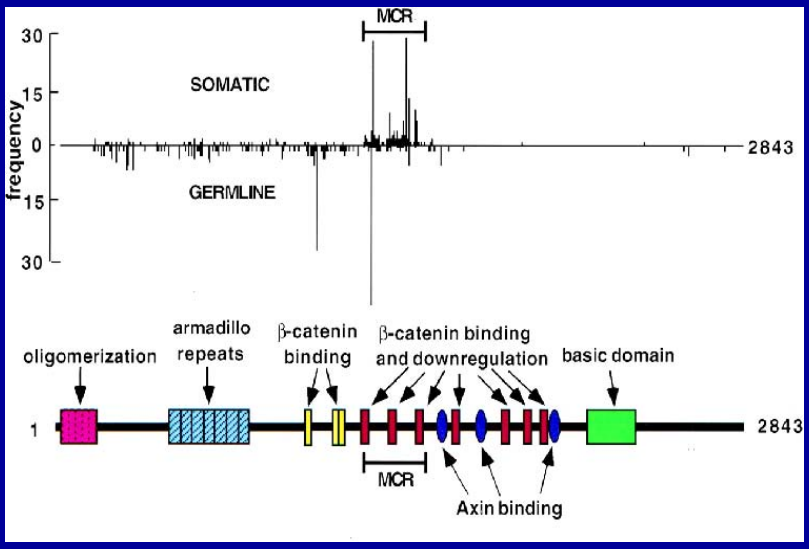
Familial Adenomatous Polyposis

- 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

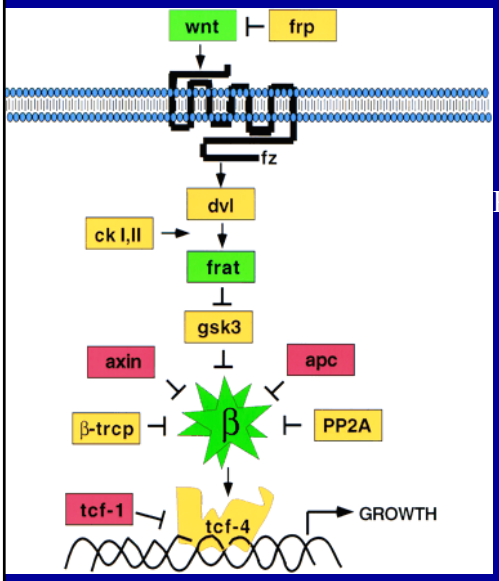
Beta-catenin

Wnt signaling pathway

Drosophila genetics

Mammalian cells

Xenopus development

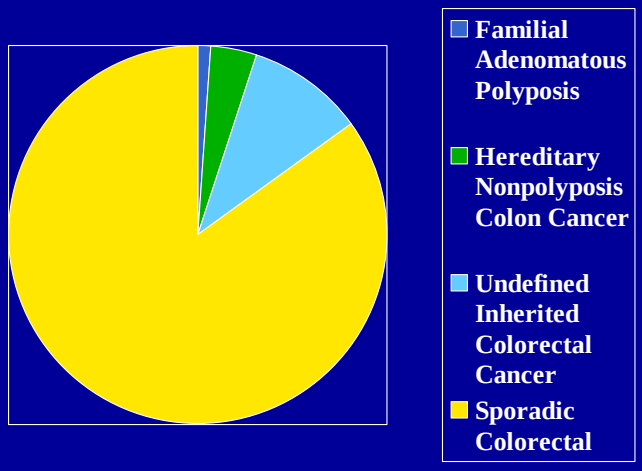


Wnt/APC/β-catenin Pathway

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

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

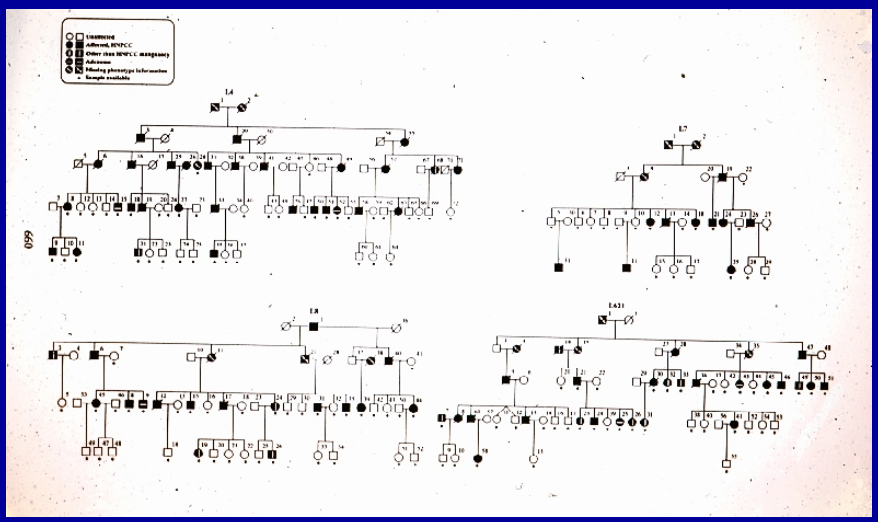
Inherited Predisposition to Colorectal Cancer



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

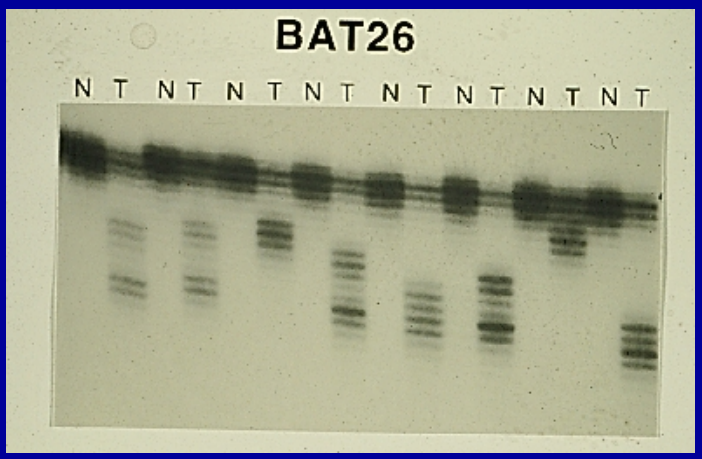
Linkage analysis in HNPCC Families

- 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



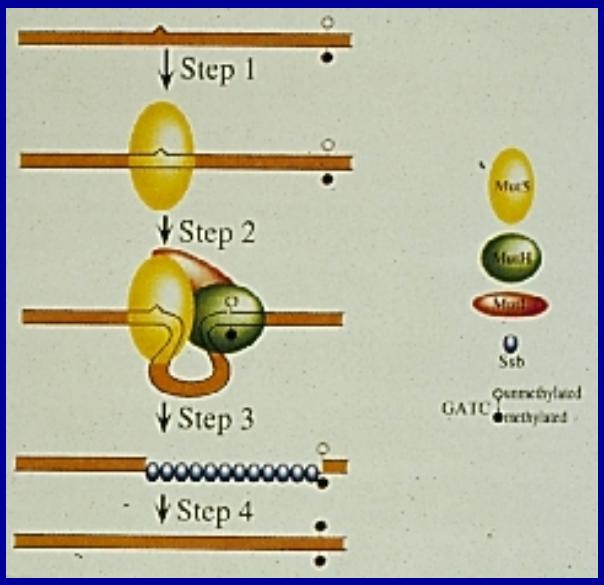
Mismatch repair and Microsatellite Instability

- 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 machinary
- MutH recognizes daughter strand
- Mismatches can be repaired with purified factors in a cell free system

Mismatch Repair



Eukaryotic Mismatch Repair

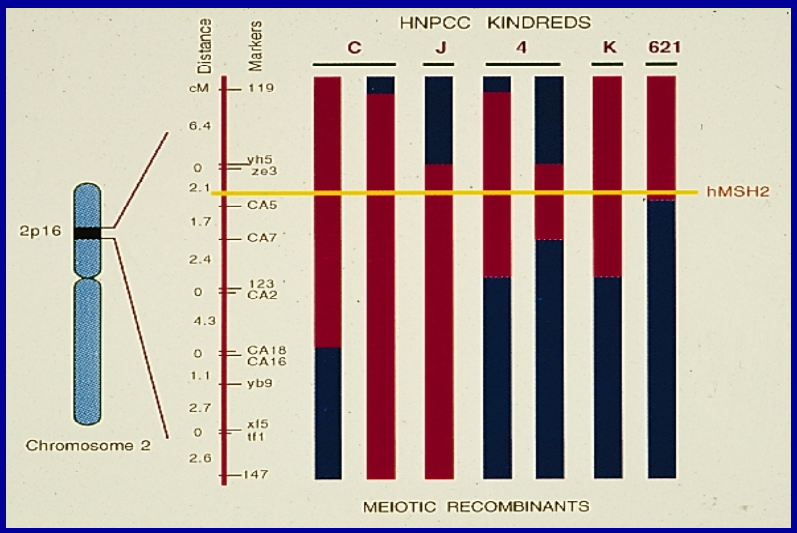
- MSH2 and (MSH3 or MSH6) bind DNA
- MLH1 binds MSH complex and recruits DNA repair machinery
- Mutation of one subunit inactivates repair
- Purified factors able to repair mismatches

Mismatch Repair Defects in Yeast Cause Microsatellite Mutations

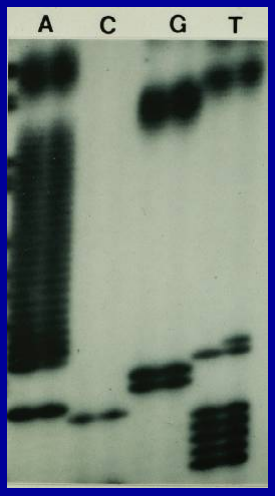
TABLE 1 Rates of alteration in lengths of poly(GT) tracts in yeast strains with mutations affecting DNA mismatch repair

Strain	Relevant genotype	Tract location	Rate of tract alterations	Rate relative to wild type
MS85	Wild type	pSH31	3.1×10^{-6}	1
MS57	*pms1*	pSH31	2.4×10^{-3}	774
MS90	*mlh1*	pSH31	1.7×10^{-3}	548
MS94	*msh2*	pSH31	1.1×10^{-3}	355
MS97	*pms1 mlh1*	pSH31	1.7×10^{-3}	548
MS86	*pol2*	pSH31	2.2×10^{-6}	0.7

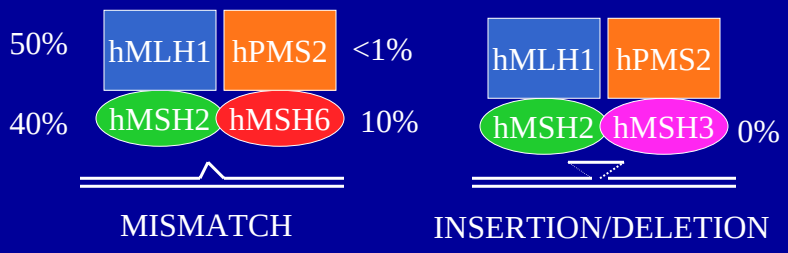
HNPCC linked to 2p16 and MSH2



Mutations of hMSH2 occur in HNPCC

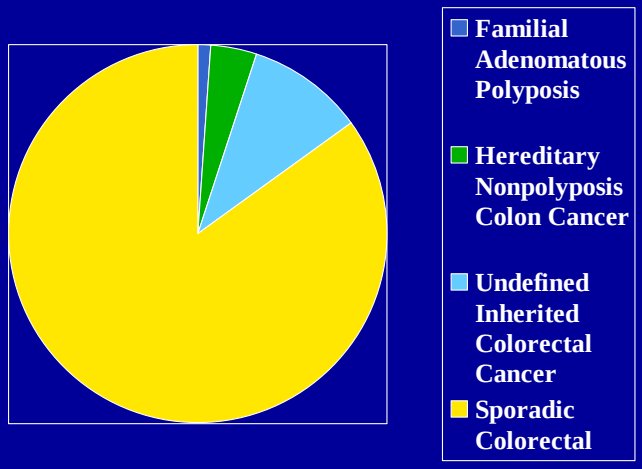


Human Mismatch Repair Germline Mutations in HNPCC



The critical subunits are hMLH1 and hMSH2

Inherited Predisposition to Colorectal Cancer



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)

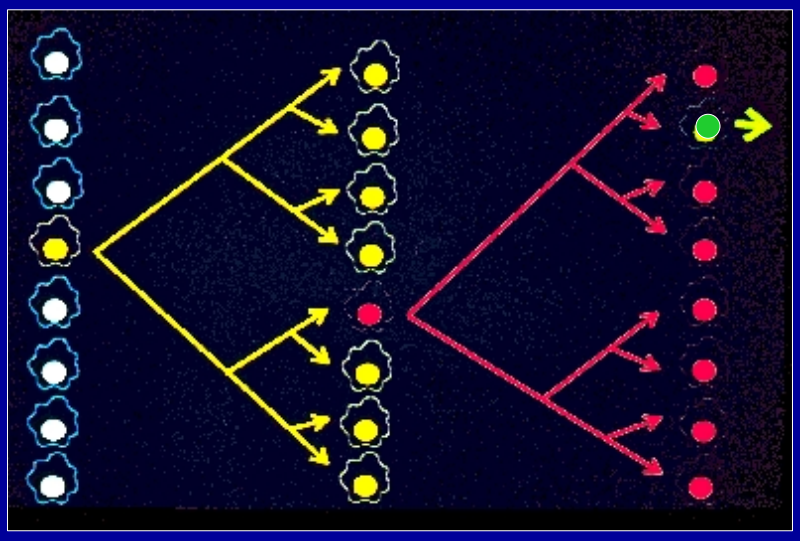
↓

Growth inhibition

Genetics of Sporadic Colorectal Cancer

Progression of neoplasia of the Colon and rectum

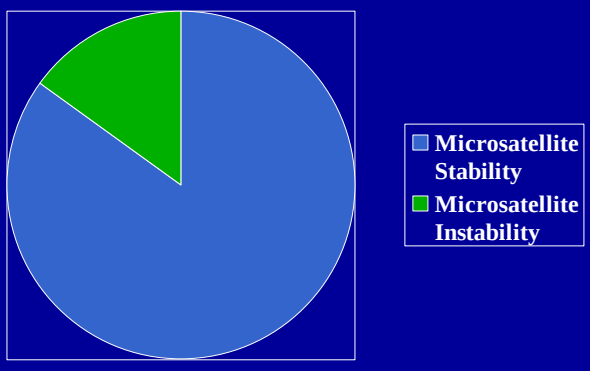
Nowell's Hypothesis of Tumor Development: Multiple Waves of Clonal Expansion



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

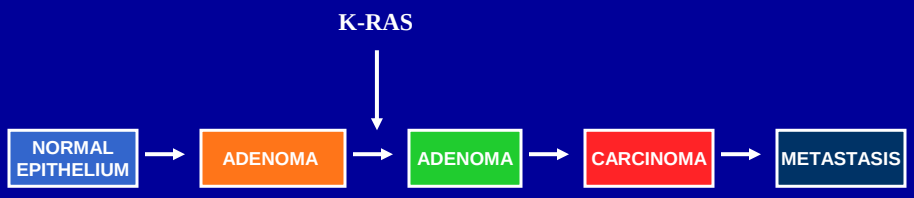


- Instability due to lack of hMLH1 expression associated with promoter methylation.
- Inactivation is somatic.

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

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

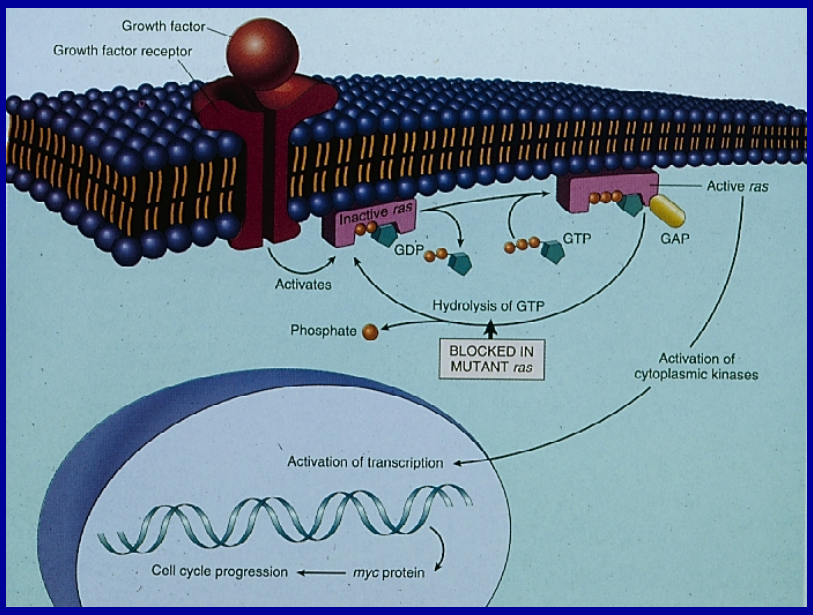
K-RAS Mutations are selected after adenoma development



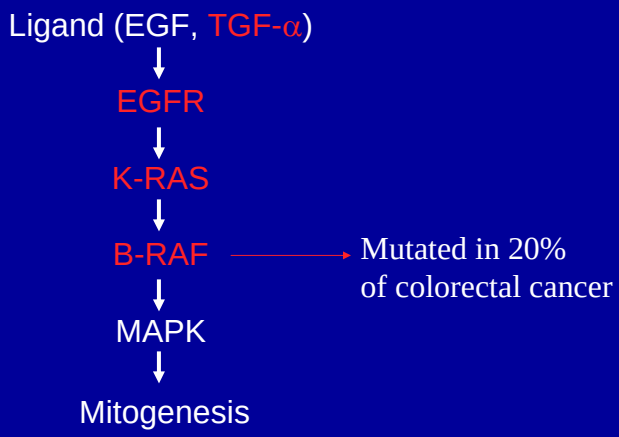
K-RAS Mutations

- 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

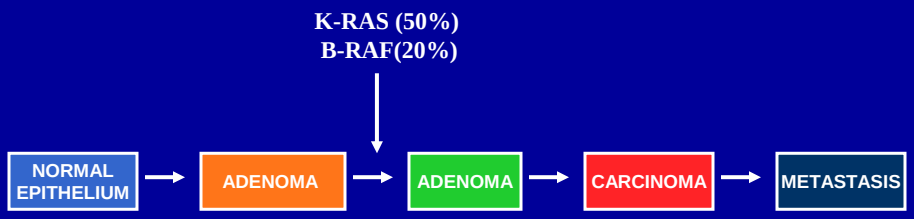
RAS Signaling



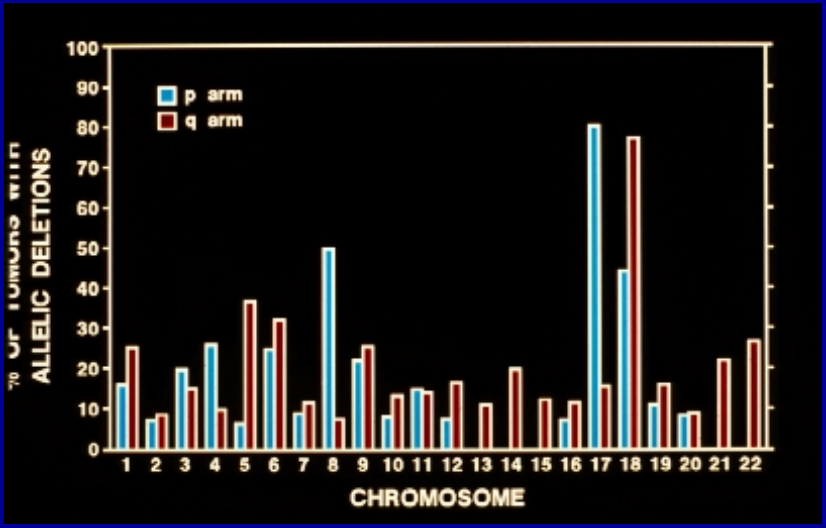
Epidermal Growth Factor Receptor/Mitogen Activated Protein Kinase Pathway-an oncogenic pathway



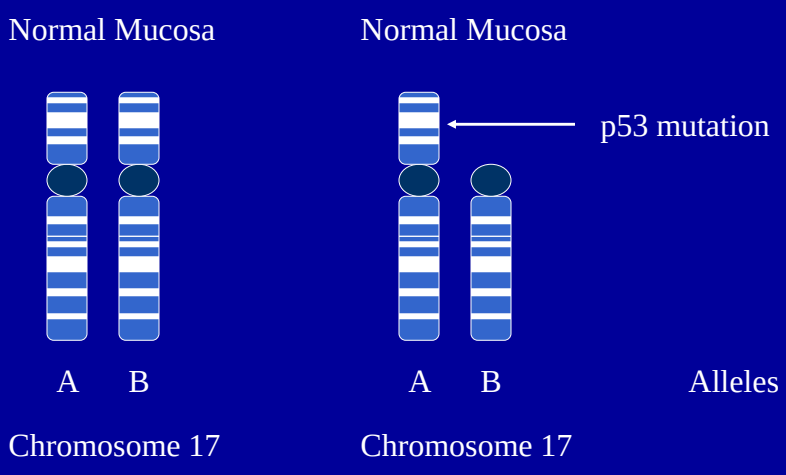
EGFR Pathway mutations in colon cancer



Loss of Heterozygosity in Colorectal Cancer



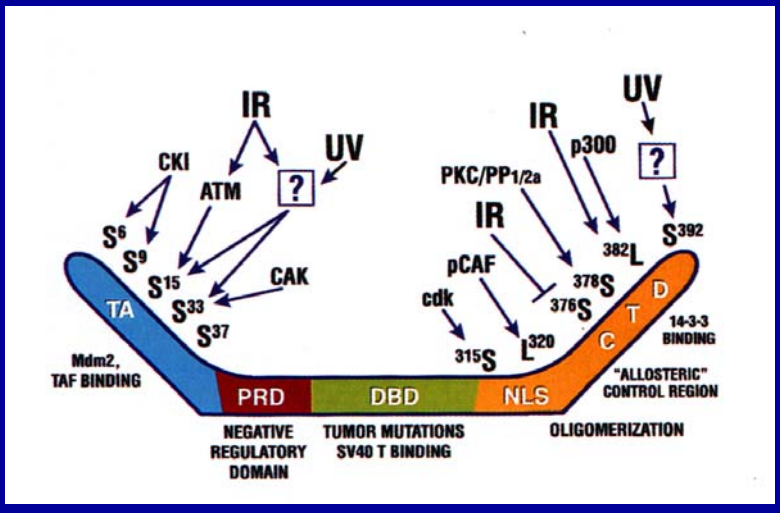
P53 is a Tumor Suppressor



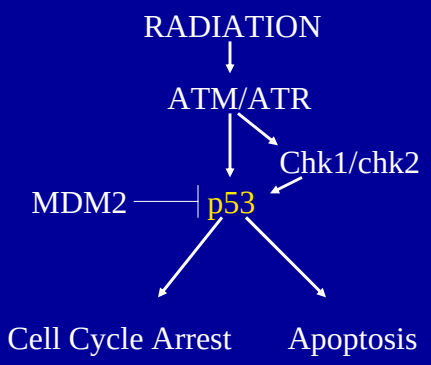
p53 Mutations

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

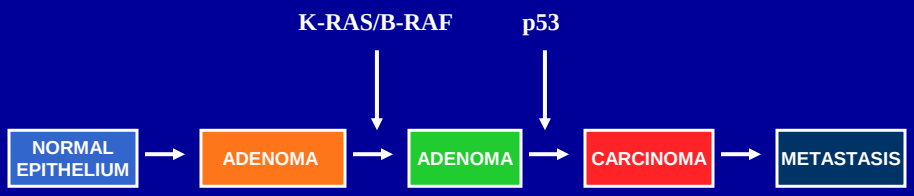
p53 Domains

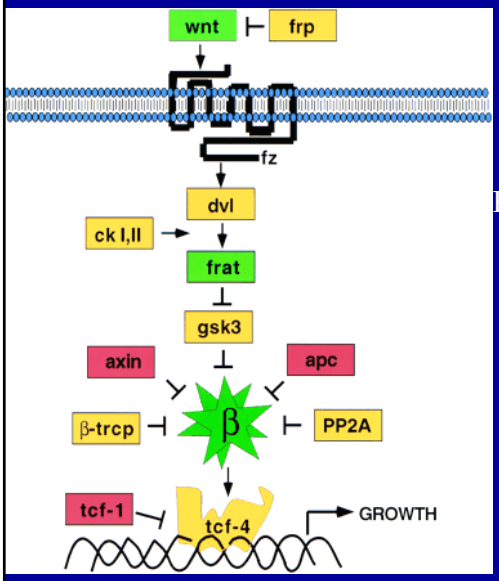


The p53 Tumor Suppressor Pathway



p53 Mutations Occur During Carcinoma Development



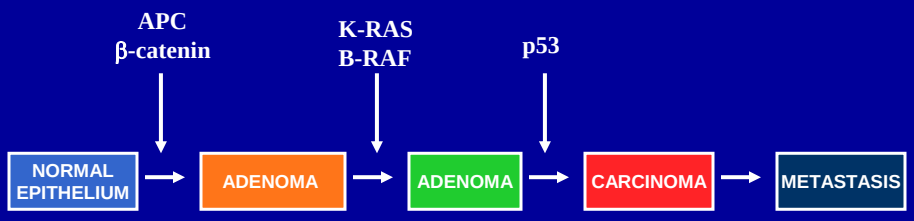


Wnt/APC/β-catenin Pathway

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!



TGF-β Pathway

TGF-β

↓

Receptor types I and II

(TGFβ and BMP receptors)

↓

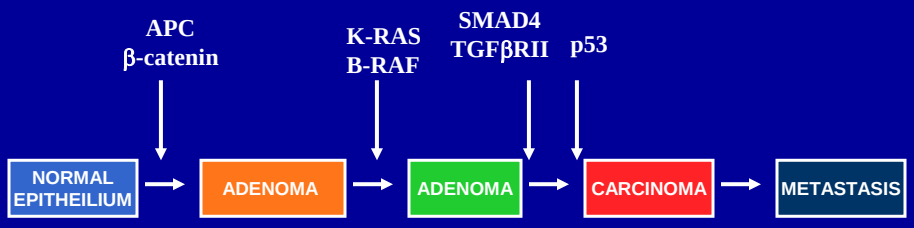
SMADs

(SMAD4)

↓

Growth inhibition

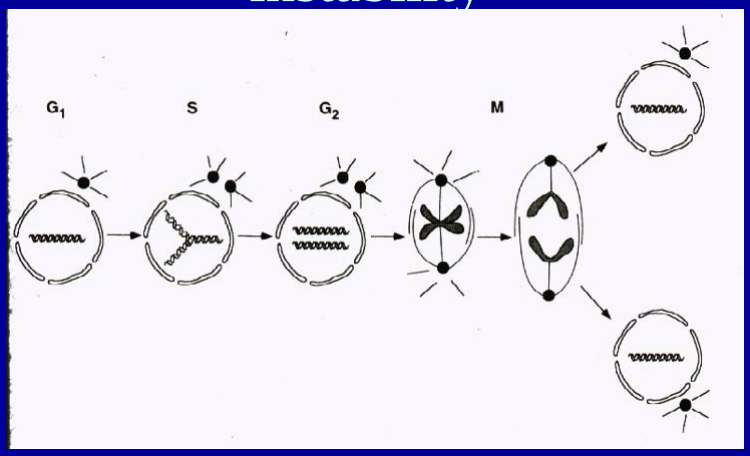
TGF-β Pathway altered in sporadic Cancer



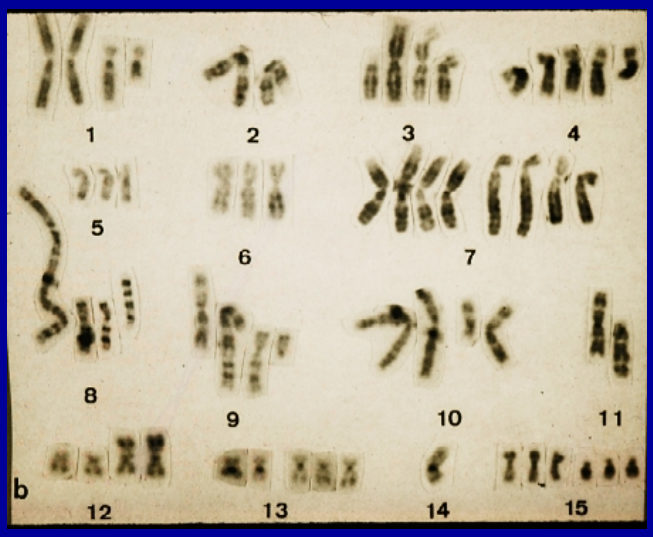
Genomic Instability in Colon Cancer

- 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

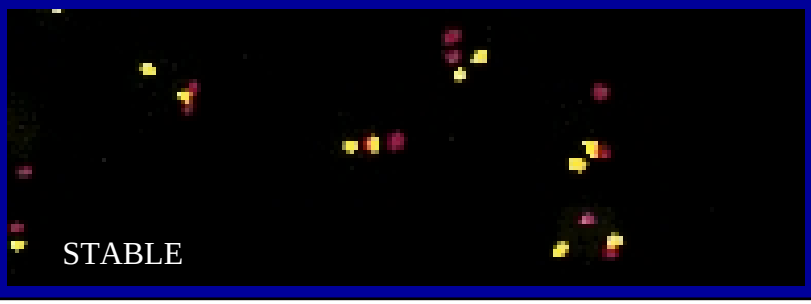
Checkpoint inactivation can lead different forms of genetic instability



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.

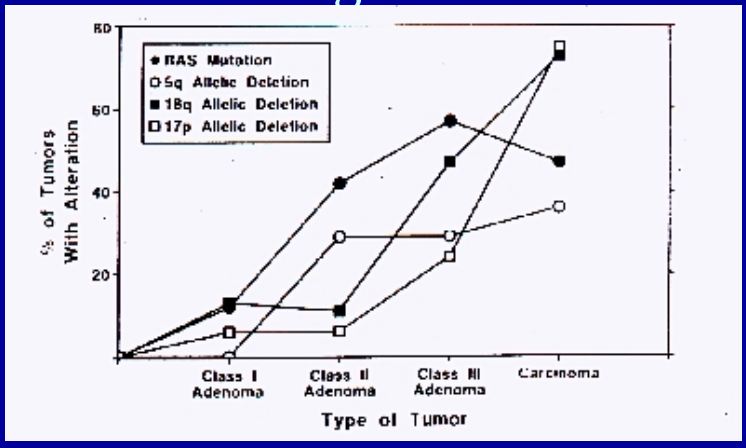
Comparison of WNT pathway Alterations (MMR + v. MMR -)

- 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

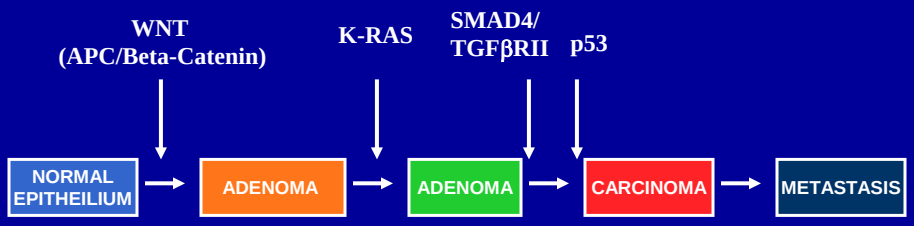
Therefore:

- 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



Increased Genetic Instability:
MMR pathway (hMLH1 and hMSH2)