Molecular Profiling & Tumor Biology

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Molecular Profiling

Method of testing each person's-

- cancer and
- genetic characteristics
- any unique biomarkers.

Aim:

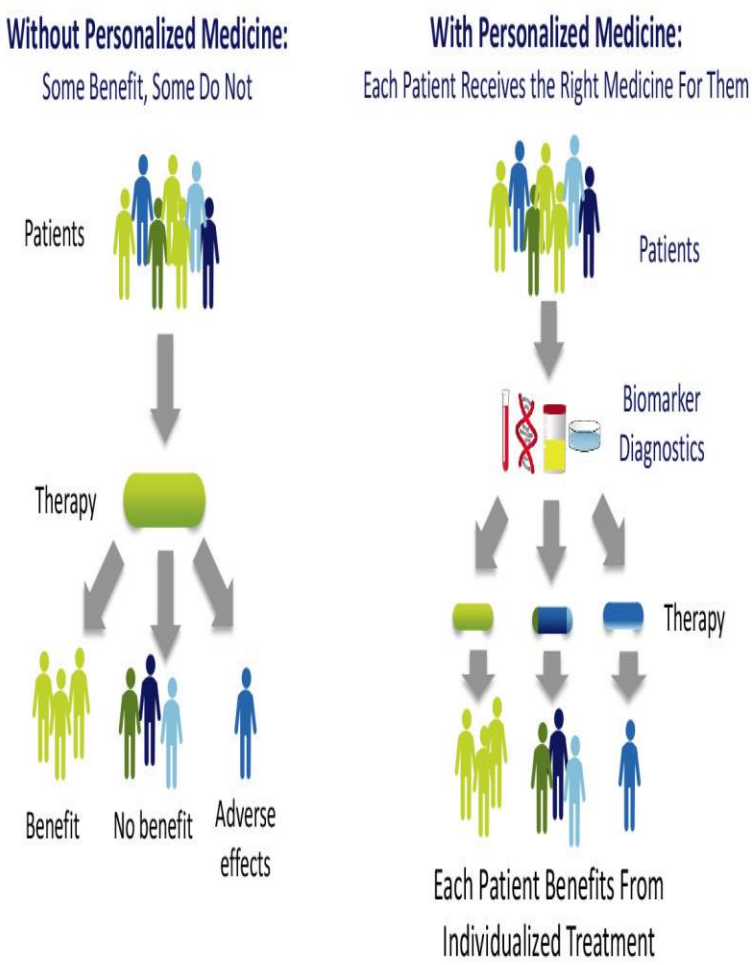
- To identify and create targeted therapies.

Specimen:

- **Needle biopsy or surgical specimen.**

Importance:

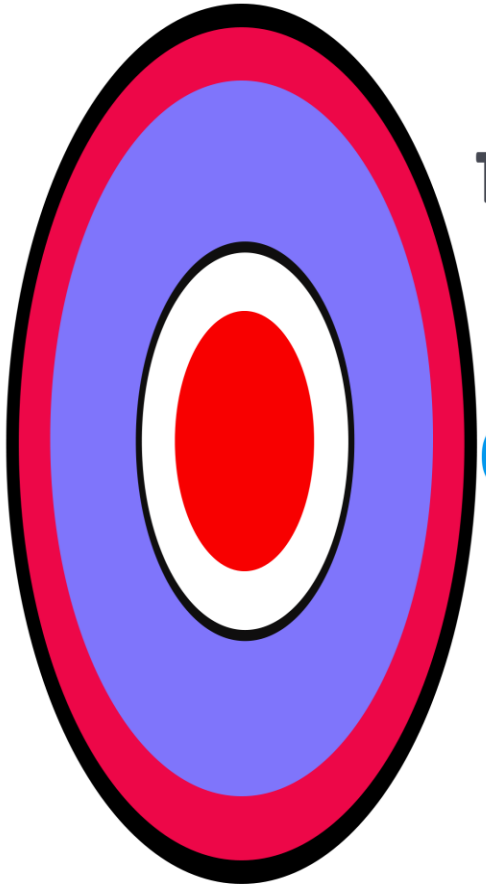
- If initial treatment fails, about 95% will not respond to the next conventional treatment.
- Biomarkers provide clues which treatments-
  - Will be more likely to be effective, &
  - Which will be more likely to fail.
- Personalized therapy for maximum effectiveness that targets
  - specific biomarkers or
  - genes.



Targeted therapies

Advantages:

- More effective,
- Fewer S/E
- Better chance of curing or controlling the tumor.



TARGETED THERAPY

Avoids Normal Cells & Goes Directly to the Cancer Cells

Example:

- MSI high --chemoresistance to 5-FU and irinotecan.
  - Predict poor response to CT,
  - May not benefit from adjuvant therapy.

- Similarly, polymorphisms in the enzymes that synthesize and metabolize folate may affect both efficacy and toxicity of 5-FU-based therapy.

- k-ras status predicts response to EGFR-targeted therapy in metastatic CRC.

Conclusion:

- Area of research
- Evolving rapidly, and
- Knowledge on molecular characteristics will certainly change the recommendations for adjuvant treatment in the future.

Personalized Cancer Therapy

Therapy to each patient's needs.

How personalized medicine is different

- Conventional treatment--some worked well and not for others.
- Genetic differences in tumors--different responses to treatment.

Examples of personalized medicine

Targeted treatments- targets a cancer's specific

- genes,
- proteins, or
- the tissue environment that contributes to cancer growth and survival.

Available targeted therapies-

- Breast cancer,
- colorectal cancer,
- GIST, RCC,
- lung cancer, melanoma,
- MM, Leukemia and lymphoma, and
- some types of childhood cancers .

Principle:

- Must presence of specific target.
- Sample of the tumor obtained through a biopsy or during surgery.

Pharmacogenomics

Looks at—

- how the body processes and
- responds to drugs.
- Influence how effective and safe a drug is for a person.

Example—

- some process a medication more quickly, so that person would require a higher dose.

- someone's does not process quickly, so will stay in the bloodstream for a longer time and may cause more severe side effects.

Example :

CRC with specific gene variation---life threatening S/E with irinotecan. This altered gene makes it harder to break down. So prescribe lower amounts of irinotecan for fewer side effects.

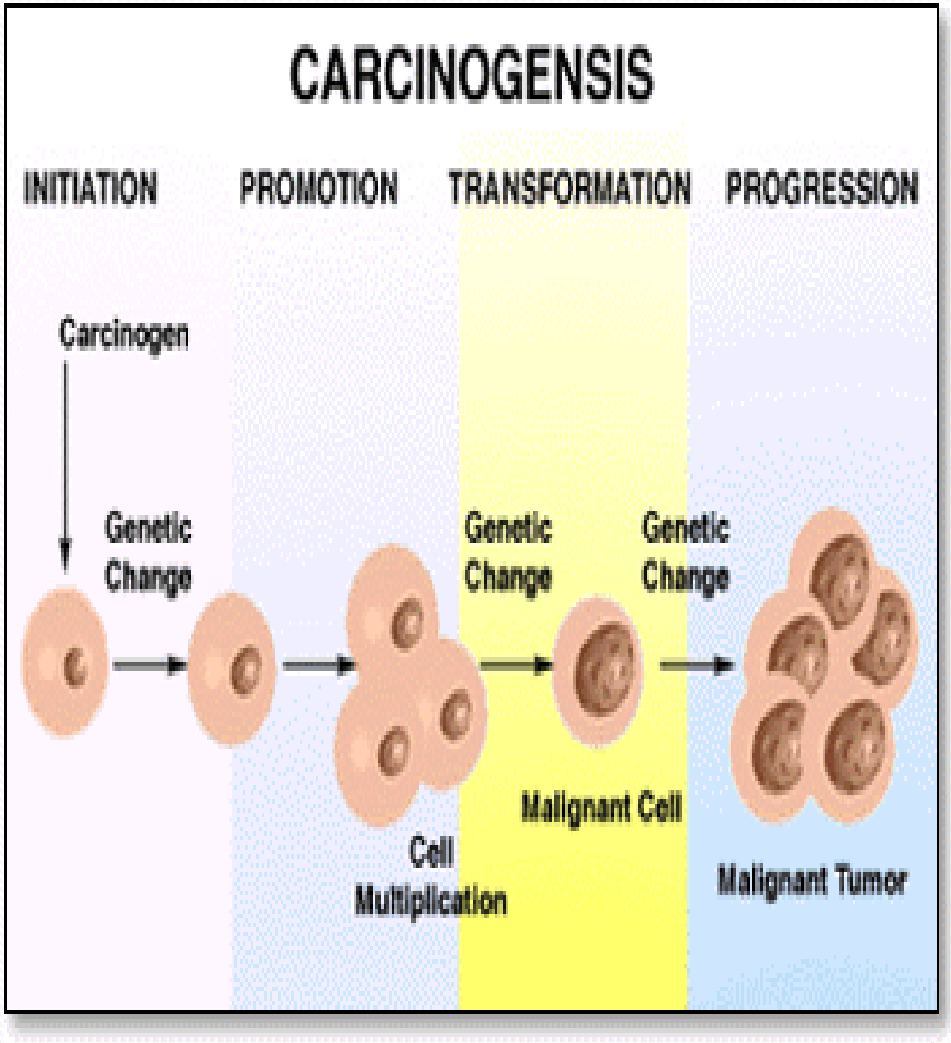
Future directions-

- Not all cancer have personalized treatment.

- Some are only available through a clinical trial.

Molecular Basis:

- All cancer has a genetic basis.
- Carcinogenesis is a multistep process, requires-
  - inherited and
  - acquired genetic alterations.
- In normal cells, growth and replication is a highly regulated process.
- Defects in genes that code for important proteins in the regulation of the cell cycle leads to carcinogenesis.



6 alterations identified in cancer cells-

- *Self-Sufficiency in Growth Signals*
  - proliferate autonomously.
- Insensitivity to antigrowth signals—
- Evading Apoptosis—
- Limitless Replicative Potential—
  - unlimited capacity for replication.
- Sustained Angiogenesis-- Virtually, all cells must reside within 1cm of a capillary for oxygen and nutrients.
- Ability to Invade and Metastasize—loss normal cell to cell adhesion to permit metastasis.

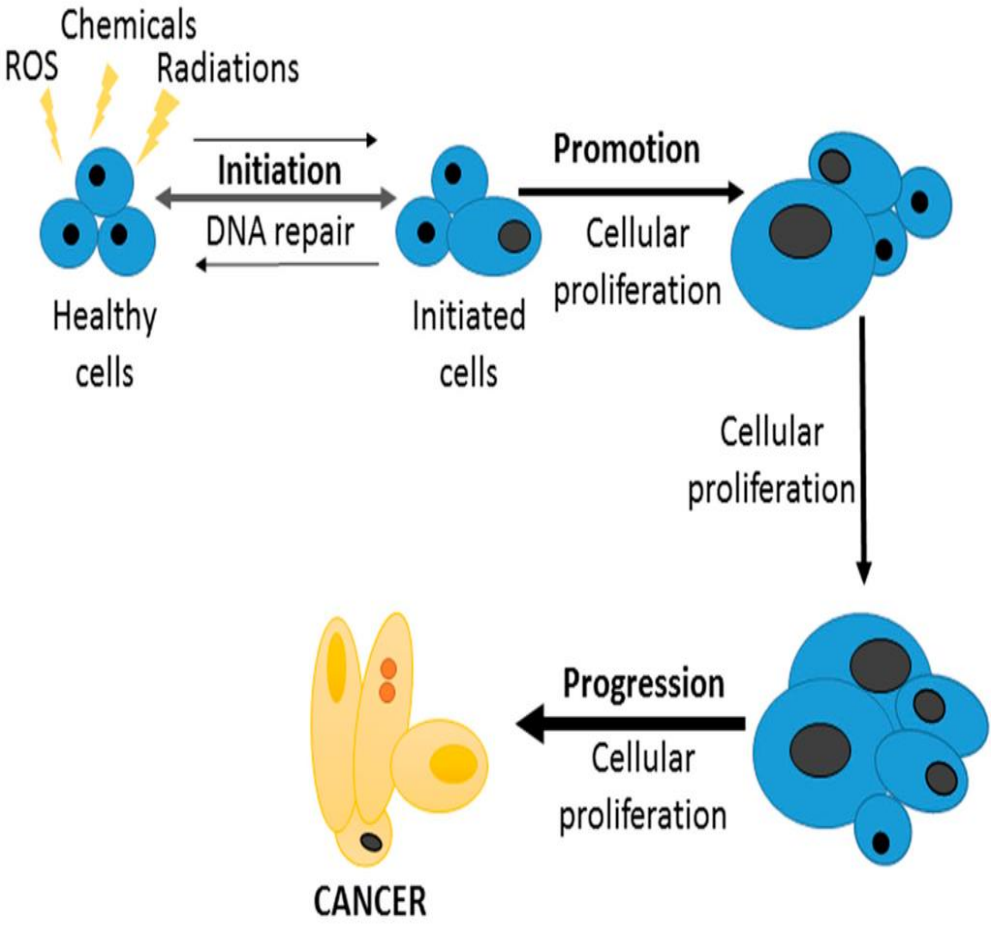
Carcinogenesis

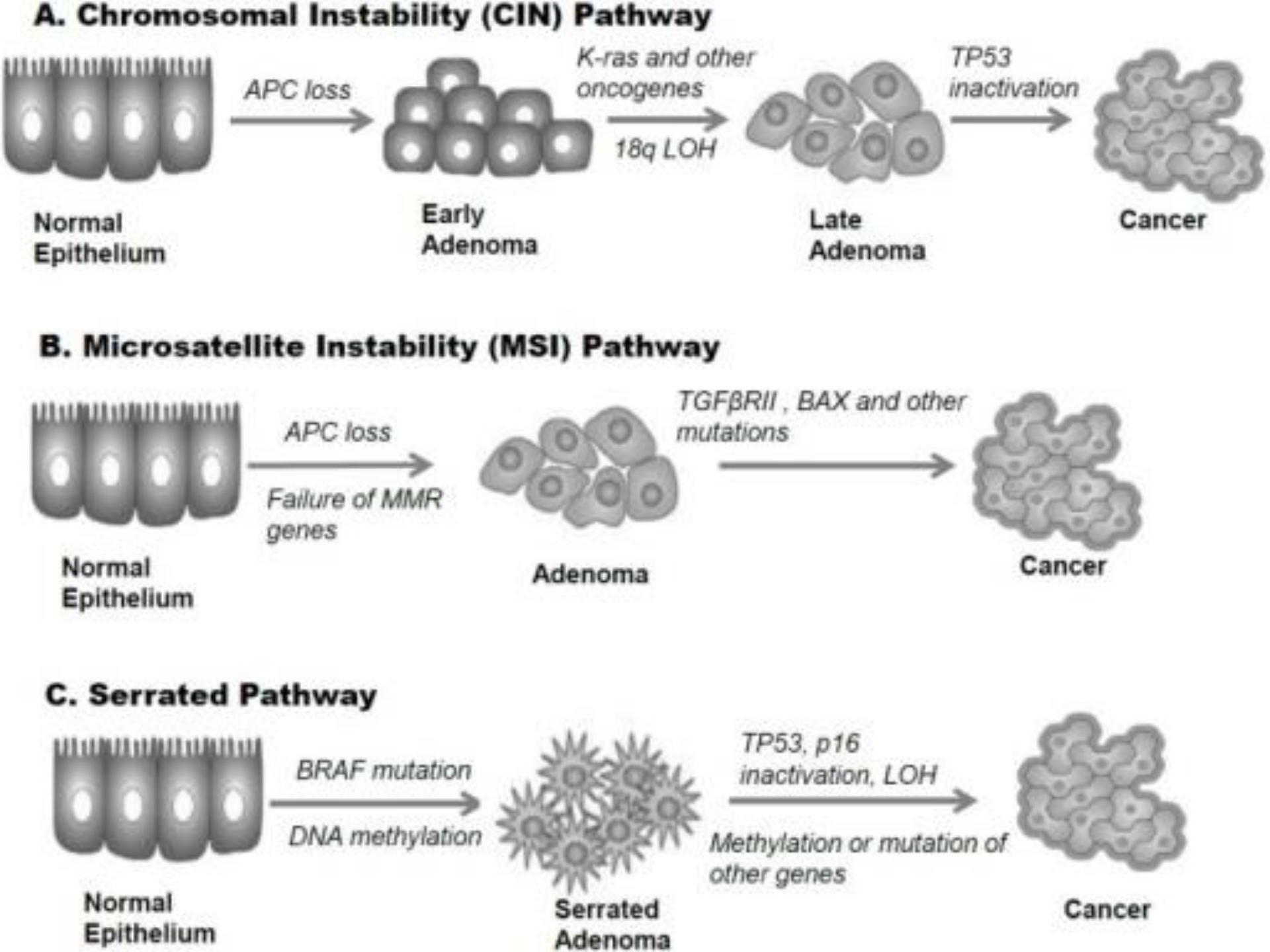
- Mutations occur early in the neoplastic process--- initiators.
- Mutations occur later---promoters.

2 broad categories of genes involved in carcinogenesis;

- Oncogenes and
- Tumor suppressor genes.

- Additionally, caretaker genes– prevent accumulation of somatic mutations. Abnormalities greatly increase the risk of cancer development, independent of environmental influence.

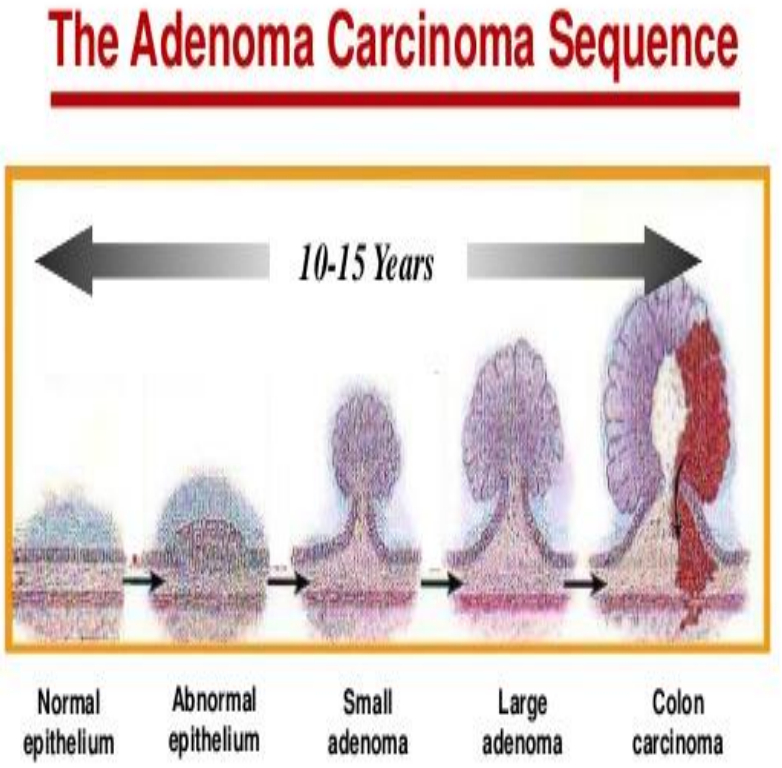



A. Chromosomal Instability (CIN) Pathway
APC loss
K-ras and other oncogenes
18q LOH
TP53 inactivation
Normal Epithelium
Early Adenoma
Late Adenoma
Cancer
B. Microsatellite Instability (MSI) Pathway
APC loss
Failure of MMR genes
TGFβRII , BAX and other mutations
Normal Epithelium
Adenoma
Cancer
C. Serrated Pathway
BRAF mutation
DNA methylation
TP53, p16 inactivation, LOH
Methylation or mutation of other genes
Normal Epithelium
Serrated Adenoma
Cancer

Adenoma to Carcinoma Sequence

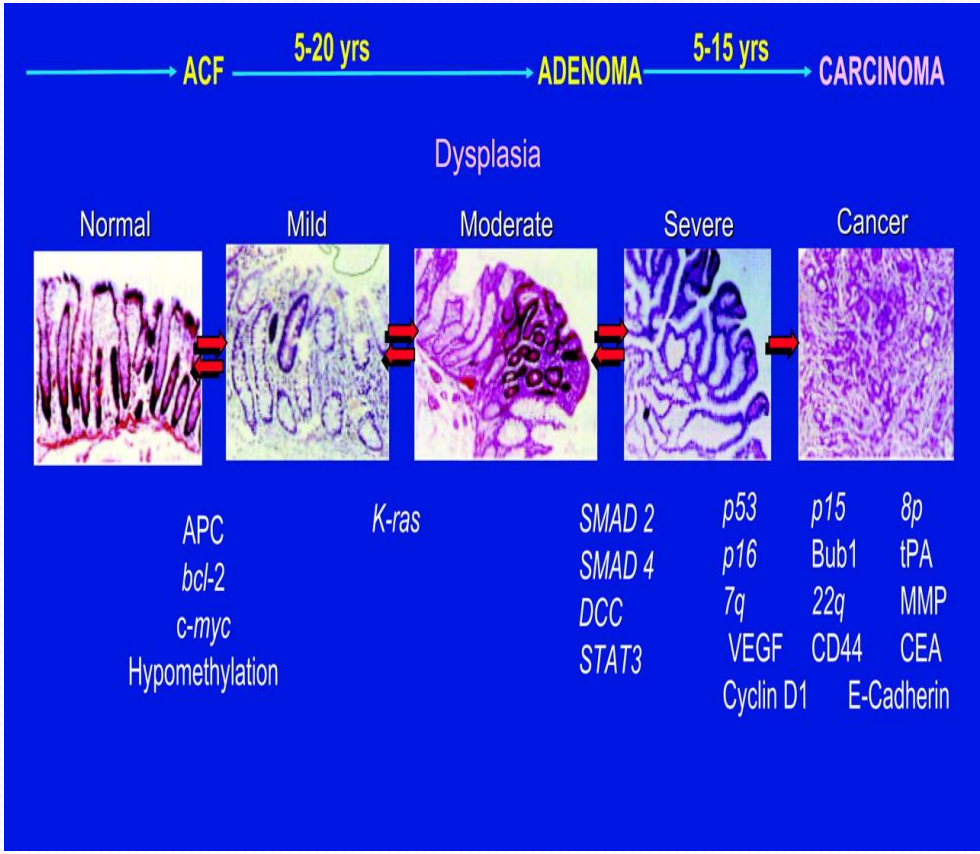
"traditional" pathway from adenoma to adenocarcinoma (also known as the
- "loss of heterozygosity" (LOH) or
- "chromosomal instability" (CIN) pathway)

- 80–85% sporadic CRC develop from adenomas.



A mathematical model suggested----

- Slow process.
  - 2–3 years for an adenoma <5 mm to grow to 1 cm,
  - Another 2–5 years– for 1 cm to cancer.
  - Mean age of adenoma to carcinoma is 7 years.



ACF--Aberrant crypt foci

Factors influencing

- Gender--- usually no effect .
- Age ---2.6% at age <60 to >5% annually at age >80 years).
- Adenoma size & type ---
  - Tubular adenoma—very low.
  - <5 mm – 3.4%,
  - 5–10 mm – 13.5%, and
  - >10 mm – 38.5%.

Advanced histologic features—

- Villous change,
- left-sided lesions, and
- age >60 years.

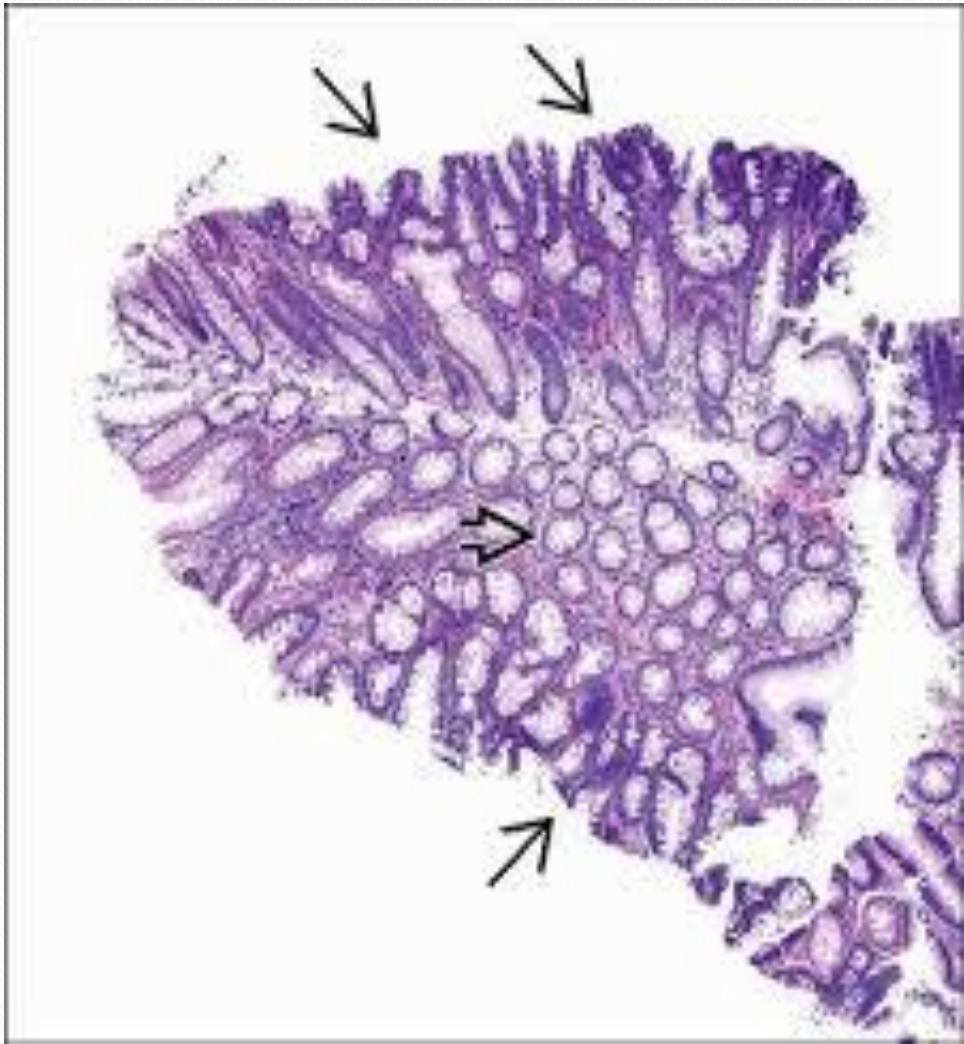
LOH---Loss of the normal, functional allele at a heterozygous locus

Advanced adenoma

- Villious adenoma of any sizes.
- Any adenoma of >1cm size.
- With/ without high grade dysplasia.

Predictors of incident of advanced adenoma-

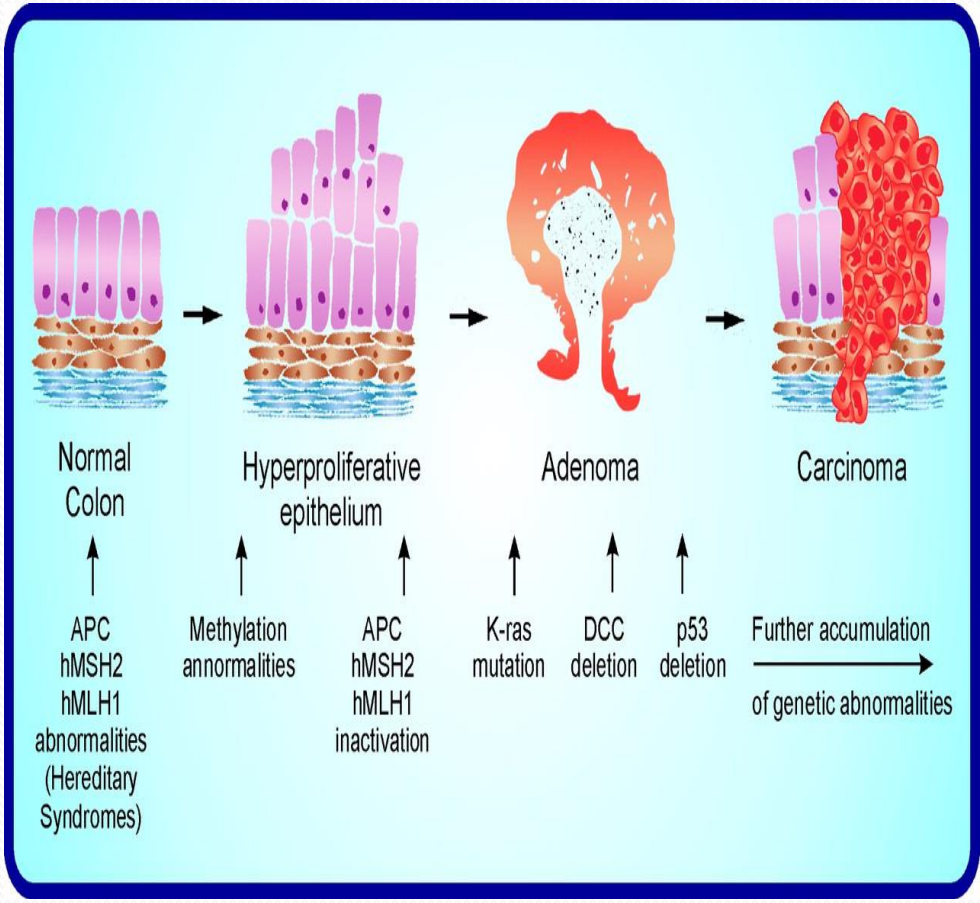
- ≥3 polyps at initial colonoscopy.
- Proximal location of adenoma.
- Age > 60 yrs.
- Family H/O parent with CRC.



Pathogenesis:

- Starts with a single CR epithelial cell---inactivation tumor suppressor *APC* gene on 5q occur very early in the process.
- *k-ras* oncogene—Mutation leads to an "intermediate" adenoma.
- *DCC* gene mutation leads to advanced adenoma.
- In 75% of CRC-- mutation in p53 gene.

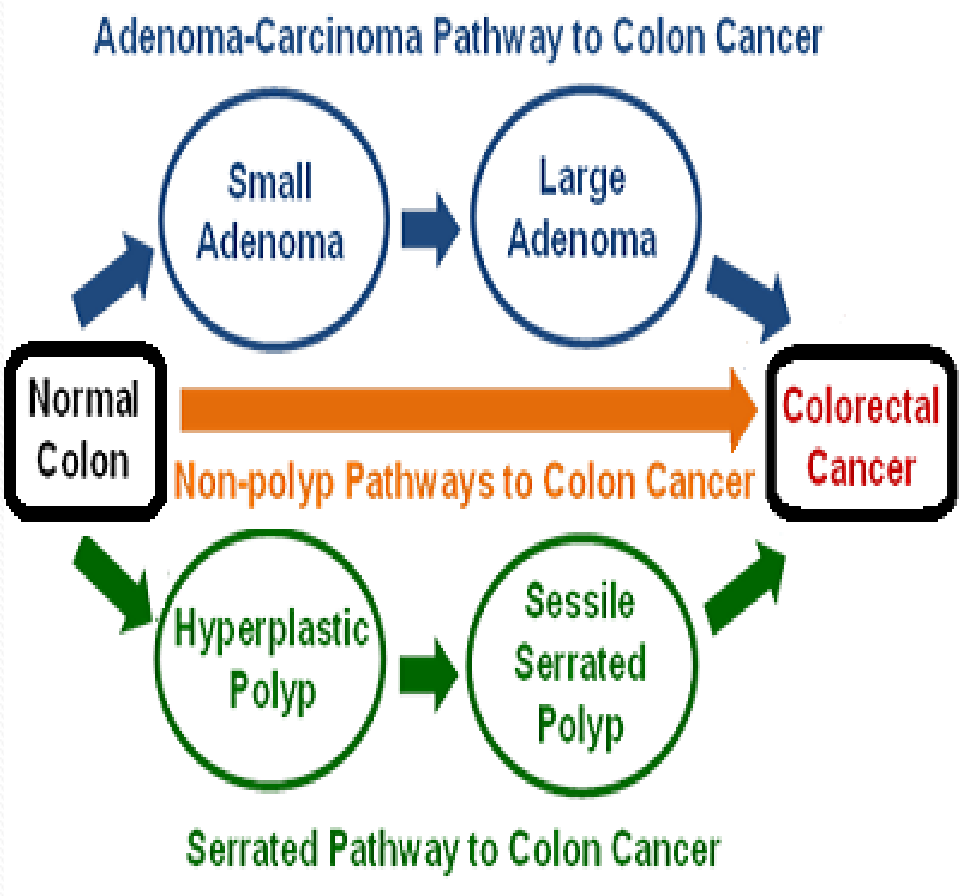
The accumulation of some or all of these molecular abnormalities is associated with invasive colorectal cancer.

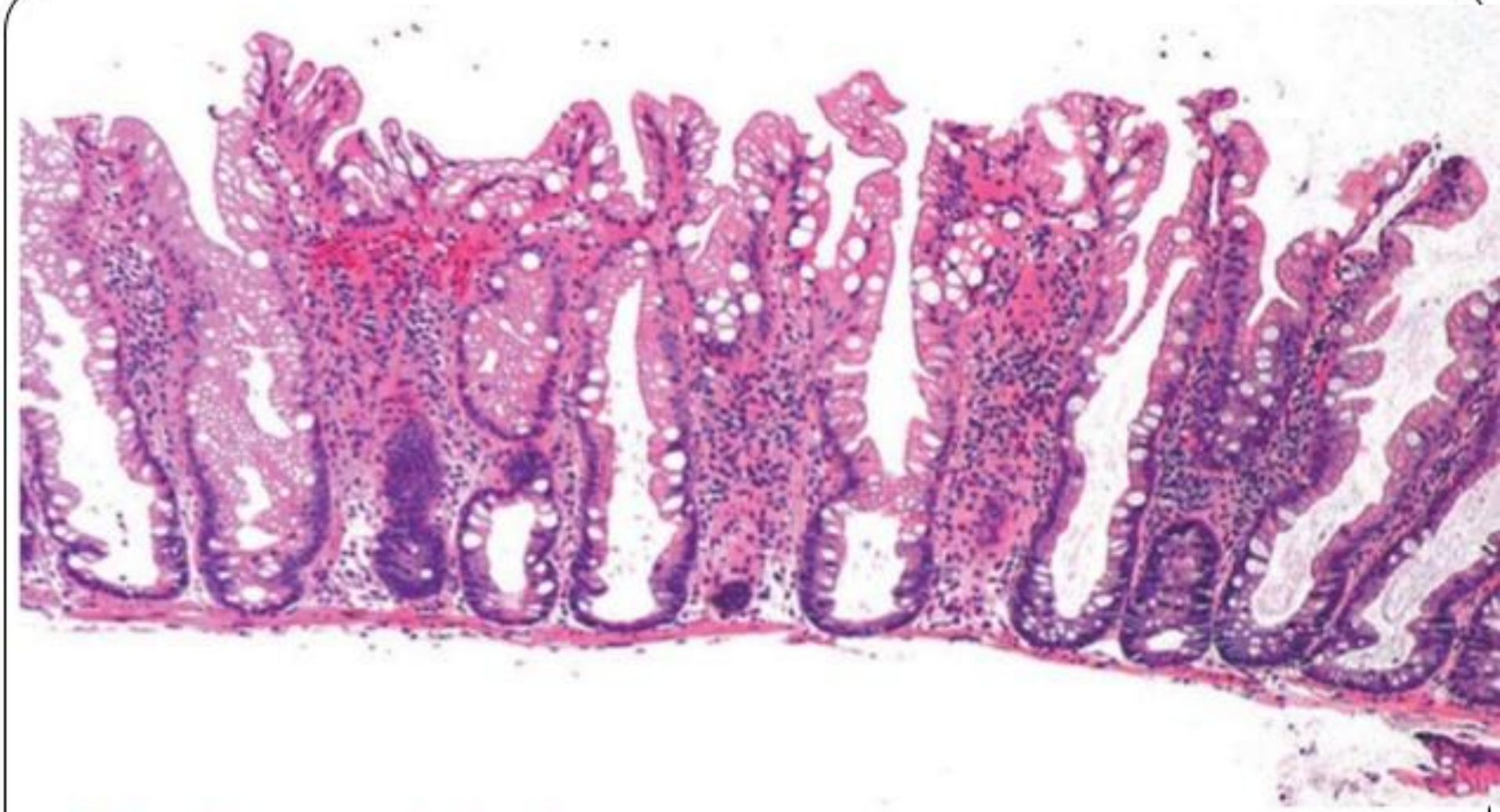


Serrated pathway syndrome

Serrated neoplasia" pathway—

- 10–15% of sporadic CRC.
- Serrated polyps progress to cancer faster than do adenomas.
- Characterized by showing MSI.
- Morphologically and pathologically similar to the MSI cancers associated with the germline MMR mutations seen in HNPCC.
- The adenomas are more likely the SSAs.





- Sessile serrated adenoma:Superficial resemblance to hyperplastic polyps. Hyperserration and prominent mucin cells at the base of the crypt,crypt dilatation.

Tumor suppressor genes

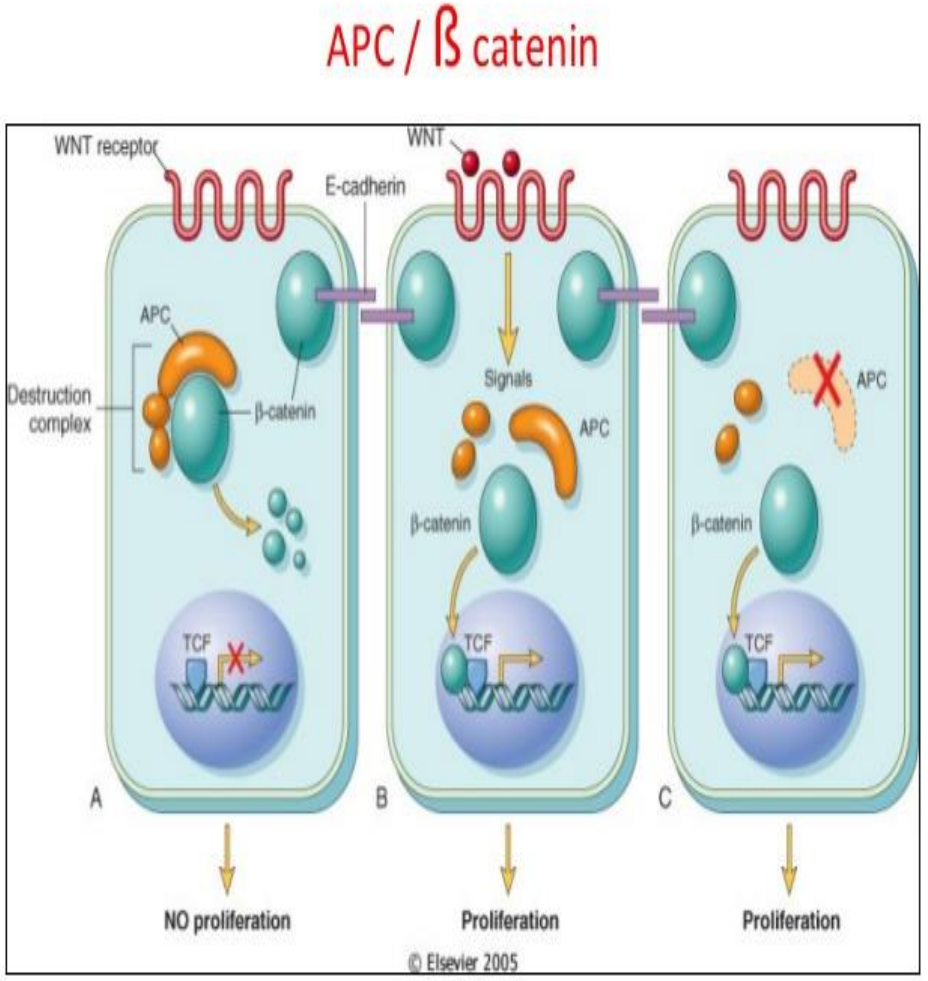
- Inhibit cellular proliferation or
- Promote apoptosis.

Number of tumor suppressor genes-

- *APC*,
- *DCC*,
- *p53*, and
- *MCC* genes.

APC

- On the 5q.
- Gatekeeper gene—as its mutation---initiators of disease.
- In 50% of sporadic adenoma.
- 75% of sporadic CRC.
- FAP---germ line mutation in the *APC* gene.

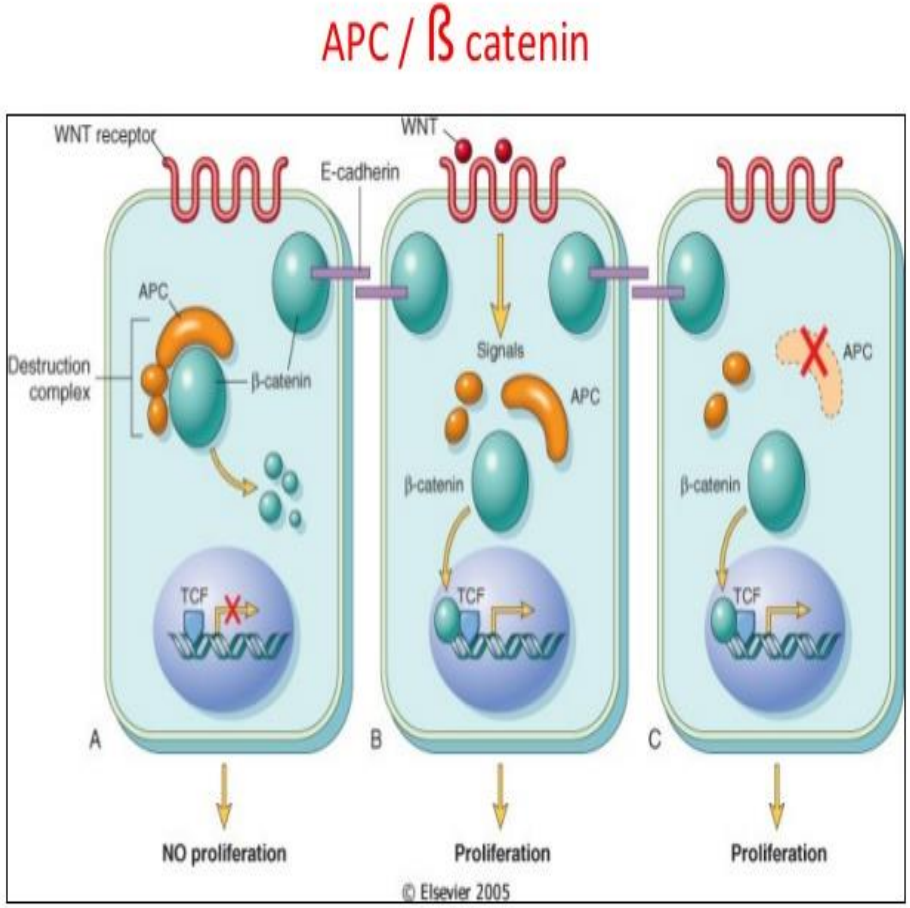


APC protein

- Binds ß-catenin intracellularly that inhibits ß-catenin function.

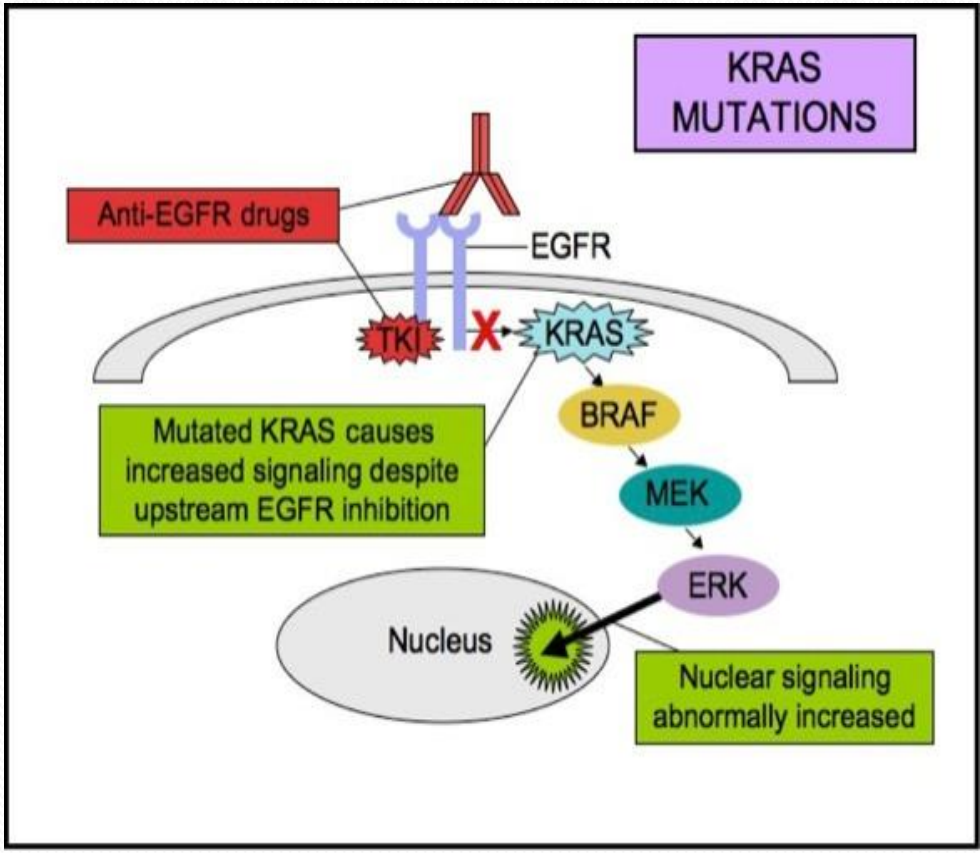
- Altered *APC* protein---increased functional levels of ß-catenin leads to
  - cell proliferation, and
  - enhances cell-to-cell adhesion,
  - limiting cell migration.

Hyperproliferating cells accumulate & result in aberrant crypt foci, the earliest phase of colorectal neoplasia.



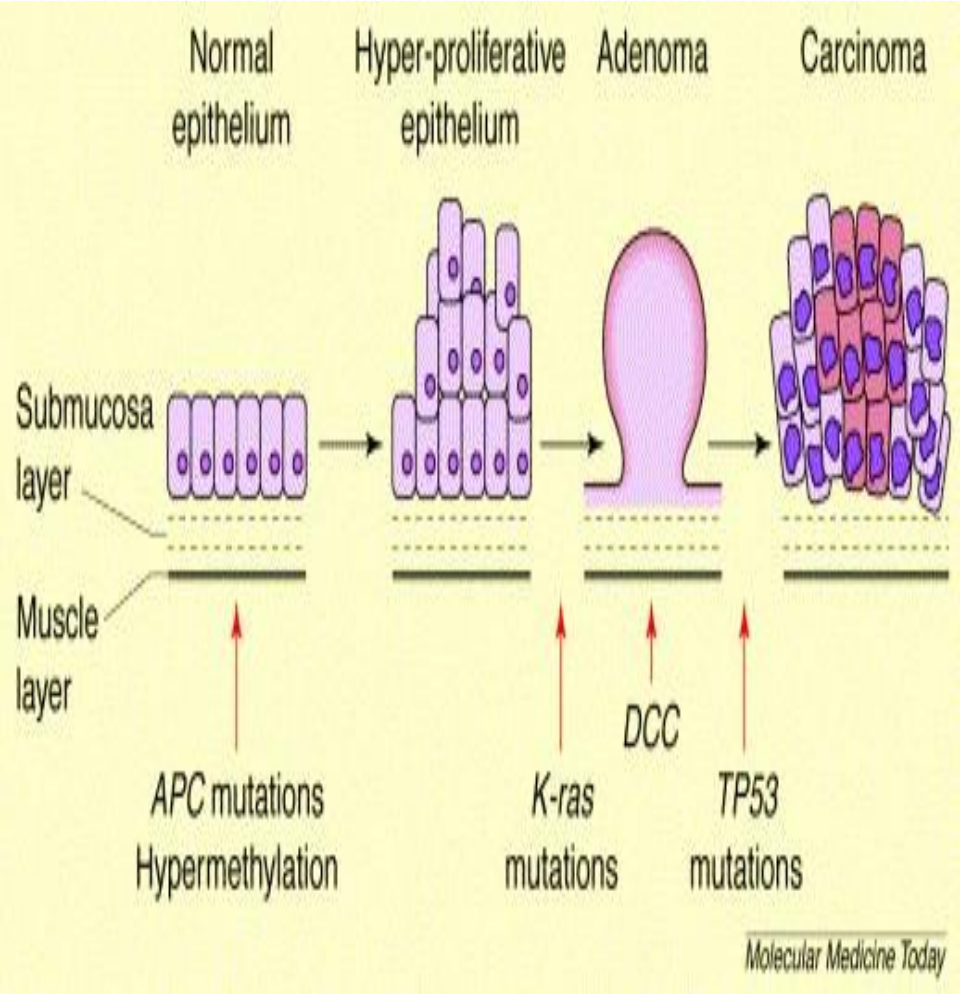
KRAS

- *Oncogene*
- Drive uncontrolled cell growth.
- Mutant KRAS---upstream blockage of EGFR is not effective in blocking MAPK.
- In nearly 40 % of CRC.
- Should be tested for KRAS mutation if consider anti-EGFR therapy.
  - (e.g., cetuximab), and such therapy should not be instituted if the patient is found to have mutated KRAS CRC.



The “deleted in colorectal cancer” (*DCC*) gene

- on 18q.
- in the majority of CRC.
- The gene product is important in cell–cell adhesion, and therefore inactivation of *DCC* may –
  - Enhance metastatic potential of CRC .
  - *DCC*-positive tumors may have a better prognosis than those with *DCC*-negative (mutated) tumors.

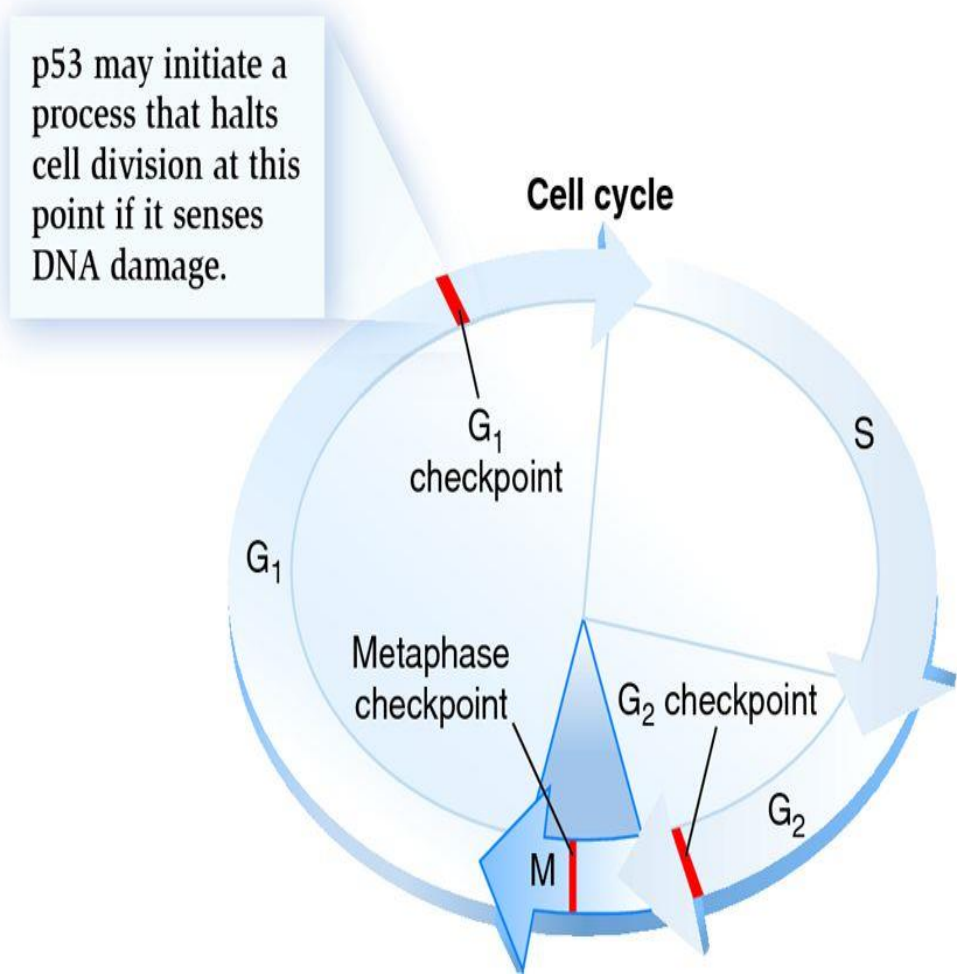


p53

- Tumor suppressor--stops the cell cycle in G1/S phase to allow mutations or replications errors to be repaired.
- If the damage cannot be repaired--may induce apoptosis.
- Present in 75 % of invasive CRC.

Clinical significance:

- Lower survival rate in patients with *p53* negative.



The MMR system

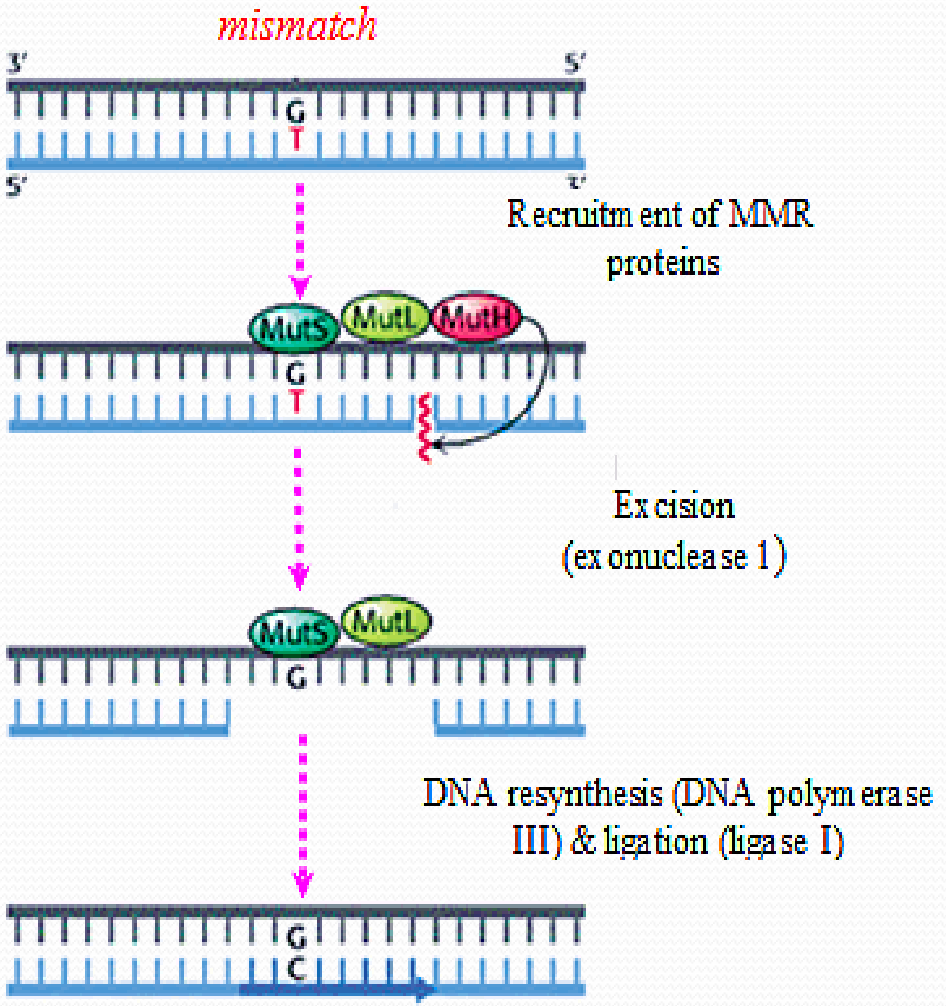
MMR genes function as spell checkers –

- base-pair mismatches are identified,
- excised, and
- correct sequence is synthesized and replaced.

Lack of MMR function results in---

- Accumulation of mutations in these genes lead to adenoma and cancer formation.
- Inherited mutations in one of the DNA MMR genes result in Lynch syndrome

Defects in the MMR system are identified by the detection of microsatellite instability.



Microsatellites

- Microsatellites are noncoding segments of DNA that contain repetitive sequences of 1-4 nucleotides.
- 100s to 1000s of microsatellites in the genome.
- Importance:
  - Microsatellite patterns provide a unique DNA fingerprint.
  - When these errors are not repaired due to MMR deficiency, the length of the microsatellite regions are altered and the fingerprint changes.
- MMR mutations--replication errors accumulate, leading to MSI.

Microsatellite Instability

Ms –Ja-17

PCR can detect microsatellite instability.

The National Cancer Institute recommends the testing of 5 microsatellite sequences to determine the MSI status of a tumor.

- If ≥2 --MSI-high (MSI-H).
- If only 1 MSI-low (MSI-L).
- If no markers are changed, the tumor is microsatellite stable.

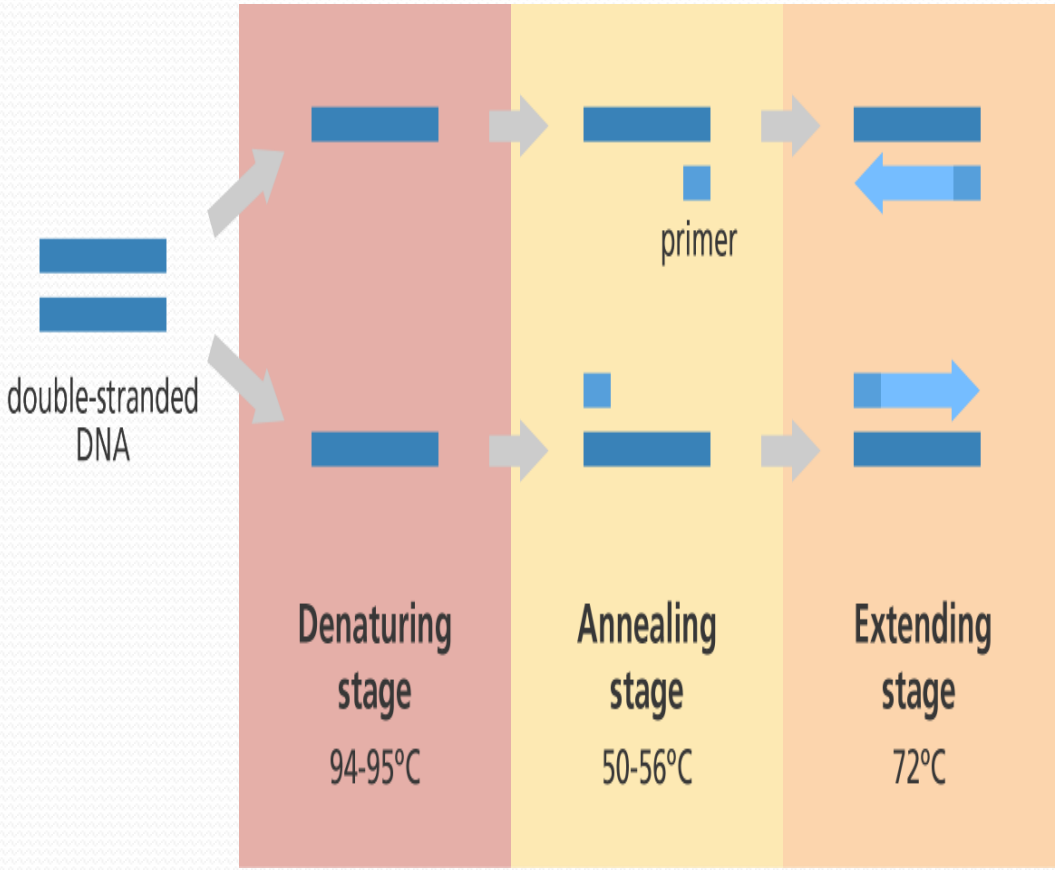
Approx.15% of CRC demonstrate MSI.

Importance:

- MSI-H tumors-->likely to be high grade, right-sided, mucinous, and have TIL.
- MSI tumors---better prognosis but may be less responsive to chemotherapy.

PCR

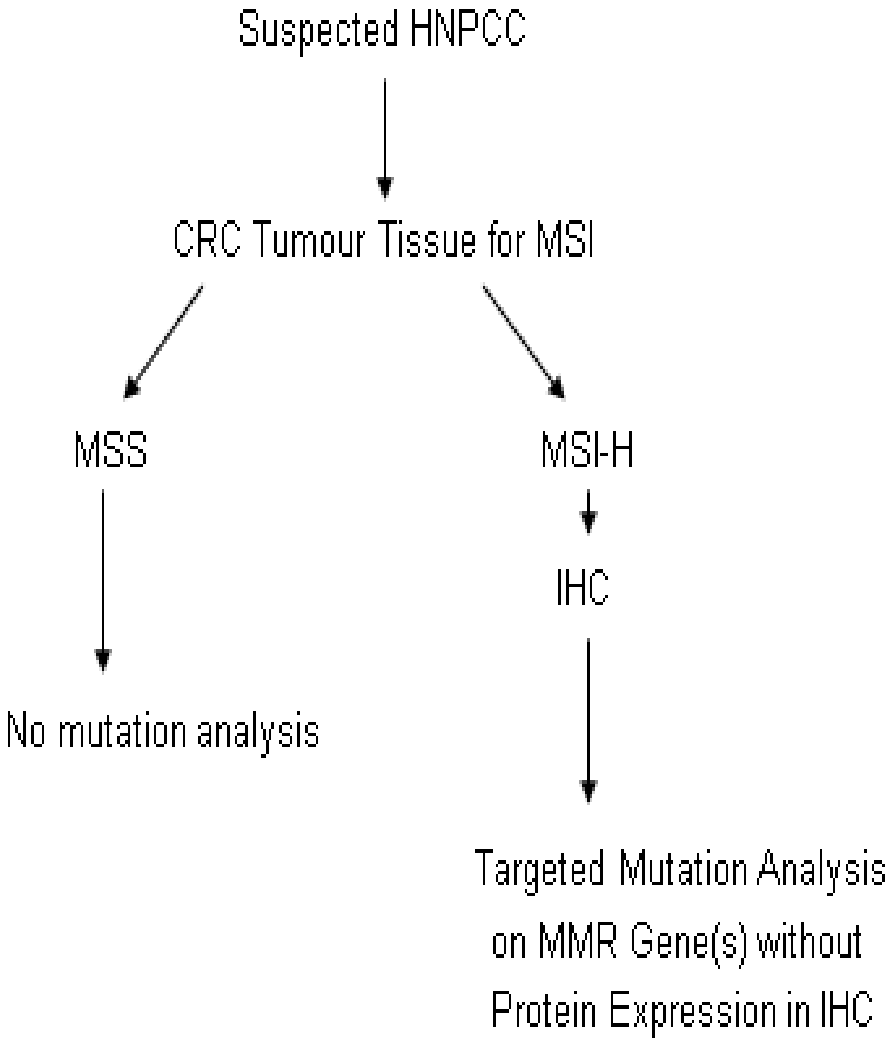
- Technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA to generate thousands to millions of copies of a particular DNA sequence.
  - easy,
  - cheap, and
  - reliable way to repeatedly replicate a focused segment of DNA.



MMR genes

- *MLH1*,
- *MSH2*,
- *MSH3*,
- *MSH6*,and
- *PMS1.*

- Germ line mutations in the *MLH1* and *MSH2* --responsible for >90% of HNPCC,
- Approx. 5–10% of HNPCC are due to mutations in the *MSH6* gene.
- Germ line mutations in other MMR genes are rare.



MYH

- DNA repair gene.

- responsible for some cases of *APC* mutation-negative FAP.

Genetic testing

- **DNA testing**

- Biochemical tests for the possible presence of genetic diseases, or mutant forms of genes associated with increased risk of developing genetic disorders.

Identifies changes in—

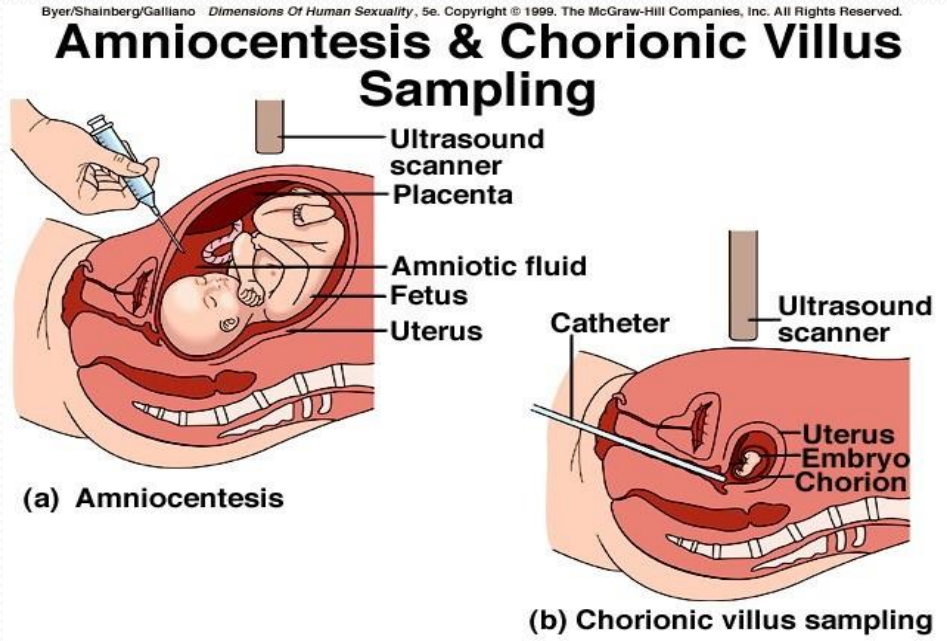
- Chromosomes
- Genes, or
- Proteins.

Medical procedure

- Done as part of a genetic consultation.
- Medical geneticist, genetic counselor, primary care doctor, or specialist can order the test .
- Obtain informed consent.

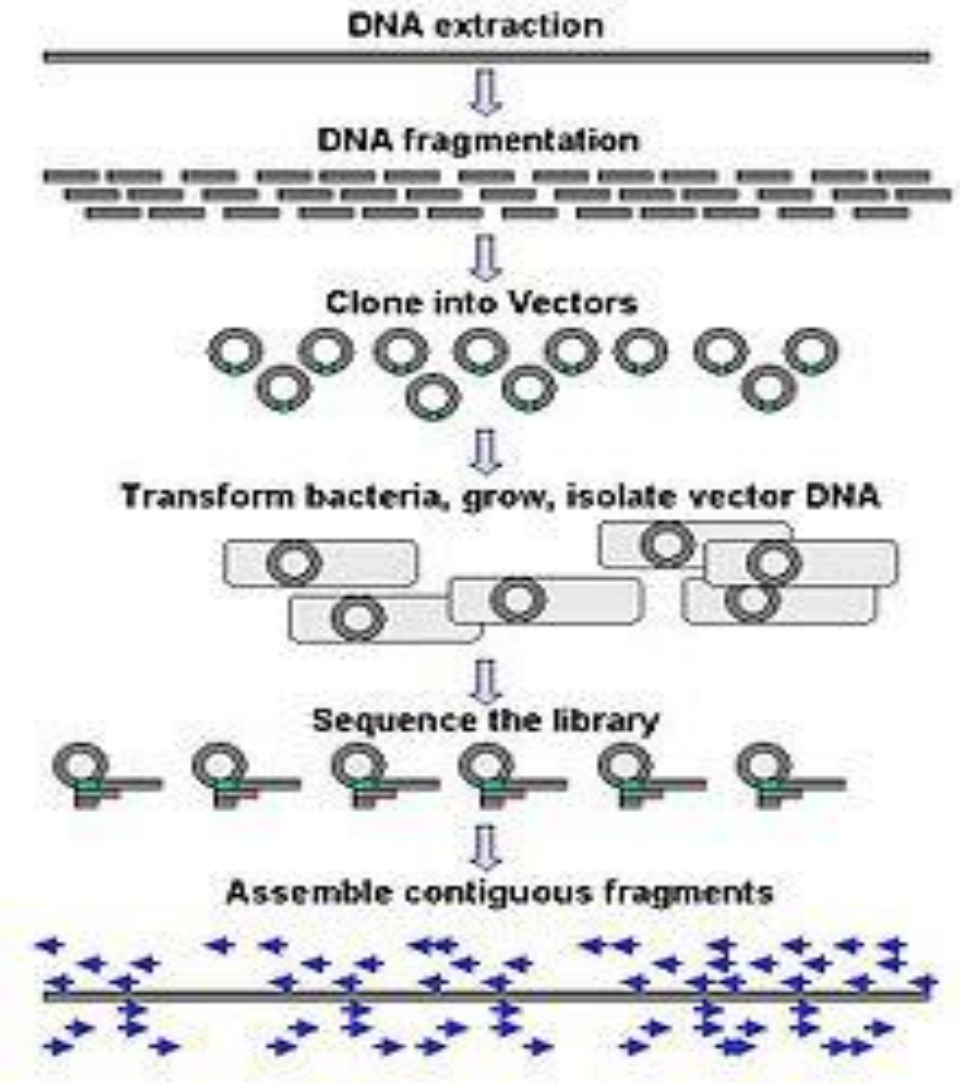
Sample-blood, hair, skin, amniotic fluid, buccal smear.

- The sample is sent to a laboratory---specific changes in chromosomes, DNA, or proteins, often using DNA sequencing.
- Test results to a person's doctor or genetic counselor.



DNA sequencing

- Process of determining the precise order of nucleotides within a DNA molecule.
- Includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA.



Genetic testing

Limitations :

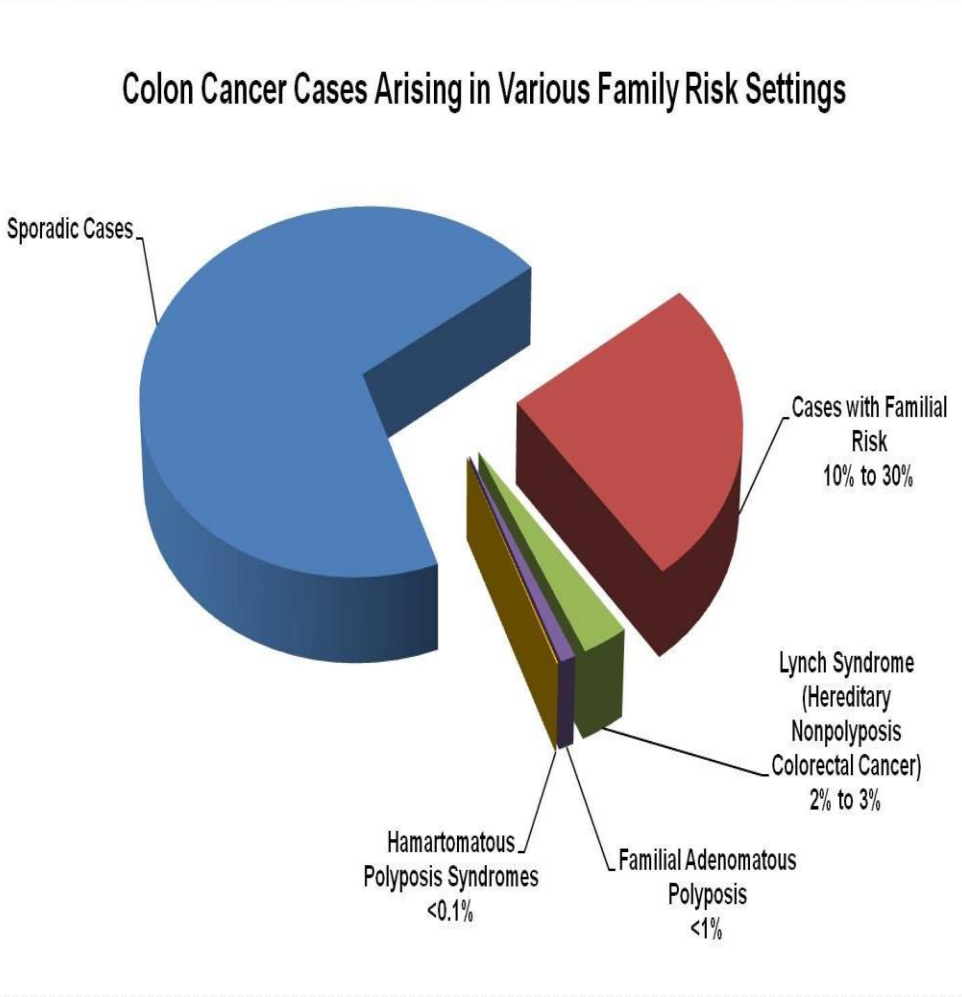
- Costly and time-consuming
- Many insurance may not cover the costs.
- Targeted therapy---also expensive.
- Not enough known yet to make personalized cancer screening and prevention.
- Don‘t know everything about the genetic changes that occur in a cancer cell and how some of these new cancer treatments work.

Risks

- physical risks-- very small,
- prenatal testing--risk of miscarriage.
- may feel angry, depressed, anxious, or guilty about their results.
- tension within a family.
- genetic discrimination in employment or insurance
- Affect ability to purchase insurance or find a job.
- accidental findings while looking for something else.[

CRC

- Environmental -65%
- Hereditary-35%.
  - Nonsyndromic-30%
  - Syndromic-5%
    - Polyposis-2%
    - Nonpolyposis-3%.



Immunotherapy, Tumor Vaccines, and Gene Therapy

Goal---

- Stimulate body's immune system to improve host defense against growing tumors.

Nonspecific immune stimulation—

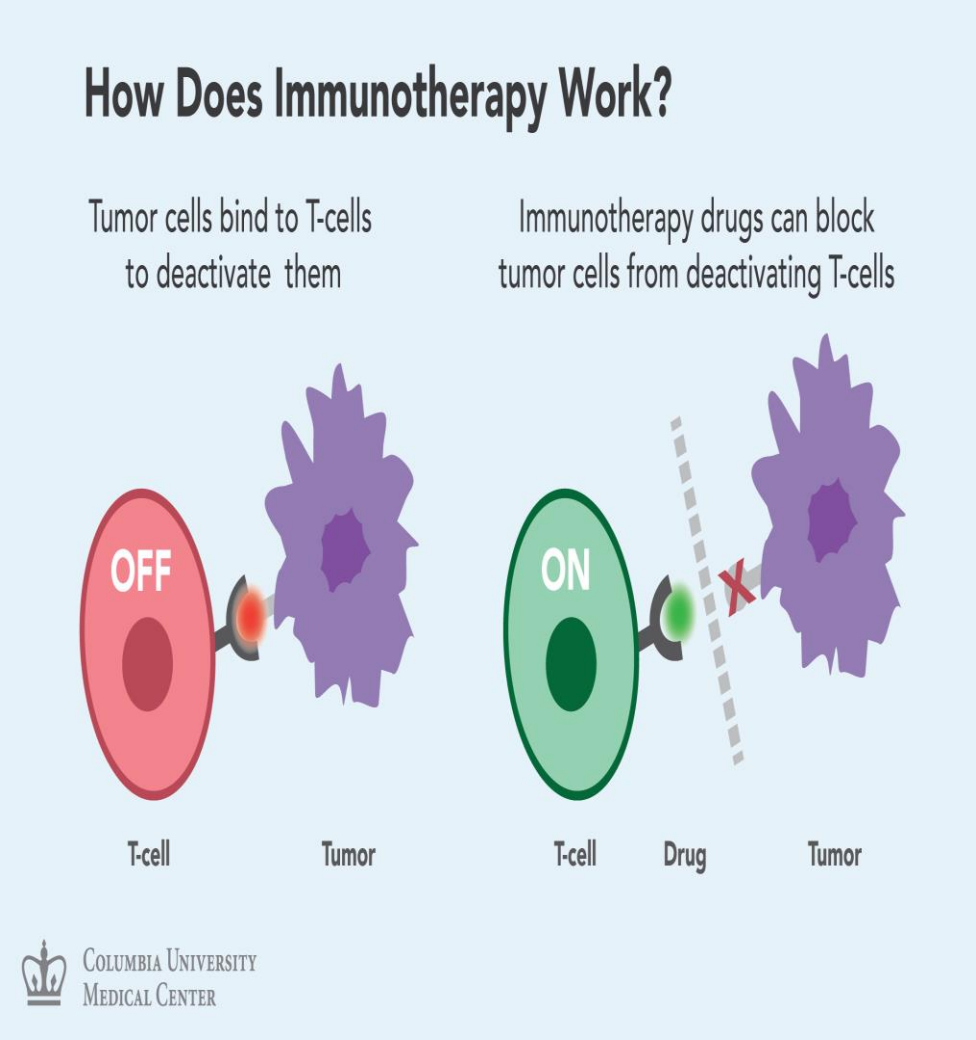
- BCG) and
- Cytokines (e.g., IL-2)

specific immune stimulation-

- target against CRC expressed antigens.

Clinical trial—

- Surgery ---vaccination with autologous irradiated tumor+ BCG---
  - immediate post-op-
  - booster 6 m postop—improve recurrance free survival & overall survival
- Further study needed to define the role.



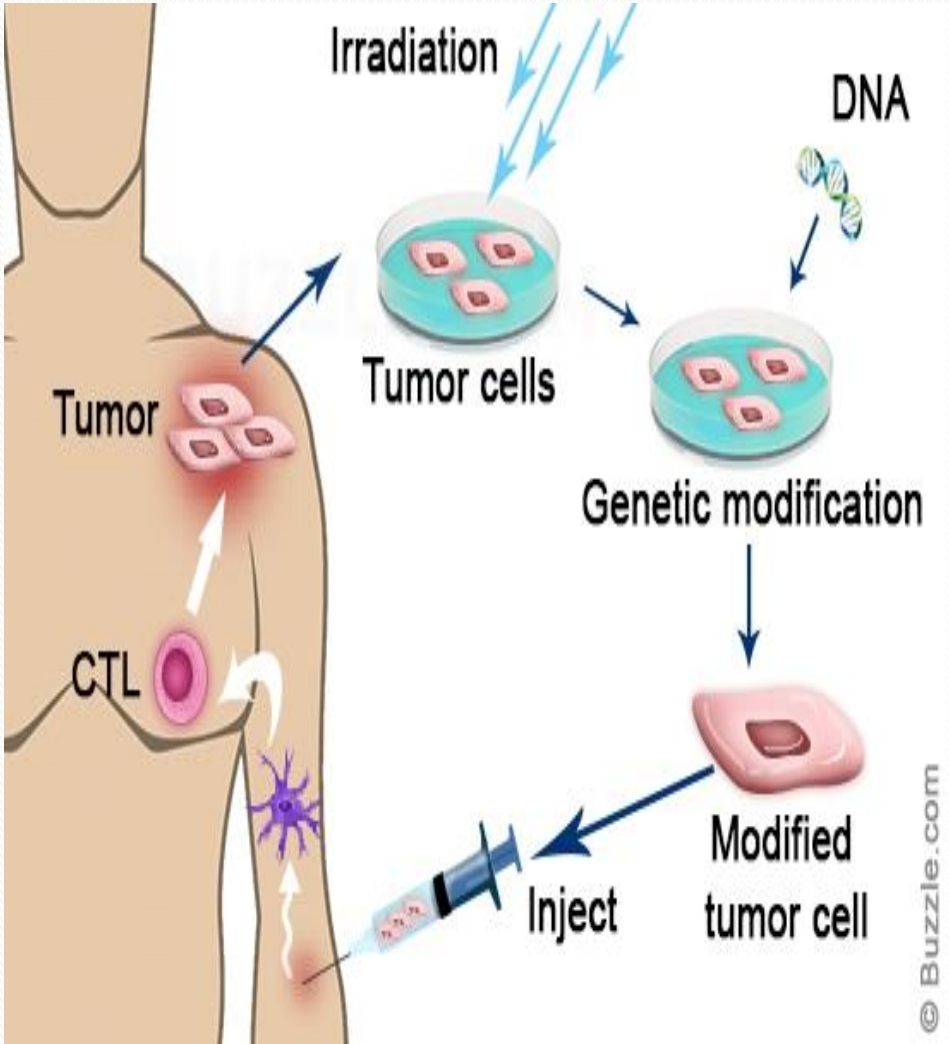
Tumor vaccine

Vaccines stimulate the immune system to-

- recognize and
- act specifically against these tumor-expressed antigens, through -
  - humoral or
  - cellular pathway.

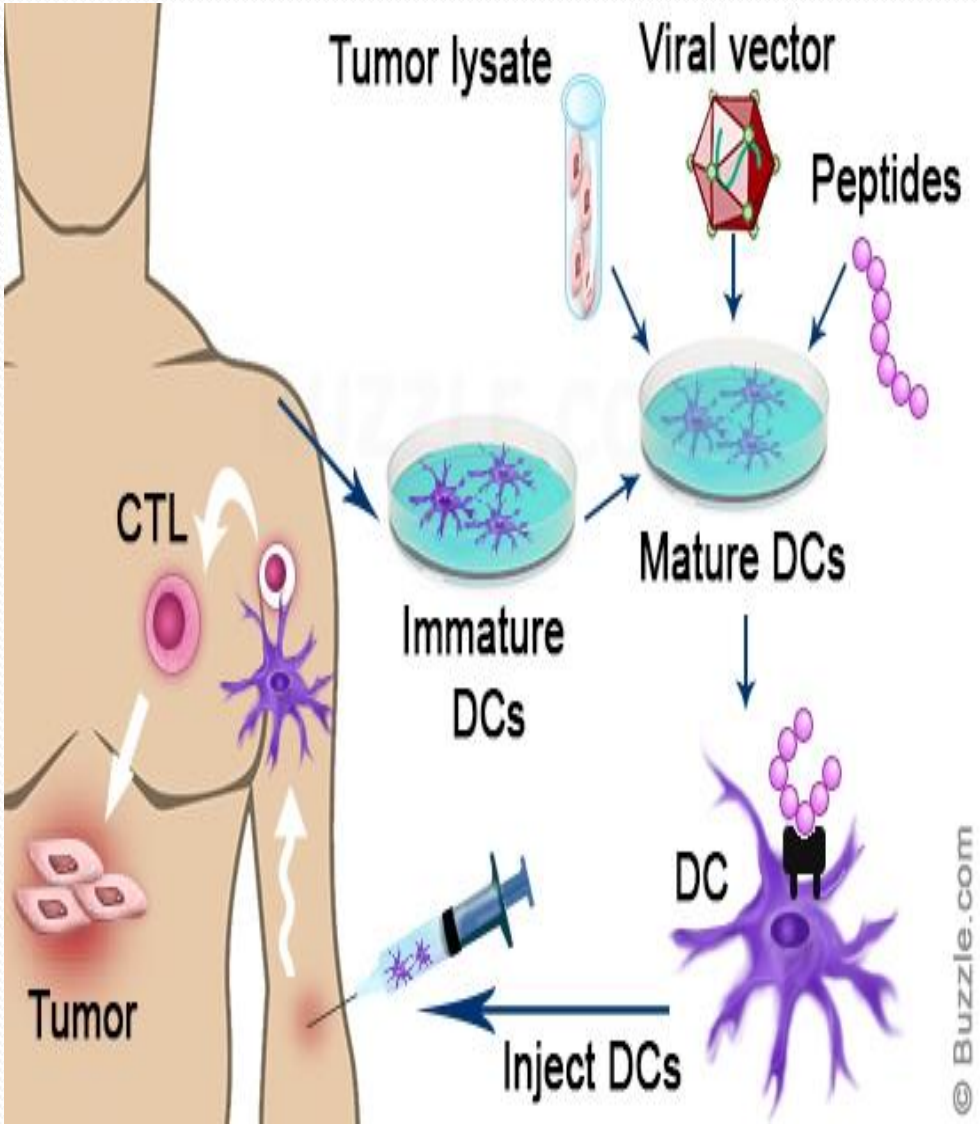
Types-vaccines based on

- Whole CRC cells,
- virus modified tumor cells,
- gene-modified tumor cells,
- tumor antigen-derived peptides,
- tumor cell lysates, proteins or carbohydrates,
- monoclonal antibodies,
- dendritic cell-based vaccines.



Result

- Promising results---in some animal models and phase I and II studies.
- Survival correlates with the patient's immune response to vaccination.



Gene therapy

Principle:

Transferring genetic material into target cells, which allow for-

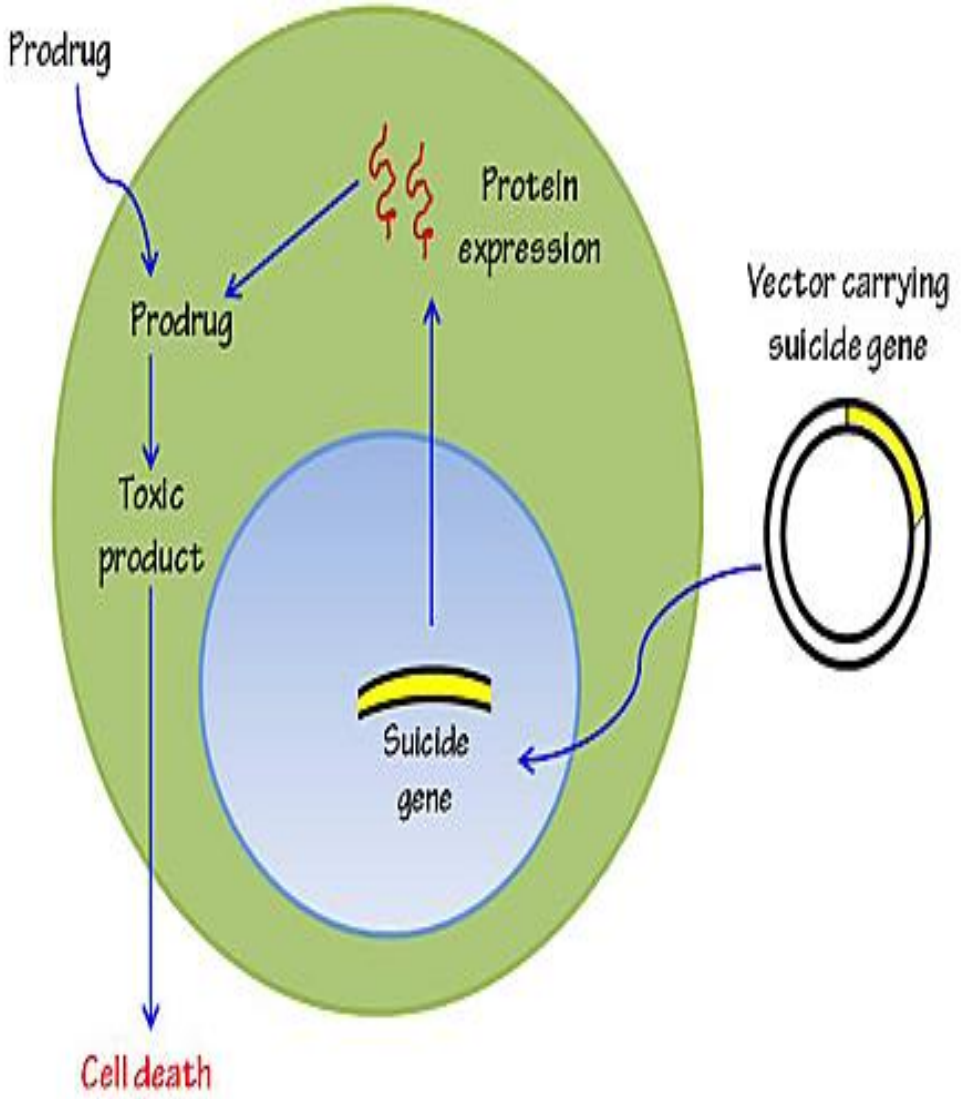
- correction of genetic defects in tumor suppressor genes,
- inactivation of oncogenes, or
- insertion of treatment-sensitizing genes (such as drug-converting enzymes) or
- Insertion of “suicide genes” into the colorectal cells.

Example:

- Correction of p53 mutations,
- inactivation of k-ras gene product
- delivery of pro-drug-converting enzymes are currently being studied.

Future:

- Long term clinical usefulness remains to be defined.



Thank You

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