

GRAND ROUND PRESENTATION

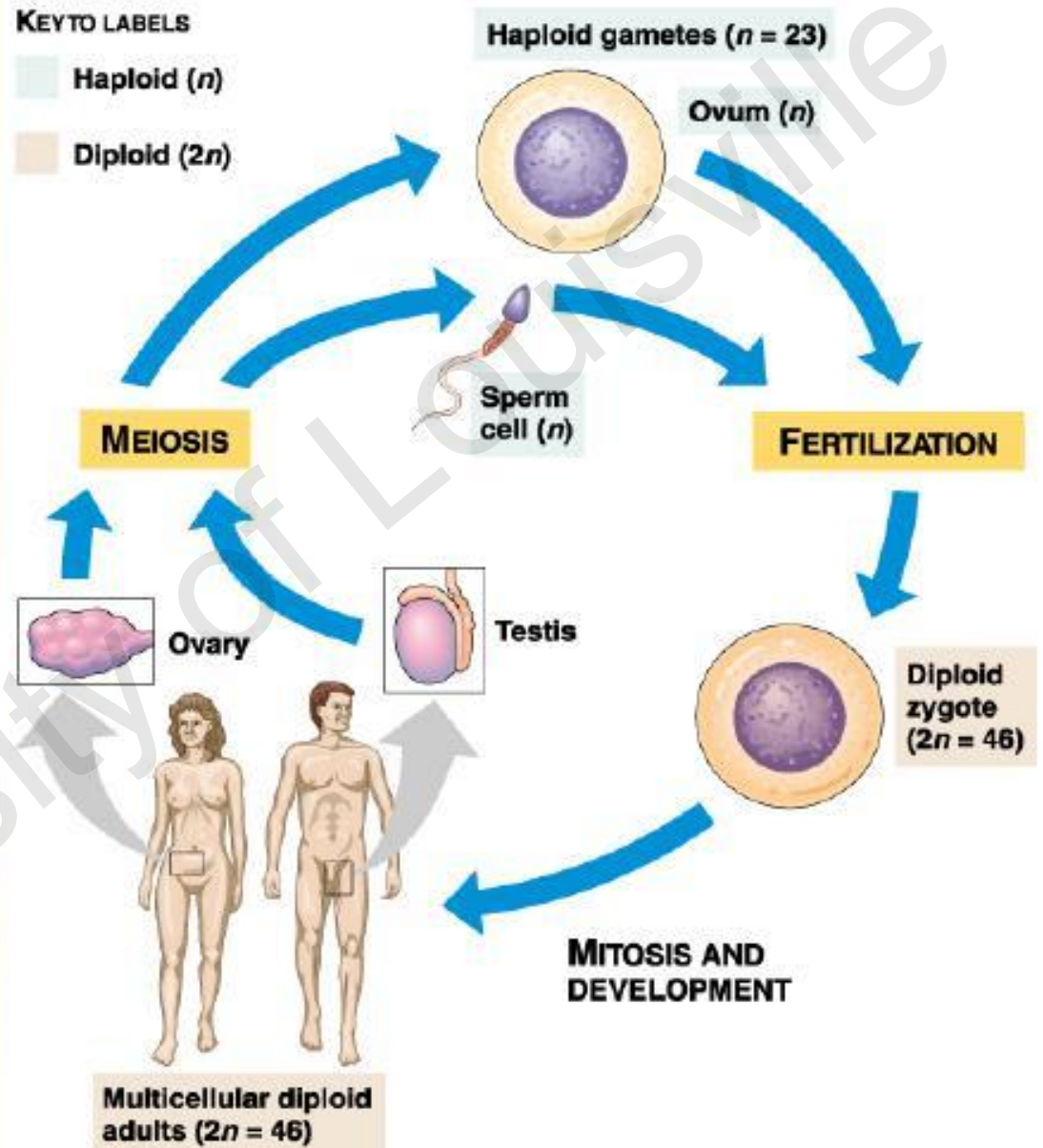
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Carcinogenesis:

Its Correlation in Gastrointestinal Cancers

The Human Life Cycle



In adult humans cell are continuously lost

Cells division = Cell loss

-two trillion cell divisions occur in an adult human every 24 hours (about 25 million per second).

Cancer

Cells division =X= Cell loss

Abnormal rate of Cell division
> Normal Cell loss

Abnormal rate of Cell death
< Normal Cell division

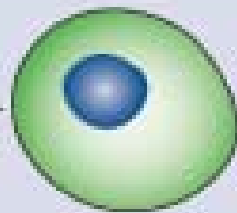
Carcinogenic stresses:

- External agents (e.g., reactive chemicals and UV light)
- Internal agents (e.g., byproducts of normal cellular metabolism, such as reactive oxygen intermediates)

CELL CYCLE

sequence of events by which a growing cell duplicates all its components and divides into two daughter cells, each with sufficient machinery to repeat the process.

New daughter cell



Mitosis (cell division)

Begin cycle

Cyclin B/cdc2

M

G₂

Cyclin A/cdc2

G₁

Cyclin D/cdk4,6

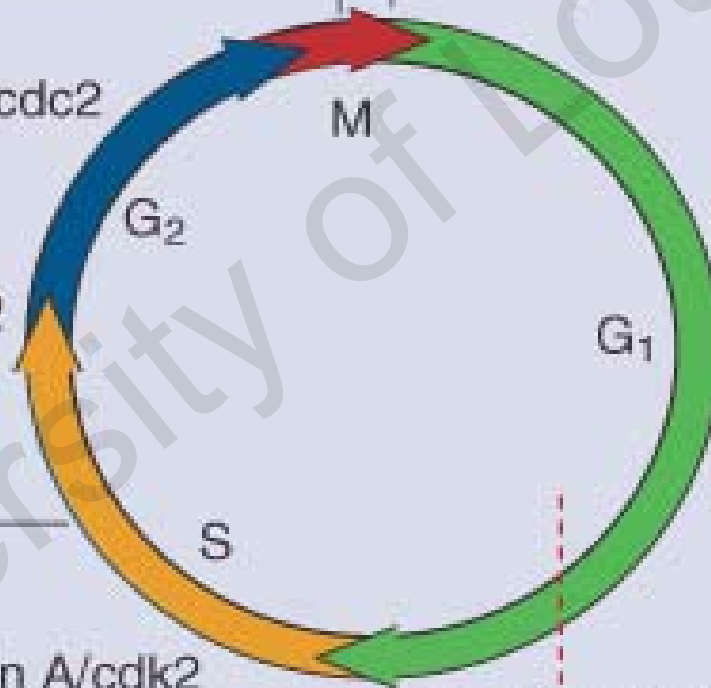
DNA synthesis
(doubling of DNA)

S

Cyclin A/cdk2

Cyclin E/cdk2

Restriction point



S phase- high-fidelity duplication of the three billion bases of DNA

M phase- proper segregation of duplicated chromosomes during mitosis

G or "gap" phases -Before and after S phase and M phase

G1 phase-

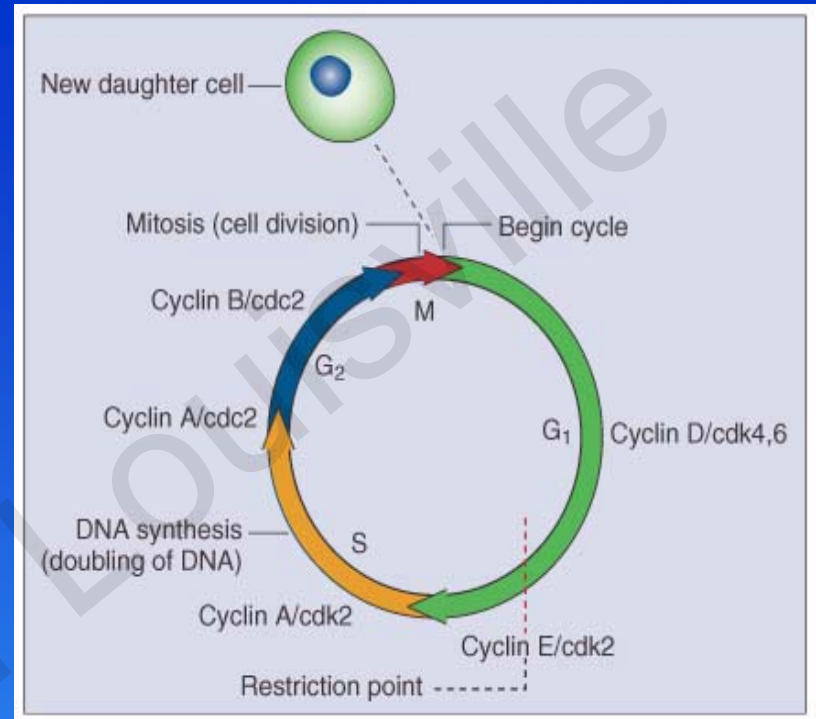
period after mitosis
when cells prepare
for DNA synthesis

G2 Phase-

period after DNA
synthesis when the cell
prepares for successful mitosis.

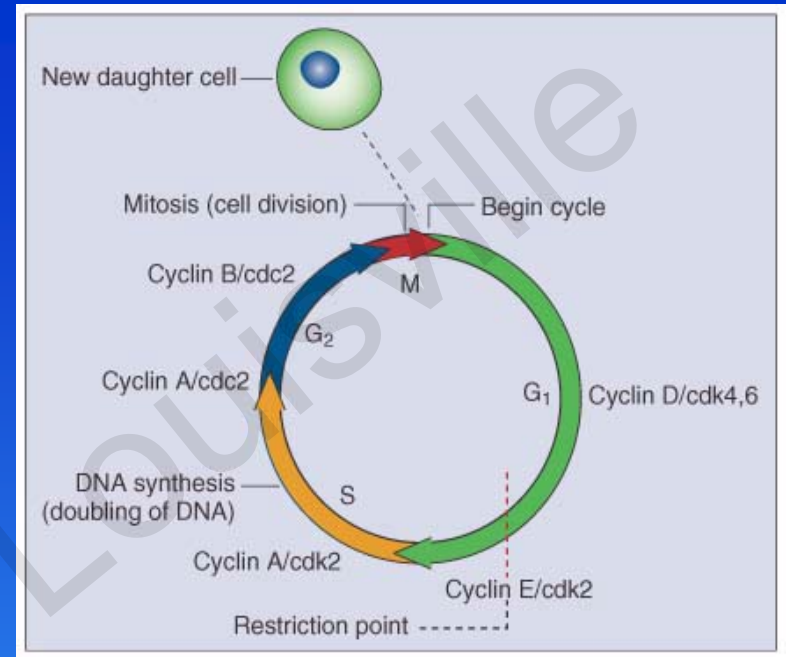
G0 Phase-

Dormant non-proliferative phase
of cells with proliferative capability



G1 phase-

- cells make decisions about the commitment to replicate & complete the cell division cycle.



- If the cellular milieu is favorable for proliferation, a decision to enter S phase is made at a time in mid-to-late G₁, called the *restriction point*

MECHANICS OF THE CELL CYCLE ENGINE

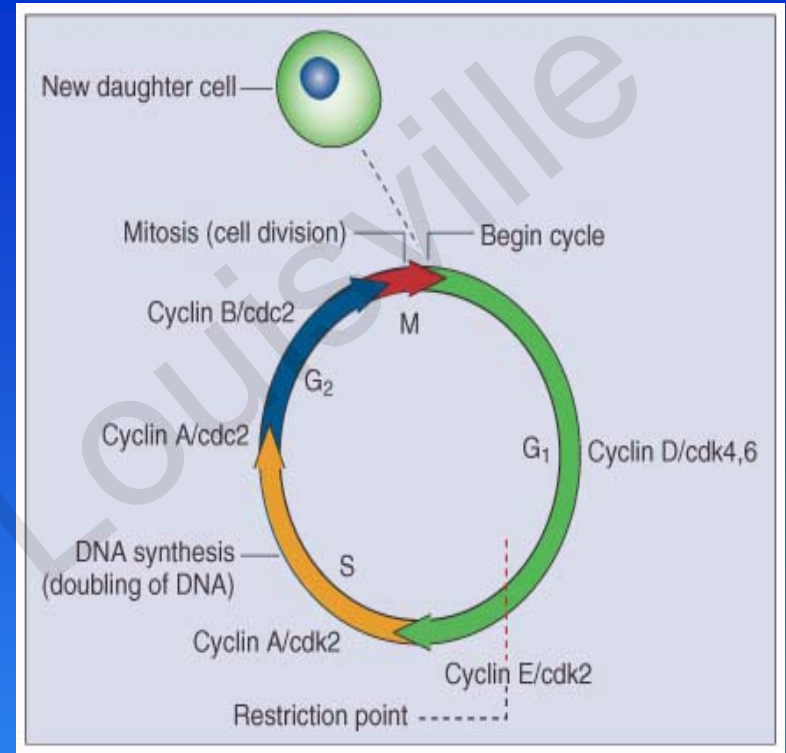
-Mediated by the activation of a highly conserved family of protein kinases, the cyclin-dependent kinases (cdks).

-Activation of a cdk requires binding to a specific regulatory subunit, termed a **cyclin**.

-Cyclins were so named because of their fluctuating levels through the cell cycle.

- The **cyclin/cdk complexes** are the central cell cycle regulators, with each complex controlling a specific cell cycle transition
- To date, at least nine cdks and 15 cyclins have been described.

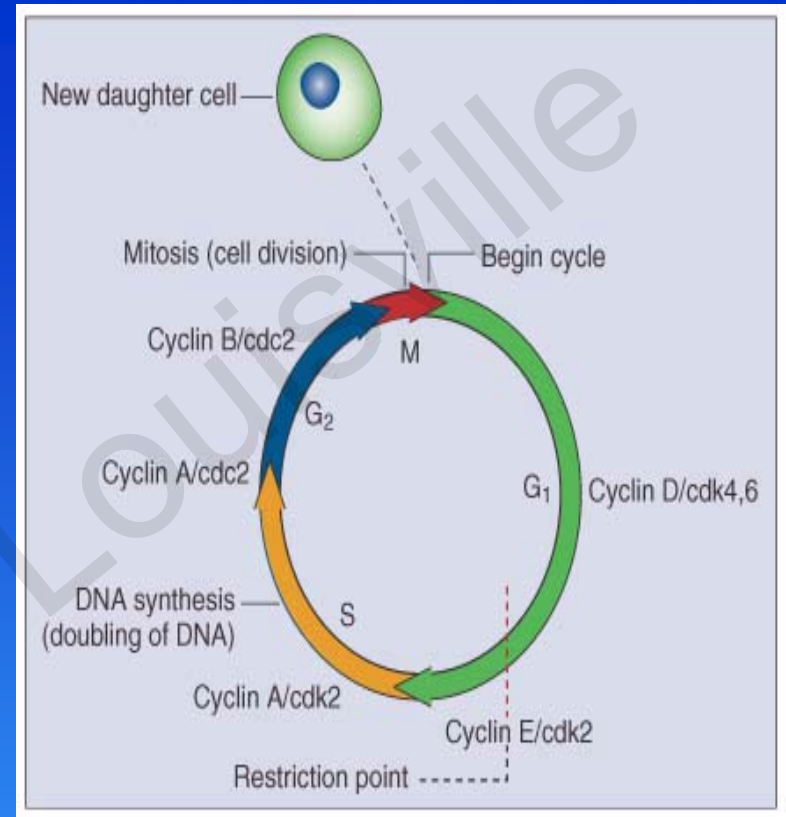
- **Extracellular stimuli**
- e.g. growth factors and hormones,
- elevate **D-type cyclins** (cyclins D1, D2, and D3)
- which bind to and activate cdk4 and cdk6
- stimulate quiescent cells to enter the cell cycle or proliferating cells to continue proliferation i.e. **G1 to S**.



-After elevation of D-type cyclins and activation of cdk4 or cdk6 in G1,

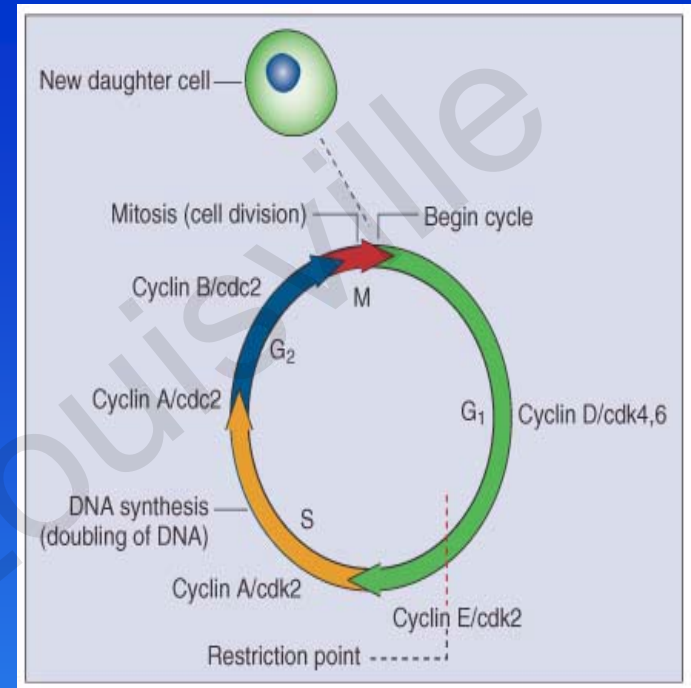
- cyclin E levels increase and bind cdk2 in cell.

-The cyclin E/cdk complexes regulate the transition from G1 into S phase.



Cyclin A

- induced shortly after cyclin E
- involved in the regulation of S phase entry, and it is also important in G₂ and M phases.



B-type cyclins-

- entry into mitosis from G₂

Regulation of the cell cycle

Regulation of CDKs

1) Phosphorylation and dephosphorylation events

Phosphorylation - cyclin-activating kinase

Dephosphorylation - members of the Cdc25 family of dual-specificity protein phosphatases.

Regulation of the cell cycle

Regulation of CDKs

2) Proteins called **cdk inhibitors**.

-INK4 family members:

p16INK4A , p15INK4B , p19INK4D ,
and p18INK4C

-Cip/Kip family members:

p21Waf1/Cip1 , p27Kip1 , and
p57Kip2 .

Regulation of the cell cycle

The levels, subcellular localization, and activity of these enzymes / inhibitors can be regulated by

- cell stress
- growth inhibitory signaling pathways.

Regulation of the cell cycle

Cell cycle checkpoints

- signaling pathways at key transitions during cell cycle
- monitor the successful completion of one phase before proceeding to the next phase.
- **Controlled by** parameters such as:
 - growth factor availability
 - cell mass accumulation

Regulation of the cell cycle

Cells can arrest at cell cycle checkpoints temporarily to allow for any of the following:

- The repair of cellular damage
- The dissipation of an exogenous cellular stress signal
- The availability of essential growth factors, hormones, or nutrients

Regulation of the cell cycle

Best studied of the cell cycle checkpoints are those that monitor the status and structure of chromosomal DNA during cell-cycle progression.

Regulation of the cell cycle

“DNA Structure Checkpoint”

- most proximal signaling elements = sensor proteins that scan chromatin for DNA abnormalities e.g breaks
- translate these stimuli into signals that modulate specific downstream target proteins that are involved in DNA repair and cell cycle arrest or cell death.

Regulation of the cell cycle

- How DNA lesions are "sensed" is not well understood
- Key transducer proteins include human ataxia telangiectasia mutated (ATM) and ATM-related (ATR).

Regulation of the cell cycle

- just a few breaks in the cell's genome results in instantaneous activation of ATM protein throughout the cell
- Two effectors, kinases Chk2 and Chk1, activated by phosphorylation in response to DNA damage and replication disturbances.

Regulation of the cell cycle

After IR or UV exposure

- Chk2 and Chk1 are activated
- rapidly phosphorylate Cdc25A
- inhibition of cdk2

Regulation of the cell cycle

P53

- a tumor suppressor protein
- Involved in checkpoint signaling in cells that are in G1 transition before the restriction point
- The most frequently altered cell cycle checkpoint signaling molecule

Regulation of the cell cycle

P53

- In normal, nonstressed cells, p53 protein is maintained at low steady-state levels
- After exposure of cells to stress (including DNA damage or oxidative stress), p53 levels increase significantly by phosphorylation .

P53

Actions

- 1) regulates the gene for the cdk inhibitor p21WAF1/Cip1
- 2) p53-dependent genes play multiple roles in apoptosis
 - several proapoptotic Bcl-2 family members are transcriptionally activated by p53
 - p53 promotes death receptor pathways

CELL CYCLE DYSREGULATION IN HUMAN CANCERS

- occur in the majority of human tumors
- include loss or mutation of the RB tumor suppressor, overexpression of cyclins, cdks, and Cdc25 phosphatases, and loss of expression of cdk inhibitors.

...most frequently altered cell cycle checkpoint signaling molecule is the p53 tumor suppressor

CELL LIFE AND CELL DEATH

Apoptosis

- One of the safeguards that prevents excess cell accumulation
- A cell-intrinsic program that can induce cell death
- appear to be impaired in virtually all tumors, suggesting that this is a required step in carcinogenesis

- concept that genes regulating cell death could play a role in cancers arose in the mid-1980s,
- a translocation commonly found in follicular lymphoma, between chromosomes 14 and 18.....

....brings a region on chromosome 18 called breakpoint cluster region 2 (Bcl-2) into close proximity with the immunoglobulin heavy chain enhancer on chromosome 14, resulting in overexpression of the Bcl-2 gene

-the Bcl-2 gene product promotes oncogenesis by inhibiting the normal programmed cell death of B cells, resulting in the failure to eliminate the clonal B cells

(The Bcl-2 family includes proteins with both antiapoptotic and proapoptotic function)

Process of apoptosis

- cell-intrinsic suicide program without inducing an inflammatory reaction.
- controlled through the activity of caspases

Caspases :-

- **Initiator**- caspases 8 and 9
activated by cellular signals and subsequently activate effector caspases
- **Effector**- such as caspases 3,6 & 7
set into motion the degradation of cellular components.

The caspase cascade is initiated through specific cellular signals.

- 1) The extrinsic, receptor-mediated pathway, or
- 2) The intrinsic, mitochondrial pathway.

Extrinsic, receptor-mediated pathway

- initiated through ligation of specific cell-surface receptors, the death receptors
- include Fas and TNFR1
- activated on binding of ligand Fas ligand (FasL) and TNF, respectively.

The intracellular domains of these receptors contain death domains which, on activation of the receptor, can recruit a **Death-Inducing Signaling Complex (DISC)**, which leads to activation of a caspase cascade.

Intrinsic, mitochondrial pathway

1996, Liu and colleagues demonstrated that cytochrome c, a component of the electron transport chain normally contained in the mitochondrial intermembrane space, could initiate programmed cell death when present in the cytosol

Events that lead to mitochondrial apoptosis

- loss of normal survival-promoting extrinsic signals
- DNA damage
- metabolic stress such as hypoxia and nutrient limitation
- toxins.

ONCOGENES AS TRIGGERS OF APOPTOSIS

- Oncogenic stresses, such as activation of Myc or loss of Rb can induce mitochondrially mediated apoptosis.
- The mechanisms by which this occurs are not clear.
- Research has demonstrated clearly the importance of apoptotic pathways in control of tumorigenesis.

PROTO-ONCOGENES AND CANCER

-Cancer cells contain genetic damage that appears to be the responsible event leading to tumorigenesis.

Genetic damage- of two types:

-**Dominant**- the genes have been termed *proto-oncogenes*

-**Recessive** - the genes variously termed *tumor suppressors, growth suppressors, recessive oncogenes or anti-oncogenes*.

Genetic damage

– Dominant (Proto-Oncogenes)

A gene whose protein product has the capacity to induce cellular transformation given it sustains some genetic insult.

Oncogene is a gene that has sustained some genetic damage and, therefore, produces a protein capable of cellular transformation.

The process of activation of proto-oncogenes to oncogenes

- retroviral transduction or integration
- point mutations
- insertion mutations
- gene amplification
- chromosomal translocation
- protein-protein interactions

Proto-oncogenes have been identified at all levels of the various signal transduction cascades that control cell growth, proliferation and differentiation

CLASSIFICATION OF PROTO-ONCOGENES

Growth Factors

c-Sis gene - encodes PDGF.

The int-2 gene - encodes FGF-related growth factor.

The KGF gene- an FGF-related growth factor (identified in gastric carcinoma.)

Receptor Tyrosine Kinases

c-Fms (*fms*) gene - encodes colony stimulating factor-1 (CSF-1) receptor

Flg (*flag*) gene - encodes a form of the FGF receptor.

Trk (*track*) genes - encodes the NGF receptor-like proteins. (first Trk gene was found in a pancreatic cancer)

- Membrane Associated Non-Receptor Tyrosine Kinases
- G-Protein Coupled Receptors
- Membrane Associated G-Proteins
- Nuclear DNA-Binding/Transcription Factors
(Myc gene)

VIRUSES AND CANCER

Tumor viruses -two distinct types.

- viruses with DNA genomes (e.g. papilloma and adenoviruses) and
- those with RNA genomes (termed retroviruses).

RNA TUMOR VIRUSES

Only currently known human retroviruses are:

- Human T Cell Leukemia Viruses(HTLVs) and
- Human immunodeficiency virus (HIV).
- Transform cells they infect by two mechanisms

RNA TUMOR VIRUSES

First Mechanism

- Retrovirus infects a cell
- RNA genome is converted into DNA (reverse transcriptase)
- DNA integrates into the host genome
- rearrangement of the viral genome & incorporation of a portion of the host genome into the viral genome. (transduction).

- Occasionally this transduction process leads to the virus acquiring a gene from the host that is normally involved in cellular growth control.
- Because of the gene being transcribed at a higher rate due to its association with the retrovirus the transduced gene confers a growth advantage to the infected cell.
- The end result = unrestricted cellular proliferation leading to tumorigenesis.

Second Mechanism

- When a retrovirus genome integrates into a host genome it does so randomly.
- Sometimes this integration process leads to the placement of the LTRs close to a growth regulating protein gene
=Termed retroviral integration induced transformation.
- LTRs (long terminal repeats)
= powerful transcriptional promoter sequences contained at the ends of the retroviral genome.

It has recently been shown that HIV induces certain forms of cancers in infected individuals by this integration induced transformation process.

Cellular transformation by DNA tumor viruses

- result of protein-protein interaction.
- Proteins encoded by the DNA viruses, (**tumor antigens** or T antigens), can interact with cellular proteins.
- This interaction effectively sequesters the cellular proteins away from their normal functional locations in the cell.
- Predominant types of proteins sequestered = tumor suppressor type
- loss of their normal suppressor functions

Colorectal cancer (CRC)

- approx 146,940 new cases/yr
- In 2004, estimated > than 56,000 Americans will die of CRC

Risk factors for CRC

- both environmental and genetic
- mode of presentation of CRC

follows one of three patterns:

- A) Sporadic
- B) Inherited
- C) Familial

A) Sporadic disease

- There is no family history
- Accounts for approx 70 % of CRCs.
- Most common in persons older than 50 years of age
- Dietary and environmental factors have been etiologically implicated.

B) Inherited

- < than 10% of pts have an inherited predisposition to CRC
- Subdivided according to whether or not colonic polyps are a major disease manifestation.

i) The diseases with polyposis

- Familial adenomatous polyposis (FAP) and
- The hamartomatous polyposis syndromes (eg, Peutz-Jeghers, juvenile polyposis)

ii) The diseases without polyposis

- Hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome I),
- Cancer family syndrome (Lynch syndrome II).

All these conditions are associated with a high risk of developing CRC, and the genetic mutations underlying many of them have been identified.

C) "Familial" CRC

- Third and least well understood pattern
- Up to 25 percent of cases
- Affected patients have a family history of CRC, but the pattern is not consistent with one of the inherited syndromes described above

- Individuals from these families are at increased risk of developing CRC
- Risk is not as high as with the inherited syndromes.
- Single affected first-degree relative (ie, parent, child, or sibling) increases the risk 1.7-fold over that of the general population
- Risk is further Increased if two first-degree relatives have CRC, or if the index case is diagnosed before 55 years of age.

Molecular events underlying CRC

A) Sporadic disease

stepwise accumulation of somatic mutations

B) Inherited

Specific germline mutations

C) Familial

genetic abnormalities remain incompletely understood

C) Familial

- In persons of Ashkenazi Jewish descent, a specific mutation in the adenomatous polyposis coli (APC) gene has been linked
- Others have postulated that these families represent a variant of HNPCC
- Loss of DNA mismatch repair genes has been reported in a small subset

GENE MUTATIONS AND COLORECTAL TUMORIGENESIS

Genetic mutations

- inherited or acquired

Inherited

- at or before fertilization of the ovum
- transmitted from parent to offspring
 - If mutation occurs spontaneously in the sperm, ovum, or zygote, the affected person's parents do not manifest the cancer phenotype

Somatic mutation

- spontaneous mutation appearing in a cell during the growth and/or development of a particular tissue or organ
- result in preferential proliferation of the cell containing the mutated genetic material (**clonal evolution**)

- Clonal nature of tumors is a critical feature of the somatic mutation/clonal evolution theory of human carcinogenesis
- Growth advantage acquired by a single mutated cell allows its progeny to outnumber those of neighboring cells

- From within this clonal population, a single cell acquires a second mutation, providing an additional growth advantage
- Subsequent waves of clonal expansion are driven by the sequential acquisition of additional mutations leading to cancer

The adenoma-carcinoma sequence

- Most human CRCs are thought to arise from adenomatous polyps that are dysplastic but nonmalignant

- Adenomatous polyps form in the colon when normal mechanisms regulating epithelial renewal are disrupted

-Surface mucosal cells are continuously lost and must be continuously replaced.

-Typically, proliferation occurs at the crypt base and as cells move towards the luminal surface, they cease proliferating, and terminally differentiate.

-This ordered process is increasingly disrupted as adenomas increase in size, become dysplastic, and eventually attain invasive potential.

Multistep process of colorectal carcinogenesis

- Specific genetic changes are thought to drive the transformation from normal colonic epithelium to cancer.
- In 1990, Fearon and Vogelstein molecular basis for CRC as a multistep process in which each accumulated genetic event conferred a selective growth advantage to the colonic epithelial cell

According to the Vogelstein model,

- germline or somatic mutations are required for malignant transformation,
- it is the accumulation of multiple genetic mutations rather than their sequence that determines the biological behavior of the tumor

-Mutations in the APC gene, which are a feature common to both inherited and sporadic tumors, occur early in the process

- mutations of the p53 suppressor gene generally occur late in the process.

Specific mutations implicated in human tumorigenesis

- **Gain of function** mutations involving activation of growth promoting pathways including oncogenes
- **Loss of function** mutations usually involve tumor suppressor genes or apoptotic pathways;
- **Epigenetic alterations** such as DNA methylation and loss of imprinting

Epigenetic alterations

- Can silence the expression of certain genes through methylation.
- Methylation of the promoter region of some DNA mismatch repair (MMR) genes and/or loss of imprinting is thought to underlie cases of sporadic colorectal cancer and some cases associated with HNPCC.

Oncogenes in CRC

-Oncogenes implicated in sporadic CRC are

- ras, src, c-myc and c-erbB-2
(HER2/neu)

Ras oncogenes

- encode a family of proteins that regulate cellular signal transduction by acting as a one-way switch for the transmission of extracellular growth signals to the nucleus
- Ras mutations, typically point mutations, leave the protein resistant to GTP hydrolysis by GTPase, resulting in a constitutively active GTP-bound protein, and a continuous growth stimulus.

Ras mutations

Found in:-

- Up to 50 % of sporadic CRCs
- 50 % of colonic adenomas > 1 cm
- Rarely seen in smaller adenomas

Potential clinical relevance for both screening and therapy:

- Detection of ras mutations in fecal material - under study as a screening method for the early diagnosis of CRC
- Therapeutic potential of agents that target the ras signal transduction pathway in patients with CRC whose tumors contain ras mutations.

Tumor suppressor genes in CRC

- first molecular evidence for the involvement of tumor suppressor genes in CRC came from the study of allelic loss

- When comparing tumor alleles with those present in normal tissue, deletions were identified as "loss of heterozygosity" or LOH

LOH in CRC

chromosome

% of cases

5q (APC)

- 36 %

8p (DCC)

- 50 %

17p (SMAD4)

- 73 %

18q (p53)

- 75 %

APC gene

- Tumor suppressor gene
- Somatic mutations in both alleles are present in 80 percent of sporadic CRCs,
- A single germline mutation in this gene is responsible for FAP

Function of the APC gene product, and the mechanism by which the abnormal gene promotes tumor formation

- APC involved in a signaling cascade - the Wnt (Wingless-type) signaling pathway.

The Wnt pathway

- an evolutionarily conserved signal transduction pathway that is necessary for embryonic development.
- also plays a role in supporting intestinal epithelial renewal, an important fact since CRC is thought to originate in the expansion of colonic crypt cells.

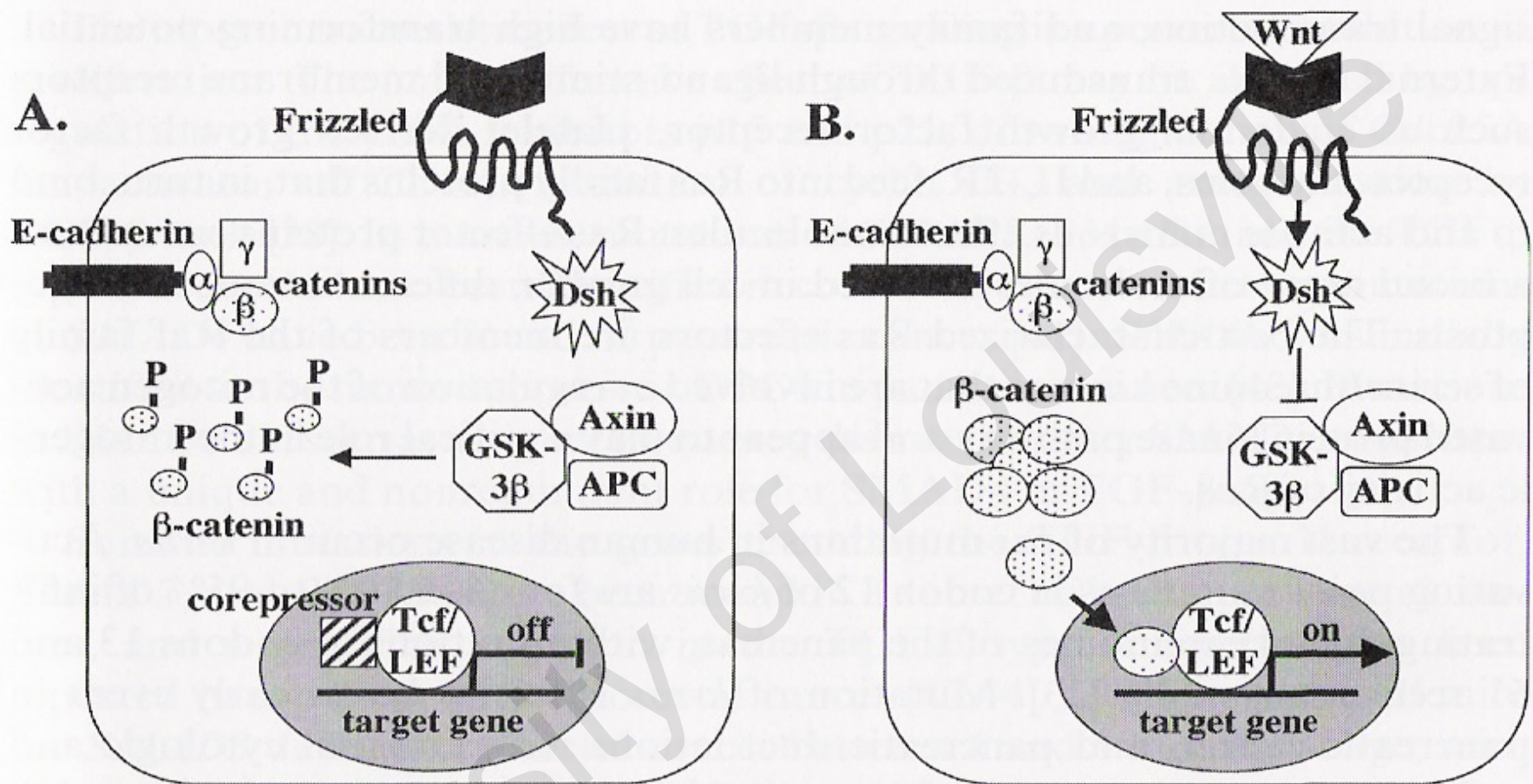


Fig. 2. Wnt signaling pathway. (A) In the absence of ligand binding and pathway stimulation, β -catenin is destabilized by a cytoplasmic complex containing the proteins Axin, adenomatous polyposis coli (APC), and glycogen synthase kinase-3 β (GSK-3 β). Phosphorylation by GSK-3 β targets β -catenin for ubiquitination and subsequent destruction. (B) When the Wnt ligand binds to its transmembrane receptor Frizzled, Disheveled (Dsh) inhibits the action of the cytoplasmic complex on β -catenin. Activation of the Wnt pathway thus stabilizes β -catenin, which translocates to the nucleus and, in conjunction with the Tcf/LEF family of transcription factors, activates target genes.

The majority of mutations in the APC gene (both germline and somatic) lead to premature truncation of the APC protein, and loss of its beta-catenin regulatory domains.

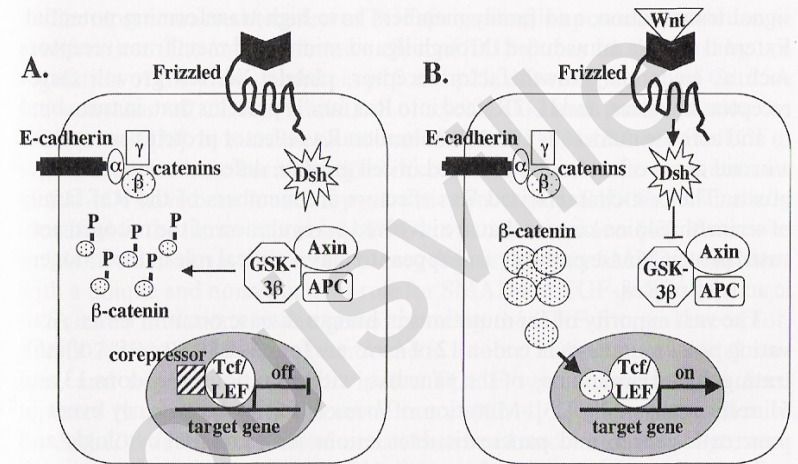
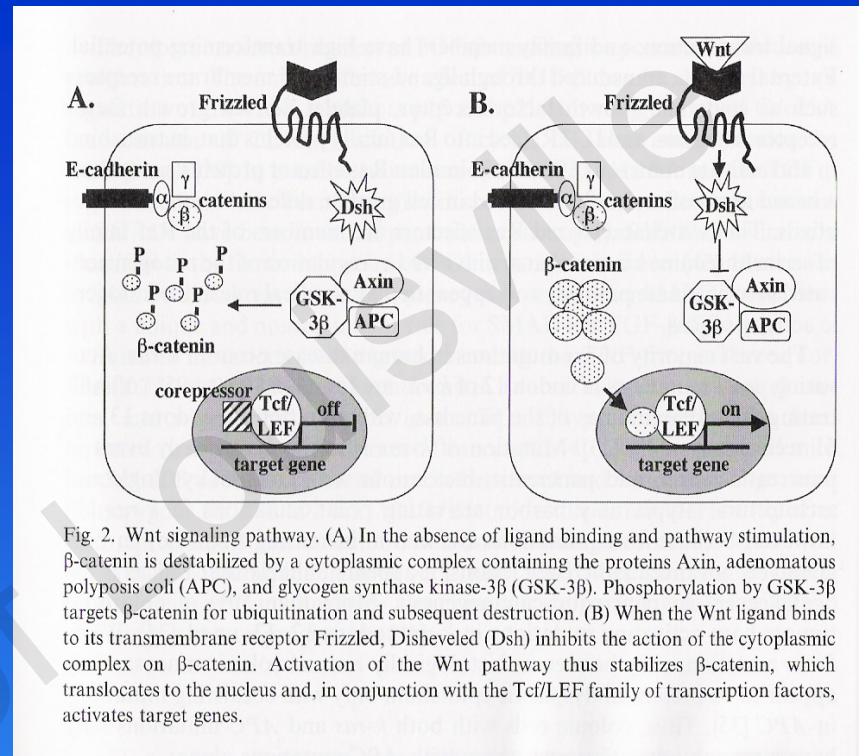


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-Loss of functional APC (as well as mutations in the beta-catenin gene) result in the nuclear accumulation of beta-catenin, which binds and activates the transcription factor T-cell factor (Tcf)-4



- Beta-catenin/Tcf-4 acts as a switch controlling proliferation versus differentiation in the intestinal crypt epithelial cells
- Activation of this pathway prevents the cells from either entering G1 arrest or undergoing terminal differentiation, and induces resistance to apoptosis
- The end result is cellular proliferation.

Other mechanisms may also contribute to the tumorigenic potential of APC mutations.

- Mutations in APC are associated with chromosomal instability predisposing the cell to "hits" in other genes that may contribute to tumor progression and malignant transformation.

p53 gene

- p53 has been referred to as the "guardian of the genome"
- most commonly mutated gene in human cancer
- lost in 75 percent of CRCs
- rarely lost in adenomas and aberrant crypt foci, suggesting that p53 loss is a relatively late event in colorectal tumorigenesis

P53

Actions

- 1) regulates the gene for the cdk inhibitor p21WAF1/Cip1
- 2) p53-dependent genes play multiple roles in apoptosis
 - several proapoptotic Bcl-2 family members are transcriptionally activated by p53
 - p53 promotes death receptor pathways

Identification of p53 mutations in an individual CRC is of potential clinical significance, prognostically and therapeutically.

-Patients whose tumors harbor p53 mutations have worse outcomes and shorter survival than those without

- From a therapeutic standpoint, it is hoped that p53 may prove to be a highly selective and effective target for intervention.

Chromosome 18q:

-the DCC, SMAD4, and SMAD2
genes

DCC protein

- thought to have a role in cell–cell or cell–matrix interactions
- Loss of DCC expression may have prognostic value
- 5-yr survival rates seem to be worse for patients with stage II (node-negative) CRCs that lack DCC expression compared to those that express it.

- Ultimate benefit of this information may be the identification of a subgroup of patients with stage II colorectal cancer who might benefit from adjuvant chemotherapy
- No prospective data yet to support the validity of this strategy

SMAD4

Previously known as **DPC4** (deleted in pancreatic cancer) gene

- encodes a protein that may be important to the signaling pathway of the transforming growth factor-beta (TGF-beta)

- TGF-beta suppresses the growth of most normal cells, and many cancer cells are resistant to this growth-suppressive effect.

- Mutations in SMAD4 have been found in a subset of sporadic CRCs .
- Germline mutations in SMAD4 have been identified in patients with juvenile polyposis.
- These patients develop multiple juvenile polyps that are distinct from adenomas, and they are at an increased risk for invasive CRCs.

Mismatch repair genes

- responsible for correcting the DNA damage that occur during DNA replication
- Several of these genes exist
 - hMSH2 (human mutS homolog 2)
 - hMLH1 (human mutL homolog 1)
 - hPMS1 & 2 (human postmeiotic segregation 1 and 2)
 - hMSH6 (human mutS homolog 6)
 - hMLH3

-HNPCC

Germline mutations in one of the MMR genes appear to be the underlying genetic defect in most kindreds

-Sporadic CRCs

MMR gene deficiencies can be found in 15 to 20 %

Epigenetic alterations affecting MMR genes

-methylation of the promoter region of some MMR genes and/or loss of imprinting is thought to underlie some cases of sporadic CRC

DNA methylation

- specifically targets CpG dinucleotides, which are present in the promoters of many genes
e.g. the MMR gene hMLH1
- methylated CpG is bound by a family of proteins which in turn, forms a multiprotein complex that silences gene expression

Modifier genes

- genes seem to be important in colorectal carcinogenesis
- exact roles and mechanisms of tumorigenesis have not been fully determined.
- **COX-2**
- **PPAR gene**

COX-2

- protective effect of ASA and other cyclooxygenase (COX) inhibitors on the development of CRC.
- Sulindac /celecoxib, can cause polyp regression in patients with FAP.
- Mechanism underlying these effects is not well understood

PPAR gene

- The peroxisome proliferator–activating receptor gene (PPAR)
- Implicated in CRC
- The PPAR gene encodes a family of nuclear receptors that function as transcriptional regulators for proteins controlling lipid metabolism and cell growth.

PPAR gene

- Activation of these receptors inhibits cell growth and promotes differentiation in a variety of epithelial cell types, including CRC cells
- Loss of function mutations in PPAR have been described in sporadic CRCs

Cellular and molecular
mechanisms responsible for
progression of Barrett's
metaplasia to esophageal
carcinoma

Barrett's metaplasia-

- found in approx. 12% to 18% of pts undergoing upper endoscopy for symptoms of reflux
- premalignant
- stepwise fashion: metaplasia to dysplasia to adenocarcinoma

Observations in Esophageal Carcinoma

1) Markers of increased cellular proliferation

- Proliferating cell nuclear antigen (PCNA)
- Ki-67

PCNA

- cofactor of DNA synthase
- indicator of cell-cycle progression at the G1/S transition .
- In BE significantly higher rate of surface proliferation in tissue with high-grade dysplasia than in tissue with low-grade or no dysplasia.

Ki-67

- a nuclear antigen expressed in proliferating but not in resting cells .
- The proliferative compartment, identified by Ki-67 staining, increases in size and expands from the basal crypts toward the luminal epithelium as Barrett's metaplasia progresses to dysplasia and Ca

What stimulates the excess proliferation of cells in Barrett's metaplasia and how it can be controlled are issues currently under study...

One study indicated that effective intraesophageal acid suppression (DeMeester score of <14.72) decreased proliferation in Barrett's esophagus biopsy samples based on PCNA immunohistochemical analysis

Tumor suppressor genes

p16

- a tumor suppressor gene located on chromosomes 9p21
- Only the frequency on *p53* gene mutation exceeds the frequency of *p16* gene mutation in human neoplasia.

p16

-also known as cyclin-dependent kinase inhibitor 2 (CDKN2), INK4a, or MTS1

-Normally, expression of p16 leads to G1-phase arrest by inhibiting cyclin-dependent kinases

- Loss of heterozygosity (LOH) at 9p21 is the predominant mechanism for inactivation of one of the p16 alleles

- LOH at 9p21

 - rarely seen in Barrett's metaplasia without dysplasia,

 - present in up to 75% to 91% of Barrett's-associated esophageal adenocarcinoma.

- Primarily, hypermethylation of the promoter region and, less often, homozygous deletion or point mutation inactivate the remaining p16 allele.

Hypermethylation of the *p16* promoter region was found in

- 3% of samples with Barrett's metaplasia without dysplasia
- 56% with low-grade dysplasia
- 75% with high-grade dysplasia

Growth factors and their receptors

TGF α

CerbB2 (Her-2/neu)

TGF α

- related to epidermal growth factor.
- TGF α binds to epidermal growth factor receptor, leading to the induction of intranuclear proto-oncogenes
- TGF α immunoreactivity occurred in the same regions of the glands as PCNA immunoreactivity

-Moreover, expression of epidermal growth factor receptor and $TGF\alpha$ in esophageal adenocarcinoma is associated with poor patient survival and metastases .

CerbB2 (Her-2/neu)

- part of a family of transmembrane tyrosine kinases involved in signaling pathways
- normal squamous epithelium, metaplastic tissue, and areas of low-grade dysplasia did not have an increase in Her-2/neu expression

Areas of high-grade dysplasia and 35% of adenocarcinomas, however, had an increased expression of Her-2/neu, based on RNA levels.

Significant correlation between
CerbB2 overexpression and depth of
tumor invasion, lymph node
metastases, distant metastases, and
status of residual tumor after
resection

CerbB2 may become a prognostic indicator for the aggressiveness of esophageal adenocarcinoma.

COX-2

- recent studies -reduction in the incidence of esophageal AdenoCa in persons taking aspirin and NSAIDs.

- Immunohistochemical studies and Western blot analysis have revealed an **up-regulation** of COX-2 protein expression in **Barrett's metaplasia**

-expression of COX-2 continues to increase in the progression from low-grade dysplasia to high-grade dysplasia.

-COX-2 remains elevated in esophageal adenocarcinoma.

...suggest that COX-2
overexpression is an early event
leading to progression from
metaplasia to adenocarcinoma

- How COX-2 expression promotes the malignant transformation of cells is currently unknown.
- The cause of up-regulation of COX-2 expression also remains to be elucidated.

COX-2 is involved in many cellular mechanisms, and overexpression is associated with decreased cell–cell adhesion, increased angiogenesis, and increased proliferation, with decreased apoptosis

- Studies have shown increased COX-2 expression in response to pulses of acid or bile acids in an ex vivo organ culture.
- In human esophageal adenocarcinoma cell lines, selective inhibition of COX-2 suppresses growth and induces apoptosis

-Studies are necessary to determine whether selective COX-2 inhibitors will be effective in decreasing the rates of progression from metaplasia to dysplasia and adenocarcinoma in patients with Barrett's metaplasia.

Chromosomal abnormalities

- No alterations were detected in Barrett's metaplasia without dysplasia
- Mean number of aberrant chromosomes, showing losses or gains, increased significantly in the transformation process.

low-grade dysplasia- losses of 5q21-23 (*APC*), 9p21 (*p16*) and 17p12-13.1 (*p53*)

high-grade dysplasia -loss of 7q33-q35 and gains of 7p12-p15, 7q21-q22, and 17q21

Invasive cancers –addl losses

DNA ploidy and aneuploidy

-significant linear increase seen in aneuploid populations in the progression from Barrett's metaplasia without dysplasia to Barrett's metaplasia with dysplasia and to adenocarcinoma.

In one study

Of 11 patients without high-grade dysplasia or adenocarcinoma on initial biopsy but showing aneuploidy and/or increased G2/tetraploid fractions...

7 developed high-grade dysplasia or adenocarcinoma.

None of the 49 patients lacking aneuploidy and/or increased G2/tetraploid fractions on initial biopsy developed high-grade dysplasia or adenocarcinoma .

- Reid et al prospective study
- Systematic endoscopic biopsy protocol with baseline histologic and flow cytometric abnormalities as predictors of cancer.

- Among patients with negative, indefinite, or low-grade dysplasia without aneuploidy or increased 4N populations, no patients developed cancer in 5 years.
- By 8 years, however, three of these patients developed esophageal adenocarcinoma .

Order of events

Many studies have been designed to determine the ordering of events in the metaplasia–dysplasia–adenocarcinoma sequence associated with Barrett's esophagus.

-A common pattern was seen in which diploid cells in metaplastic tissue developed LOH at 17p and 9p and mutations in *p16*

-These cells frequently give rise to populations that then evolve into distinct aneuploid cell populations and progress to cancer with additional LOH 53 and *p16*

