Neoplasia (III)

Molecular Basis of Cancer

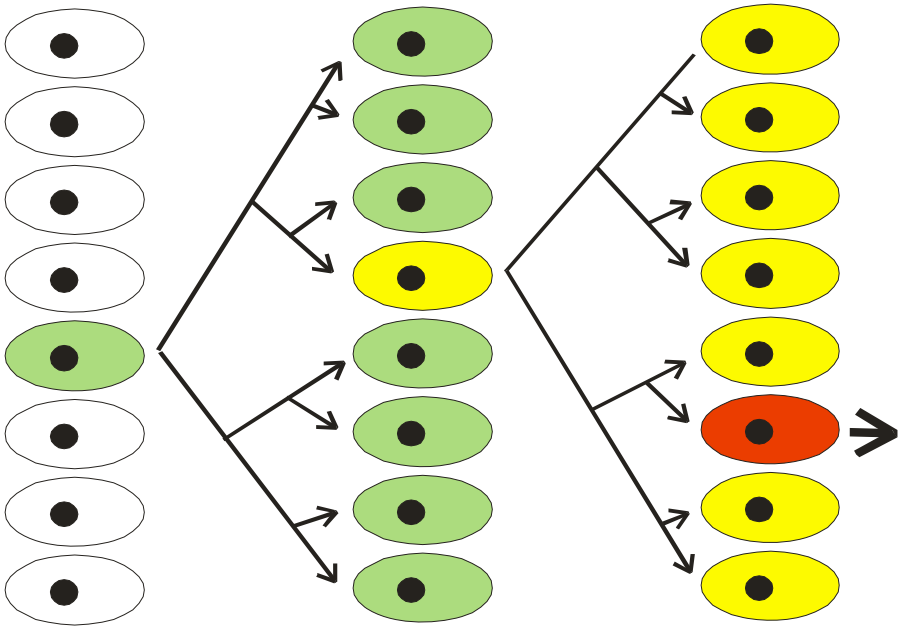
APPROVED

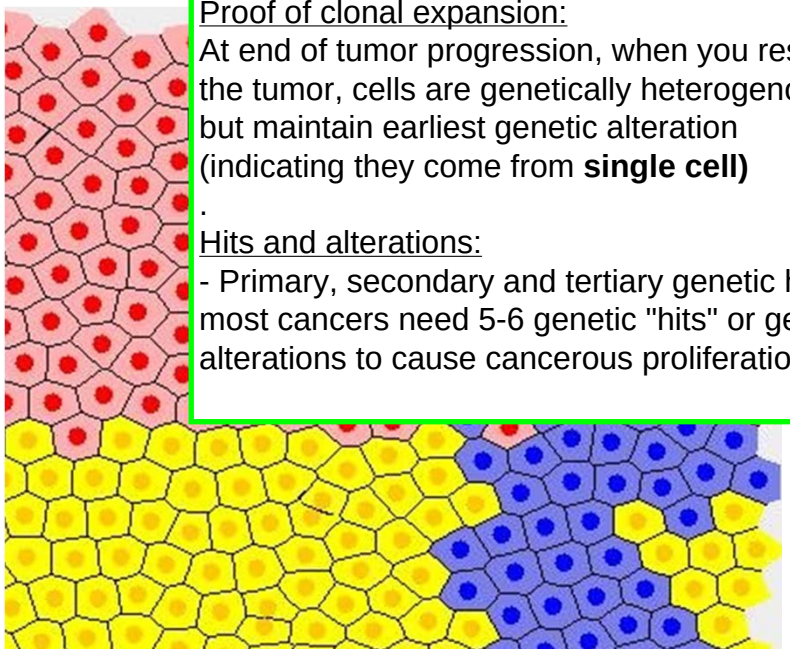
Overview

all tumors/cancer are derived from single cell that has expanded w/ multiple genetic mutations/ alteration

- **Clonal expansion**
- **Oncogenes**
- **Tumor suppressor genes**
- **Molecular based diagnosis and therapy**

Cancer Progression is Multi-stepped: Clonal Expansion & Selection





Clonal expansion:
Cancer is a big mess, but they all come from single cell

.

Proof of clonal expansion:
At end of tumor progression, when you resect the tumor, cells are genetically heterogenous, but maintain earliest genetic alteration (indicating they come from **single cell)**

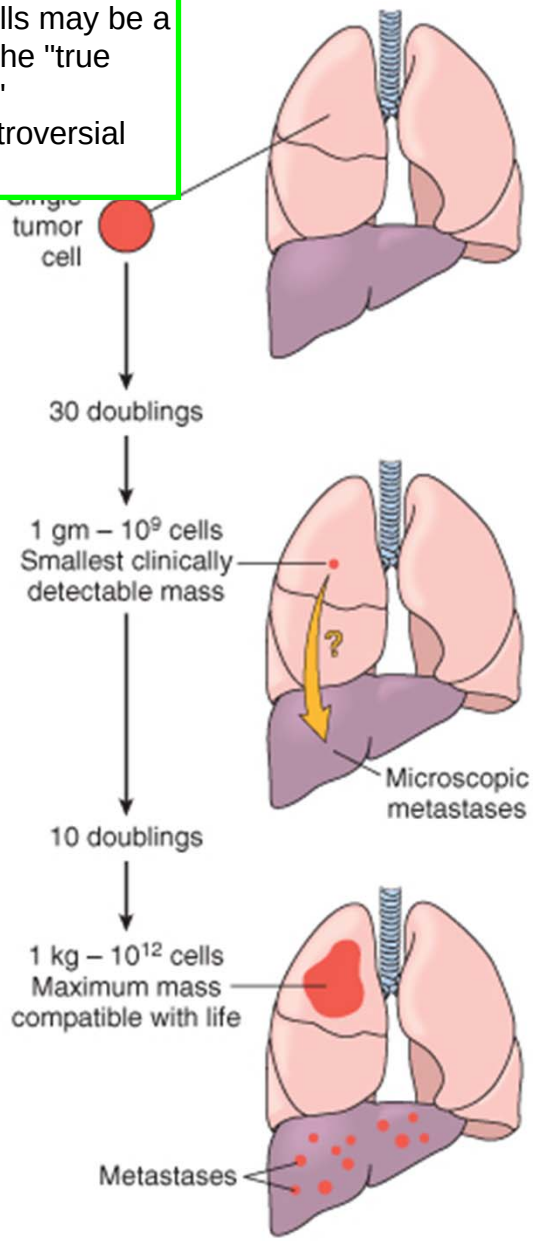
.

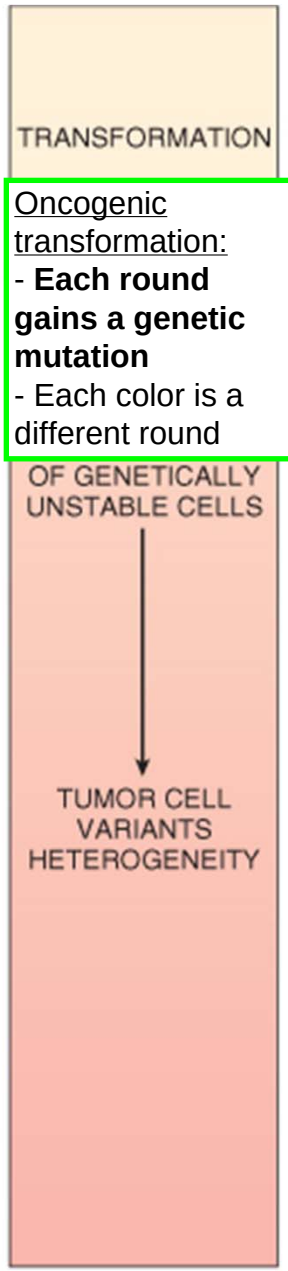
Hits and alterations:
- Primary, secondary and tertiary genetic hit, but most cancers need 5-6 genetic "hits" or genetic alterations to cause cancerous proliferation

BIG IDEA
1. **Cancer undergoes clonal expansion** - all derived from single cell
2. Tumors usually have **5-6 genetic alterations/"hits"** to cause cancerous proliferation

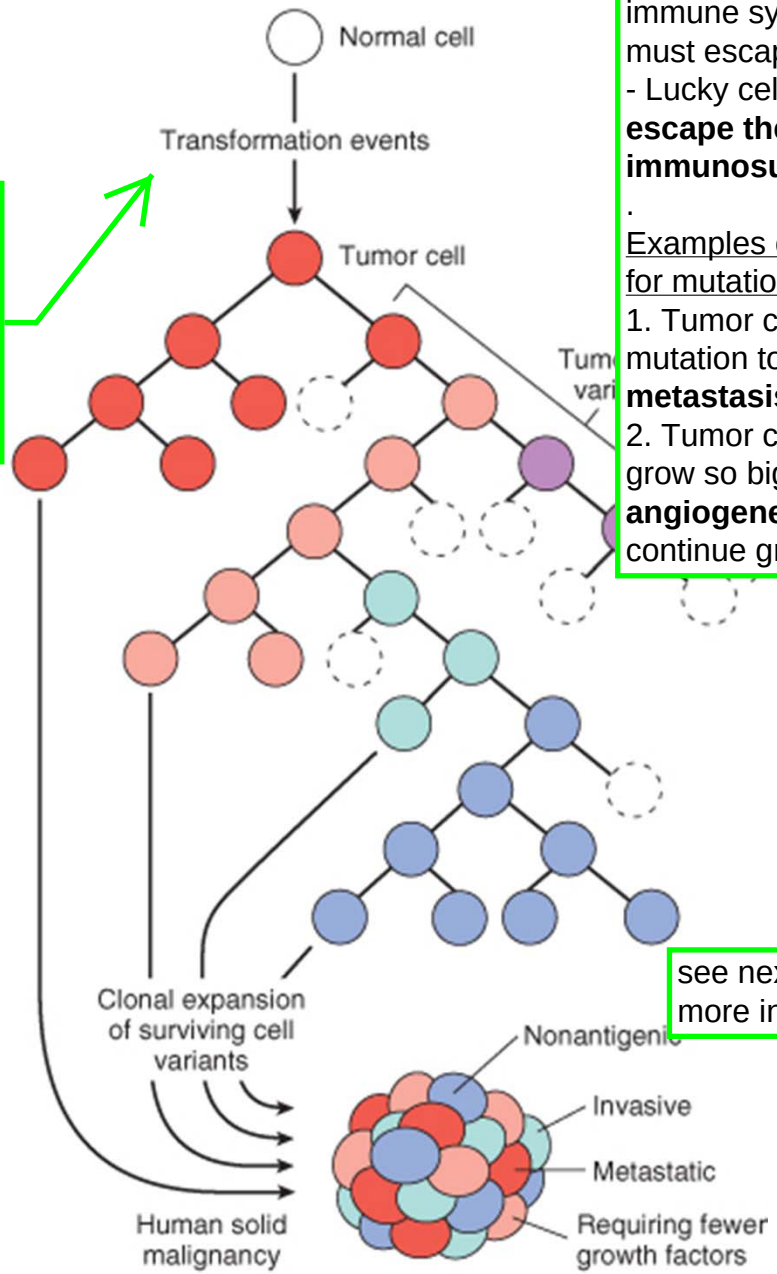
ONAL EXPANSION AND HETEROGEN

#1. What we know:
Normal cell: Mature, adult cells **CANNOT renewably proliferate**
.
What is new (research):
Cancer stem cells may be a precursor and the "true origin of cancer"
- cause still controversial and debated





Oncogenic transformation:
- **Each round gains a genetic mutation**
- Each color is a different round

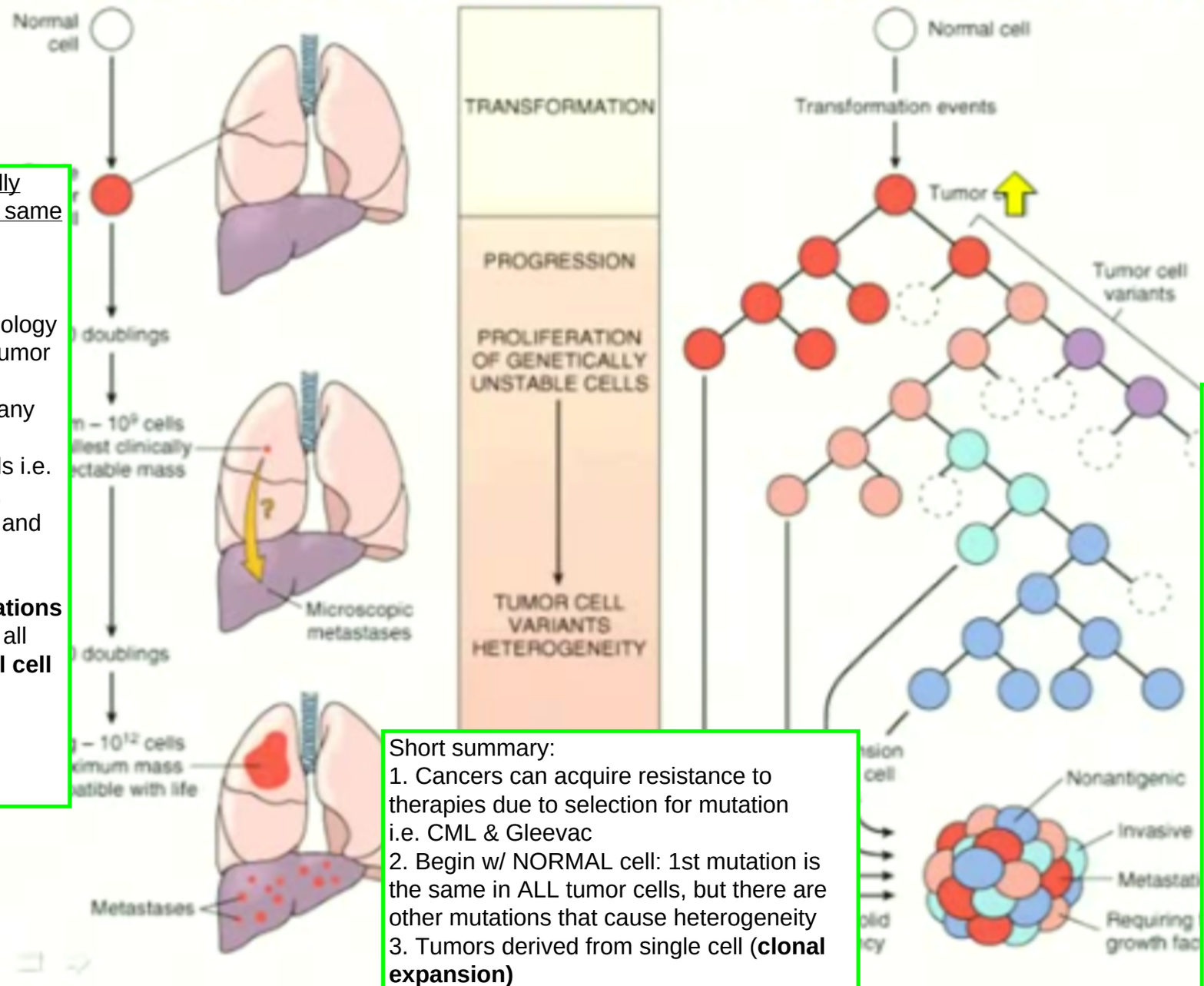


#2. Why so many mutations/genetic alterations?
- Tumor cells are "outlaws" from the immune system and must escape the body!
- Lucky cells can **escape the body & immunosurveillance**,
.
Examples of selection for mutation:
1. Tumor cell can gain mutation to allow **metastasis**
2. Tumor cells can grow so big and initiate **angiogenesis** to continue growth

see next slide for more info....

Repeated concept: Begin w/ NORMAL cell: 1st mutation is the same in ALL tumor cells, but there are other mutations that cause heterogeneity

CLONAL EXPANSION AND HETEROGENEITY



#3. Tumors are genetically heterogenous, but share same origin:
Ex. GBM (Glioblastoma)
Grade 4 histology
- Different cellular morphology in different areas of the tumor but all malignant
- Malignant cells have many characteristics which are different from normal cells i.e. immune system evasion, angiogenesis, apoptosis and tumor necrosis
- **Tumors are complex: variety of genetic alterations within same tumor,** but all stem from **same original cell (clonal expansion)**

Short summary:
1. Cancers can acquire resistance to therapies due to selection for mutation i.e. CML & Gleevac
2. Begin w/ NORMAL cell: 1st mutation is the same in ALL tumor cells, but there are other mutations that cause heterogeneity
3. Tumors derived from single cell (**clonal expansion)**

#4. Treatment:
1. Can be 100s of genetic changes, but they **share signal transduction pathways**
- Target limited # of alterations in signal transduction pathways
.
2. Can **target first genetic variation** to kill tumor cells
.
EX. **CML** is a specific leukemia subtypes that has BCR-ABL translocation fusion protein
Gleevac: Gene therapy can target variation > complete remission of disease
- **Relapse of cancer due to additional mutations in BCR-ABL**

Cancer is a Genetic Disease

At each stage, specific genetic mutations occurs Ex. 1st mutation is APC/ beta-catenin pathway **"gatekeeper genes"**

Dysplastic > "pre-cancerous cell"

"caretaker genes" repair DNA mutations

beta catenin (translocates to nucleus to activate cell proliferation) binds E cadherin, cell surface protein that mediates IC interactions

tumor suppressor gene: adenomatous polyposis coli

APC/β-catenin
FAP

RAS/BRAF

18q

p53
PIK3CA

Normal Epithelium	Dysplastic ACF	Early Adenoma	Intermediate Adenoma	Late Adenoma	Carcinoma	Metastasis

Tumor suppressor

Oncogene

Chromosomal or Microsatellite Instability (HNPCC)

Oncogene mutation is like a **stuck accelerator**! (will continue to go even if you lift your foot of the pedal)

Tumor suprressor is like the **brakes**. Problems with the brakes, forces you to go, can't stop!

"Caretaker genes" are the **mechanic.** Can't take care of the DNA repairs if they are mutated

What Gene Functions can be Altered in Cancer?

anti-apoptotic
Ex. Hypoxia, stress, radiation causes DNA damage
- Problem must be fixed or else will signal apoptosis in the normal cell

- Oncogene activation: self-sufficiency in growth signal
- Inactivation of tumor suppressor genes: insensitivity to growth-inhibitory signals
- Evasion of programmed cell death
- Defect in DNA repair: can't maintain genomic integrity
- Expression of telomerase: limitless replicative potential
- Sustained angiogenesis
- Ability to invade and metastasize

causes signal for cell death

Cancer metabolism - use different metabolic pathways

Genetic Mechanisms to activate oncogenes or inactivate tumor suppressors

Mutations. Small-scale mutations include point mutations, deletions, and insertions. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, and such an event may also result in the expression of viral oncogenes in the affected cells.

Genomic amplification occurs when a cell gains many copies of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material.

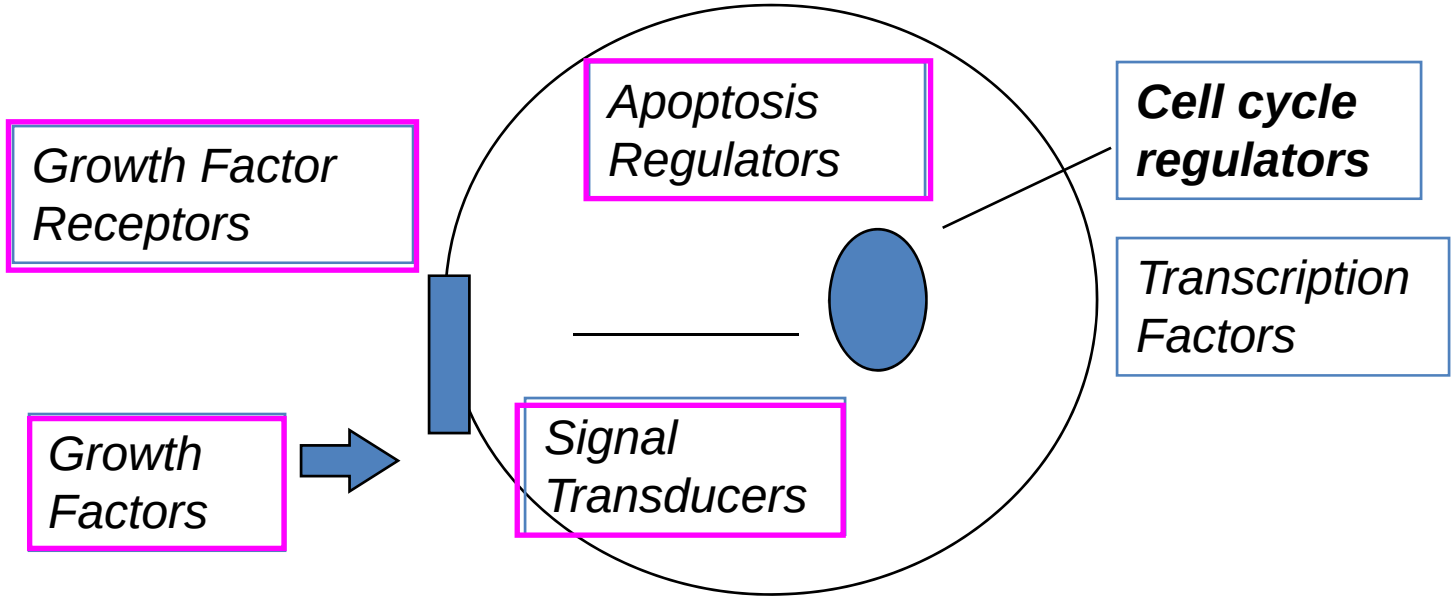
Genomic Deletion of tumor suppressor genes.

Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location.

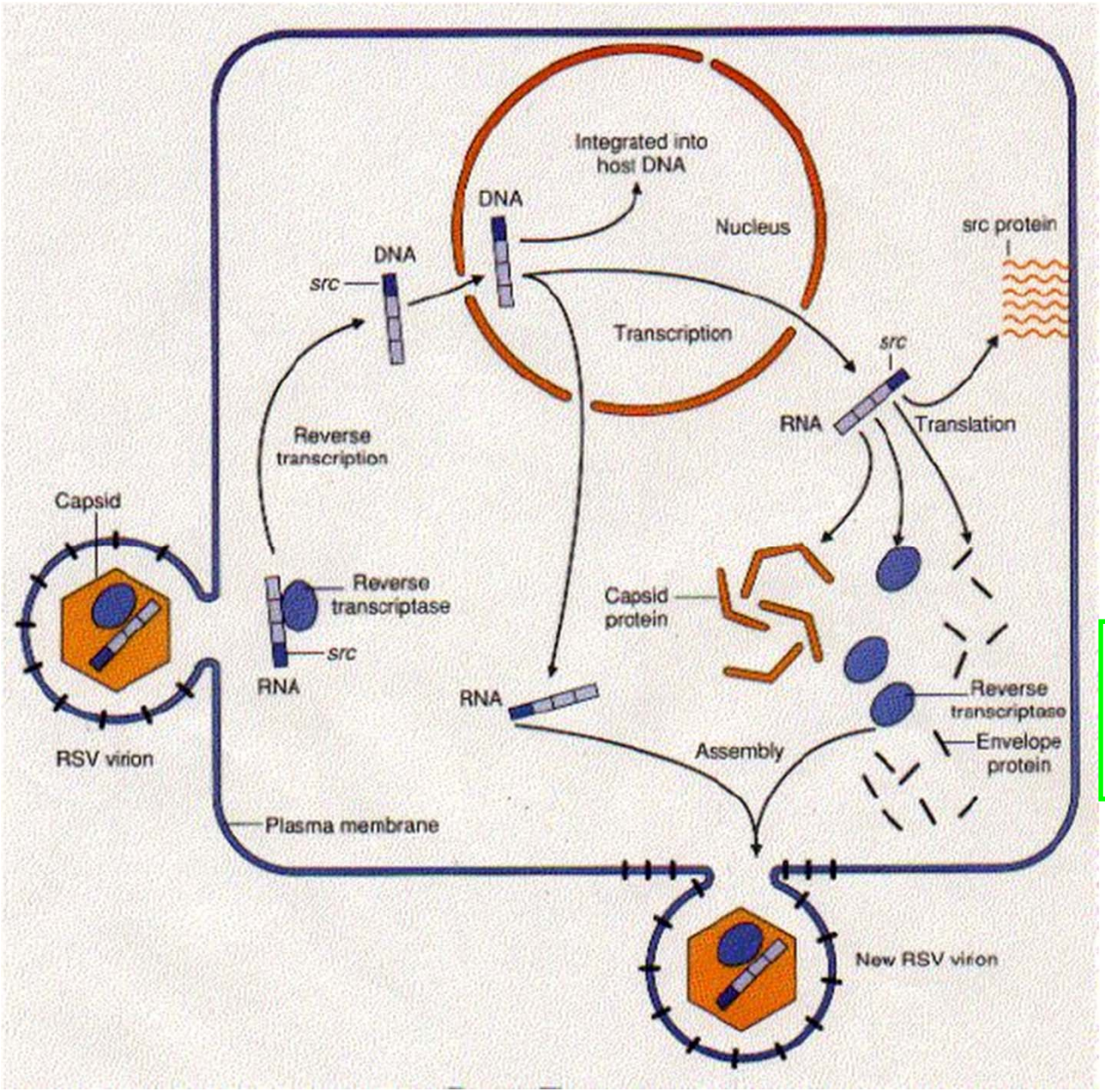
can activate or inactive gene expression

Oncogenes

Protooncogenes are normal cellular genes which are involved in growth regulation; Oncogenes result from activation protooncogenes and lead to unrestricted growth control



Oncogene Activation- Oncovirus



Rous sarcoma virus was discovered in 1911 by Peyton Rousis. It is a retrovirus and the first oncovirus to have been described: it causes sarcoma in chickens.

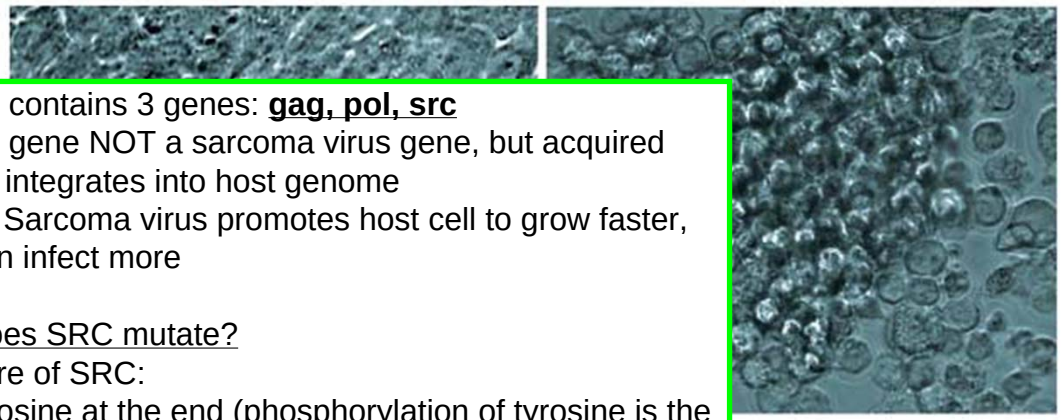
1st oncogene identified is **SARC**
- Oncovirus & oncogene
- Chicken virus causes sarcoma

Experiment: What can cause cancer?
1. Isolated tumor cells
2. Extract liquid portion + inject chickens
Result: Sarcoma
Virus in liquid portion causing cancer

BIG IDEAS:
1) Tyrosine at C terminus: phosphorylated inhibits Src gene from being expressed
2) V-src is ONCOGENE - lacks inhibitory signal
3) C-src is PROTO-ONCOGENE - mutation can remove tyrosine and cause overexpression

SRC: protooncogene and o

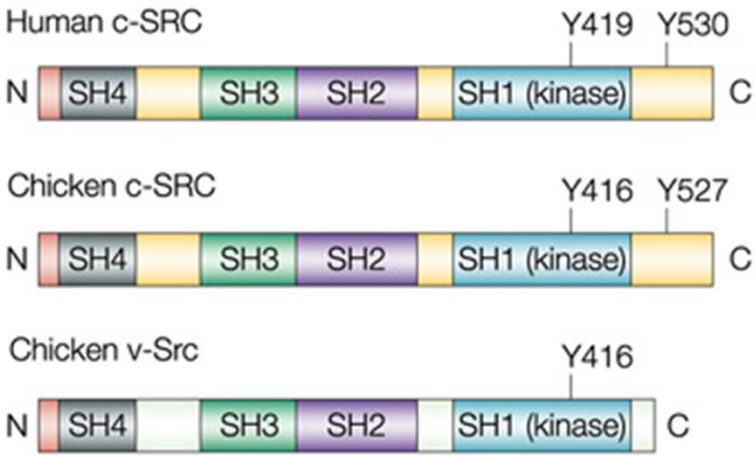
The src gene was taken up by RSV and incorporated into its genome conferring it with the advantage of being able to stimulate uncontrolled mitosis of host cells. v-src lacks the C-terminal inhibitory site, and is therefore constitutively active as opposed to c-src which is only activated under certain circumstances. v-src is an instructive example of an oncogene whereas c-src is a proto-oncogene. The c-SRC non-receptor tyrosine kinase is overexpressed and activated in a large number of human malignancies.



1) SRC contains 3 genes: **gag, pol, src**
2) SRC gene NOT a sarcoma virus gene, but acquired when it integrates into host genome
3) Fxn: Sarcoma virus promotes host cell to grow faster, so it can infect more

.

How does SRC mutate?
Structure of SRC:
Has tyrosine at the end (phosphorylation of tyrosine is the inhibitory signal of SRC)
- **v-Src** becomes **oncogene** b/c lacks inhibitory signal
- **c-src** is a **proto-oncogene** b/c it has inhibitory signal, but when mutated, can cause overexpression



Oncogene Activation- Mutation

For example: the most common oncogenes found in human cancers are Ras oncogene.
About 10-15 % of all cancers carry Ras mutations.
95% of pancreatic carcinomas carry Ras mutations.

Ras genes encode proteins with GTP guanosine-nucleotide–binding activity and intrinsic guanosine triphosphatase activity

When mutated in codon 12, 13, or 61, the RAS genes encode a protein that remains in the active state and continuously transduces signals by linking tyrosine kinases to downstream serine and threonine kinases.

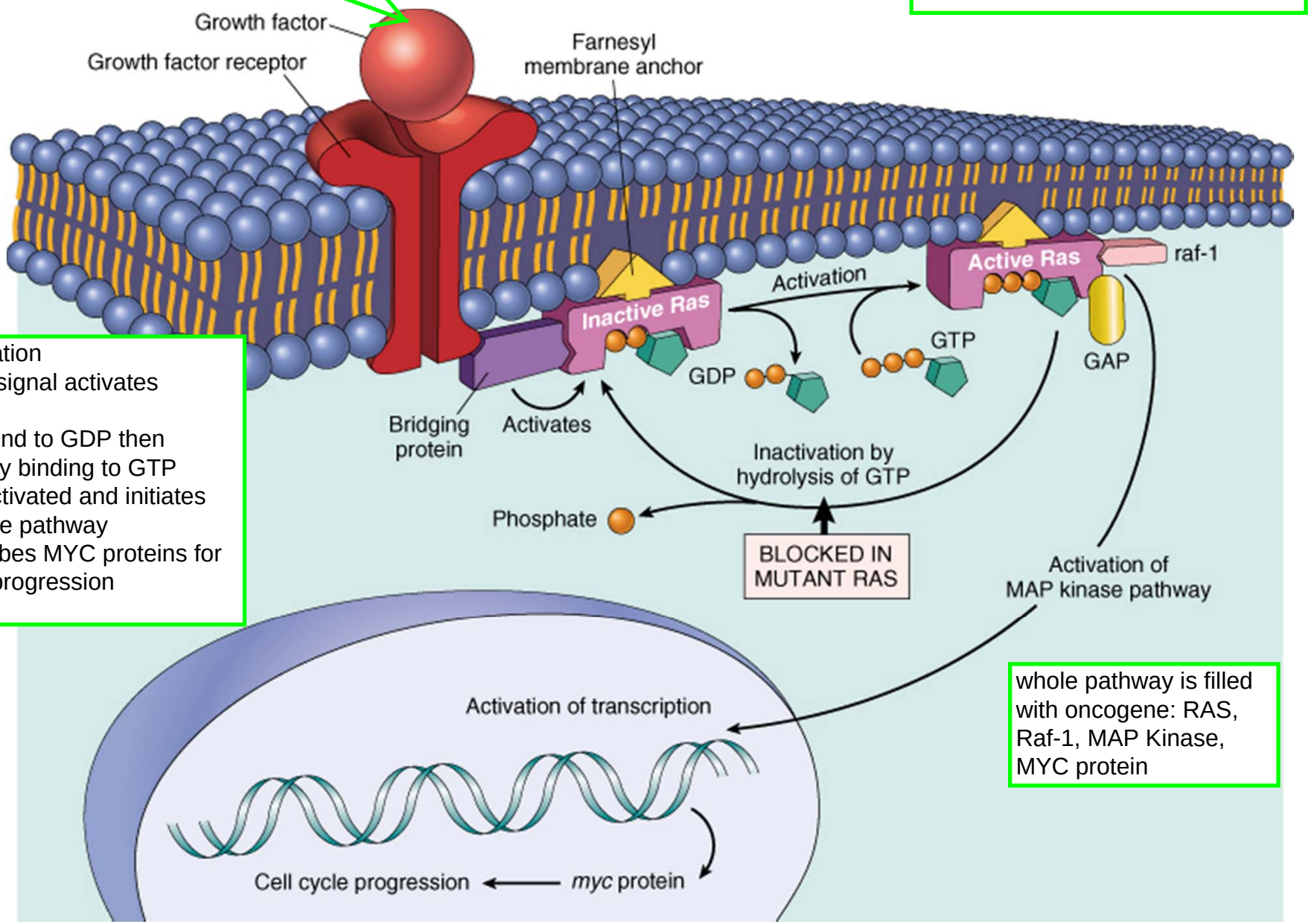
1. RAS = common oncogene
2. Frequently mutated in cancers, especially pancreatic carcinomas
3. Encodes protein that bind GTP and can degrade GTP > GDP

"Hot spot" mutations in these codons can cause activation of RAS gene

Ras Activation

growth signal

Oncogene Concept:
Mutually exclusive oncognes - only need one mutation to activate oncogene (Ras or raf-1, but not both)



RAS activation
1. Growth signal activates receptor
2. Ras bound to GDP then activates by binding to GTP
3. Raf-1 activated and initiates MAP kinase pathway
4. Transcribes MYC proteins for cell-cycle progression

whole pathway is filled with oncogene: RAS, Raf-1, MAP Kinase, MYC protein

Oncogene Activation-Amplification

Members of four different oncogene families are often amplified: MYC, cyclin D1 (or CCND1), EGFR, and RAS.

MYC is amplified in small-cell lung cancer, breast cancer, esophageal cancer, cervical cancer, ovarian cancer, and head and neck cancer.

CCND1 amplification also occurs in breast, esophageal, hepatocellular, and head and neck cancer.

EGFR (*ERBB1*) is amplified in glioblastoma and head and neck cancer. Amplification of *ERBB2* (also called *HER2/neu*) in breast cancer.

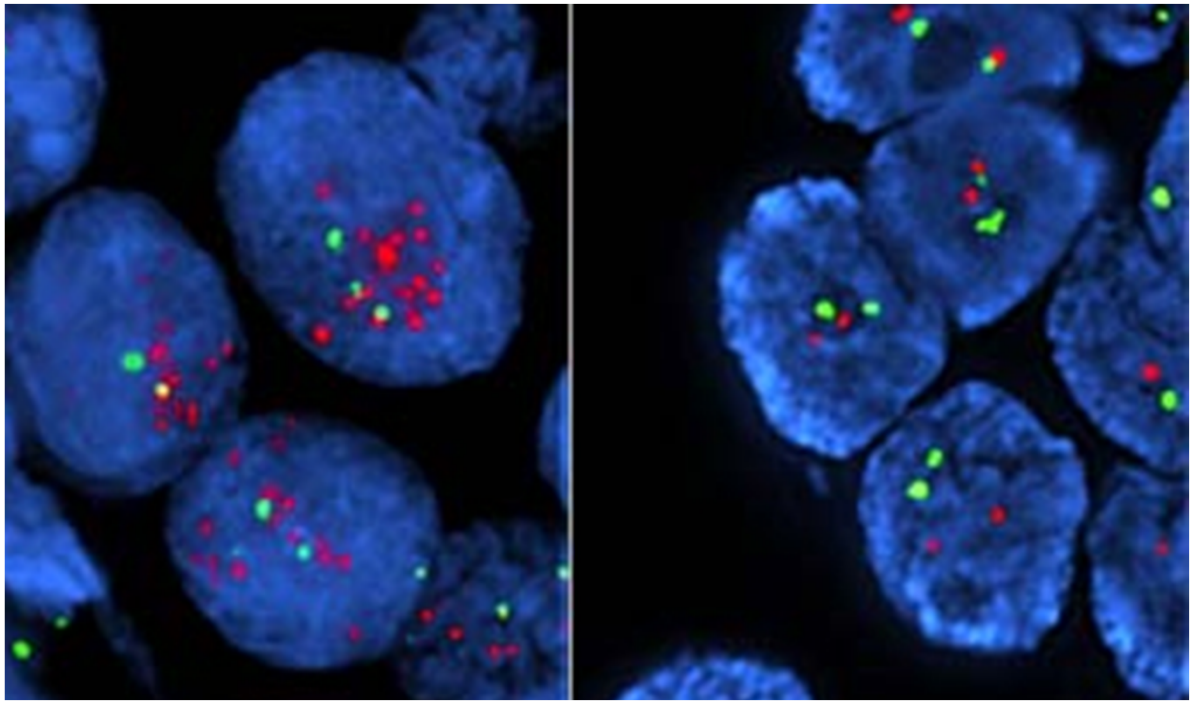
Growth factor Receptor 20% of breast cancer patients have amplication

ogene Activation

HER2/neu amplification in breast cancer cells

- Use FISH (fluorescence in situ hybridization) to determine Her2/neu amplification
- fluorescent probe to hybridize with protein of interest

HER2/neu amplified **HER2/neu nonamplified**



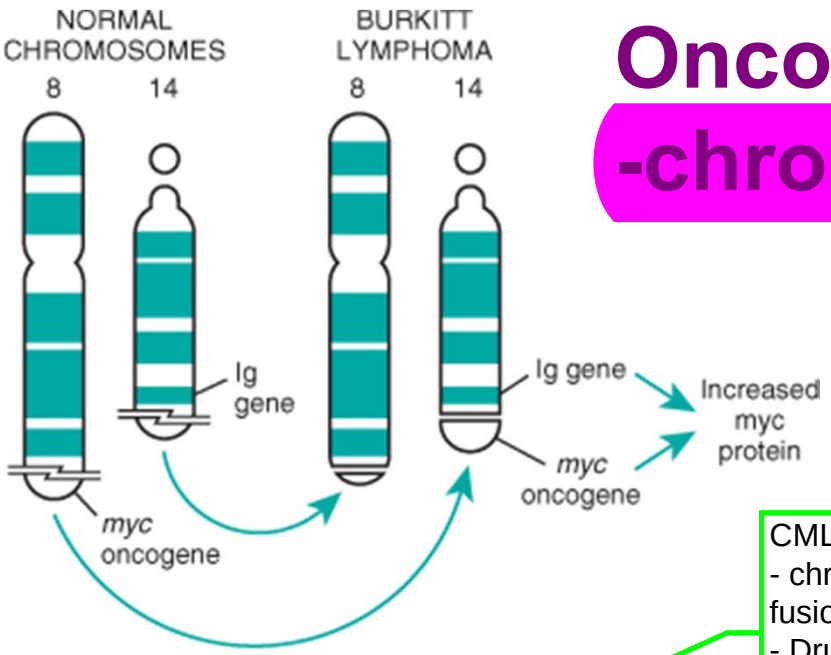
monoclonal Ab that can target HER2/neu & kill tumor cells

Amplification of *ERBB2* in breast cancer correlates with a poor prognosis.

A monoclonal antibody against the product of this oncogene (trastuzumab) is effective in breast cancers that overexpress HER2/neu.

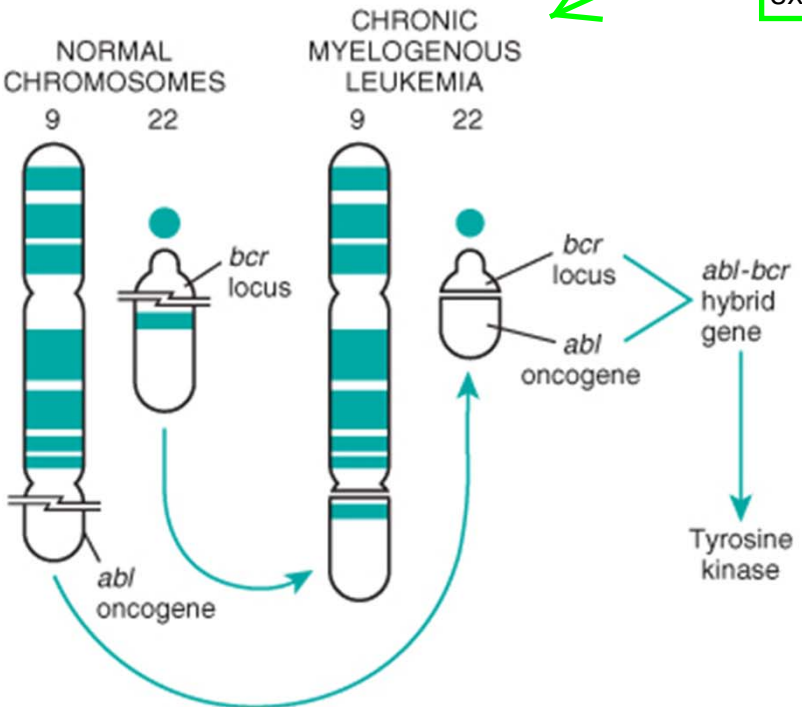
Oncogene Activation

-chromosomal translocation

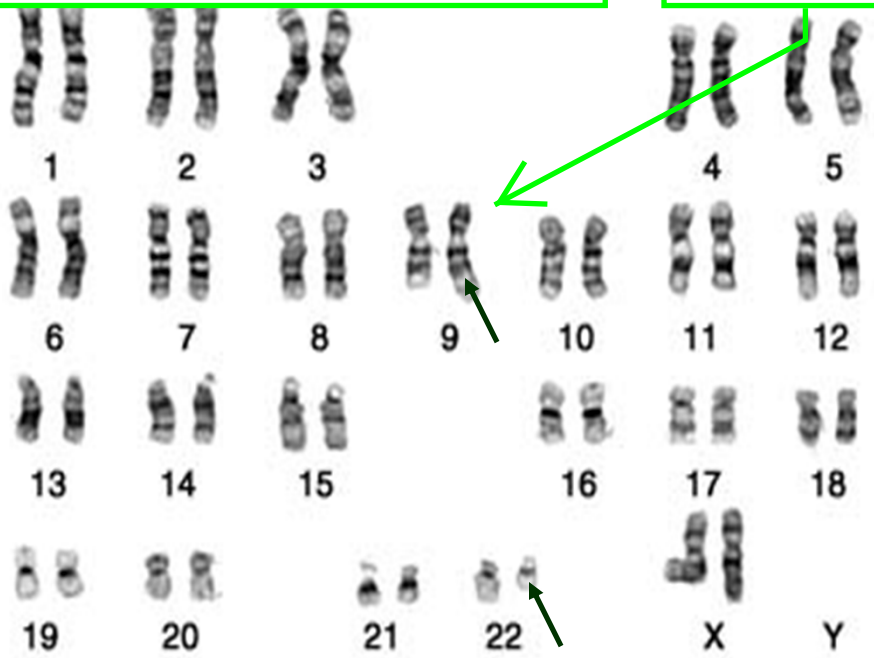


Ex. Burkitt's lymphoma w/ chromosome 8 & 14
- MYC oncogene fused with Ig gene (very active) & induces overexpression

CML chr 9,22 translocation
- chr 22/Philadelphia chromosome: ABL-BCR fusion
- Drug targets BCR kinase (that activates expression)



chr 9 longer
chr 22 shorter
(philadelphia chr)

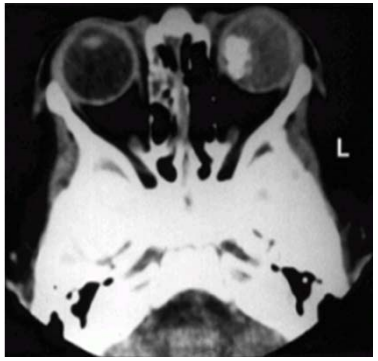


Tumor Suppressor Genes

Quick review:
How many alleles do you need to activate an oncogene? How many for a tumor suppressor?
A:
Oncogene - 1 allele **activated**
Tumor suppressor = 2 allele **inactivation (KNUDSON'S TWO HIT HYPOTHESIS)**

Normal physiologic function of the wild type gene is to slow cell growth, cause cell differentiation, activate apoptosis or repair DNA. Require mutations (or silencing) of both alleles (Knudson's two hit hypothesis).

Knudson's Two Hits Theory and retinoblastoma



Inactivation of both copies of Rb tumor suppressor gene is required for tumorigenesis

Hereditary - begin with 1 hit
Sporadic - begin with 0 hits

In hereditary cases:

First hit is inherited from an affected parent and present in all cells

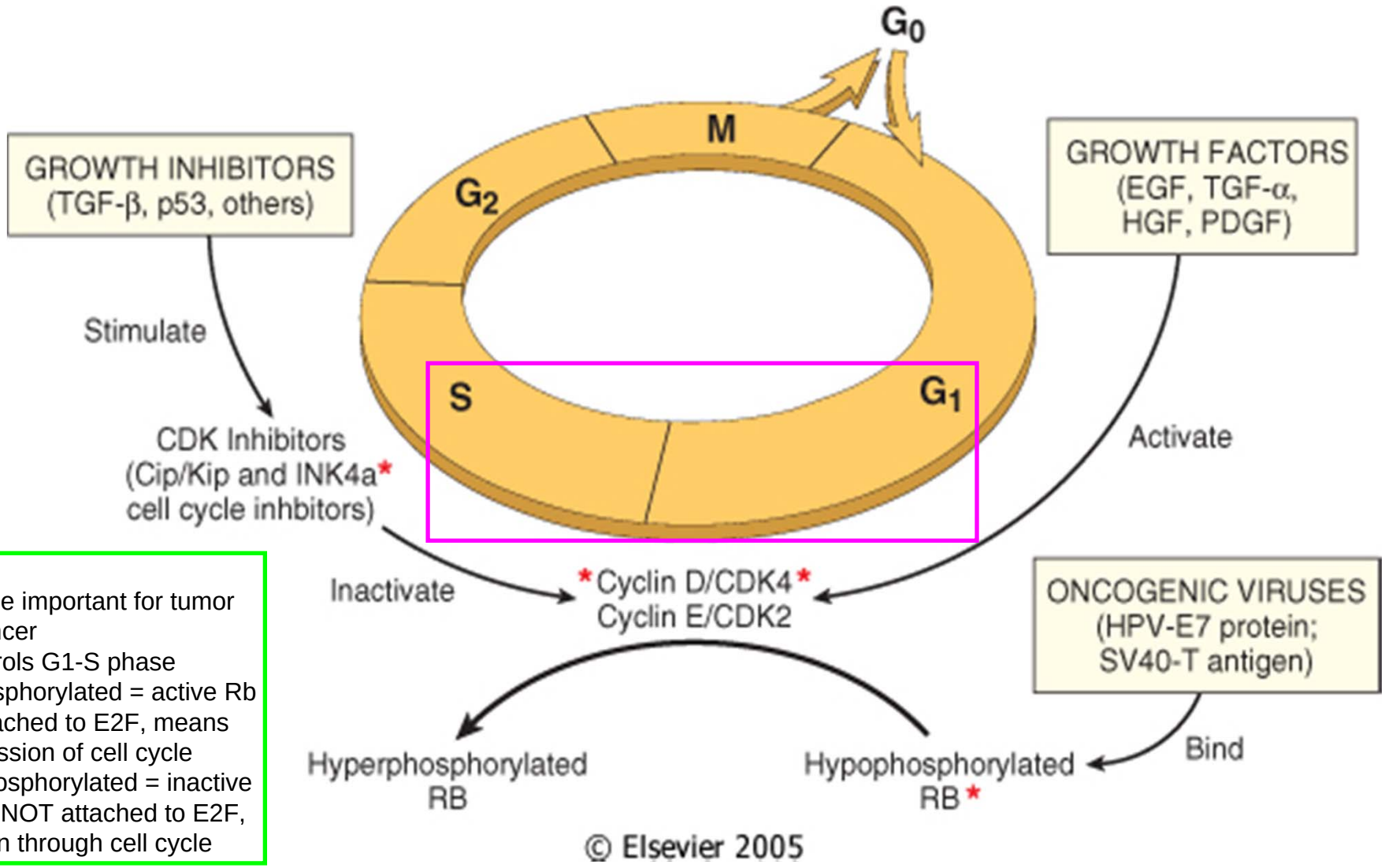
Second hit occurs in one of the many of retinal cells, which already carry the first hit.

Carriers of a mutant Rb gene have a 10,000-fold increased risk, children develop retinoblastoma at much younger age than sporadic cases, usually bilateral .

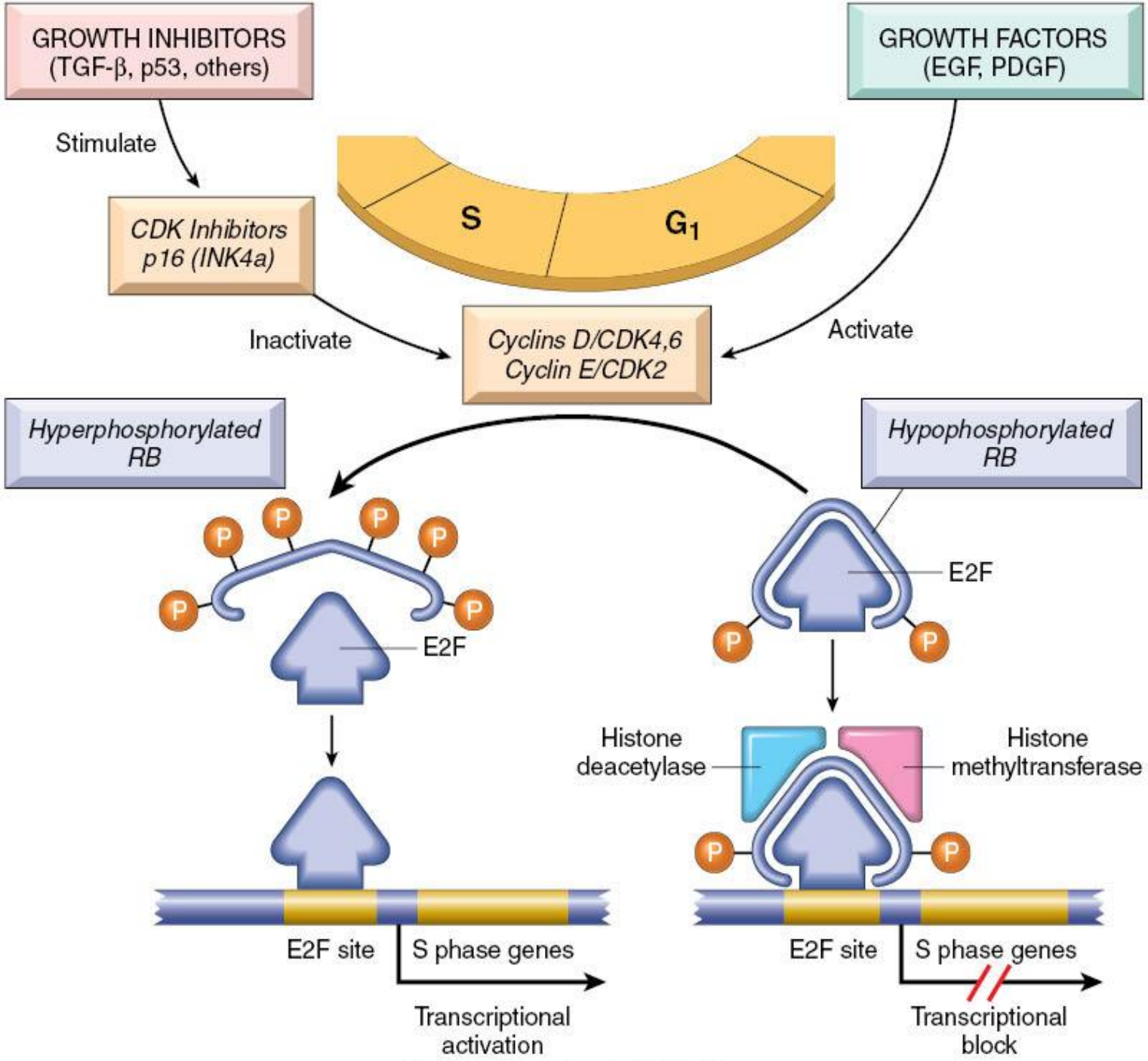
In sporadic cases:

Both hits occur somatically within a single retinal cells, whose progeny then form the tumor.

Rb functions in G1-S cell cycle control



Big ideas:
1. Cell cycle important for tumor growth/cancer
2. Rb controls G1-S phase
HYPOphosphorylated = active Rb means attached to E2F, means NO progression of cell cycle
HYPERphosphorylated = inactive Rb means NOT attached to E2F, progression through cell cycle



TP53 tumor suppressor gene

TP53 is mutated in more than half of human tumors

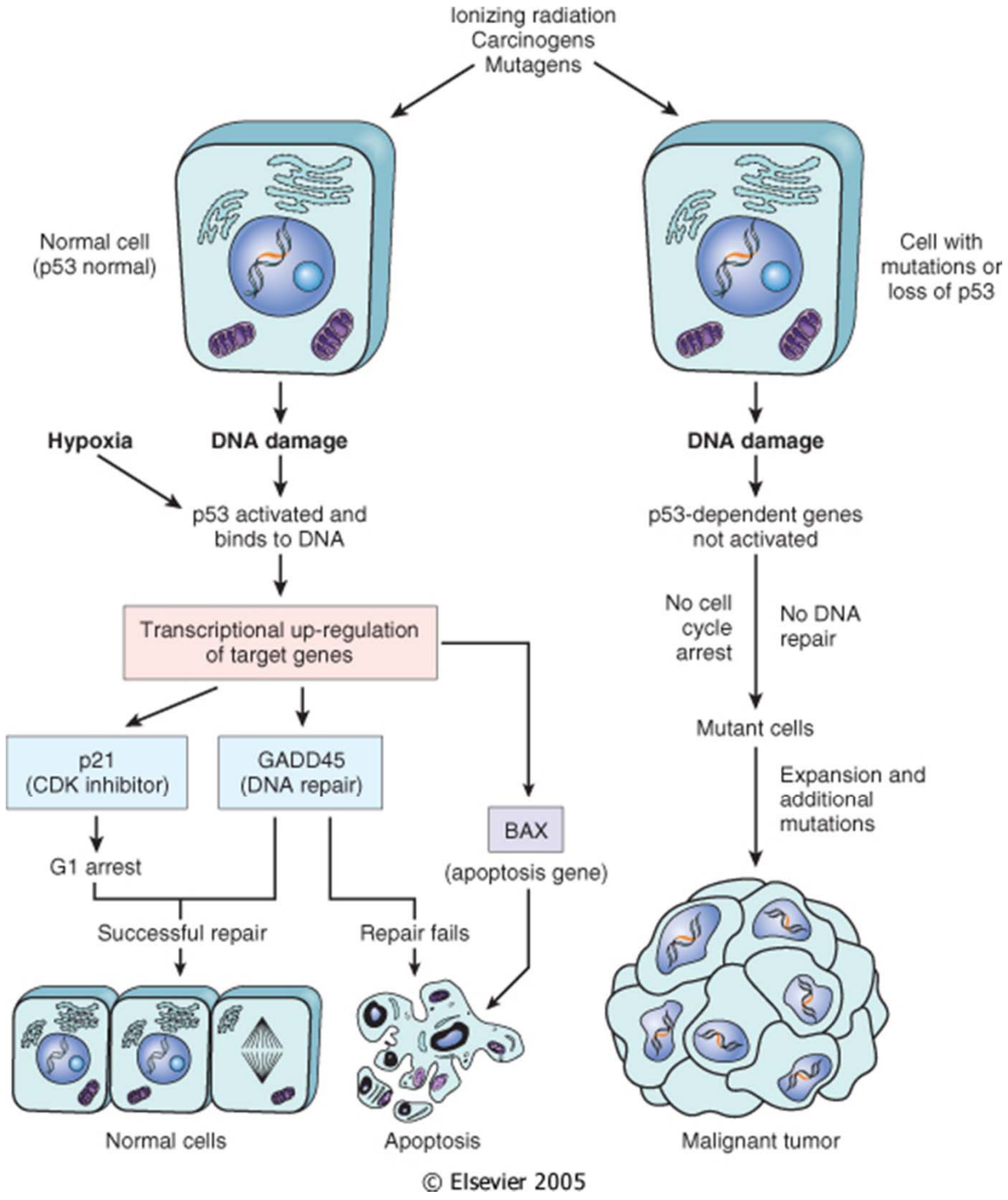
One of its normal functions is to prevent replication of DNA which has been damaged, guardian of genome

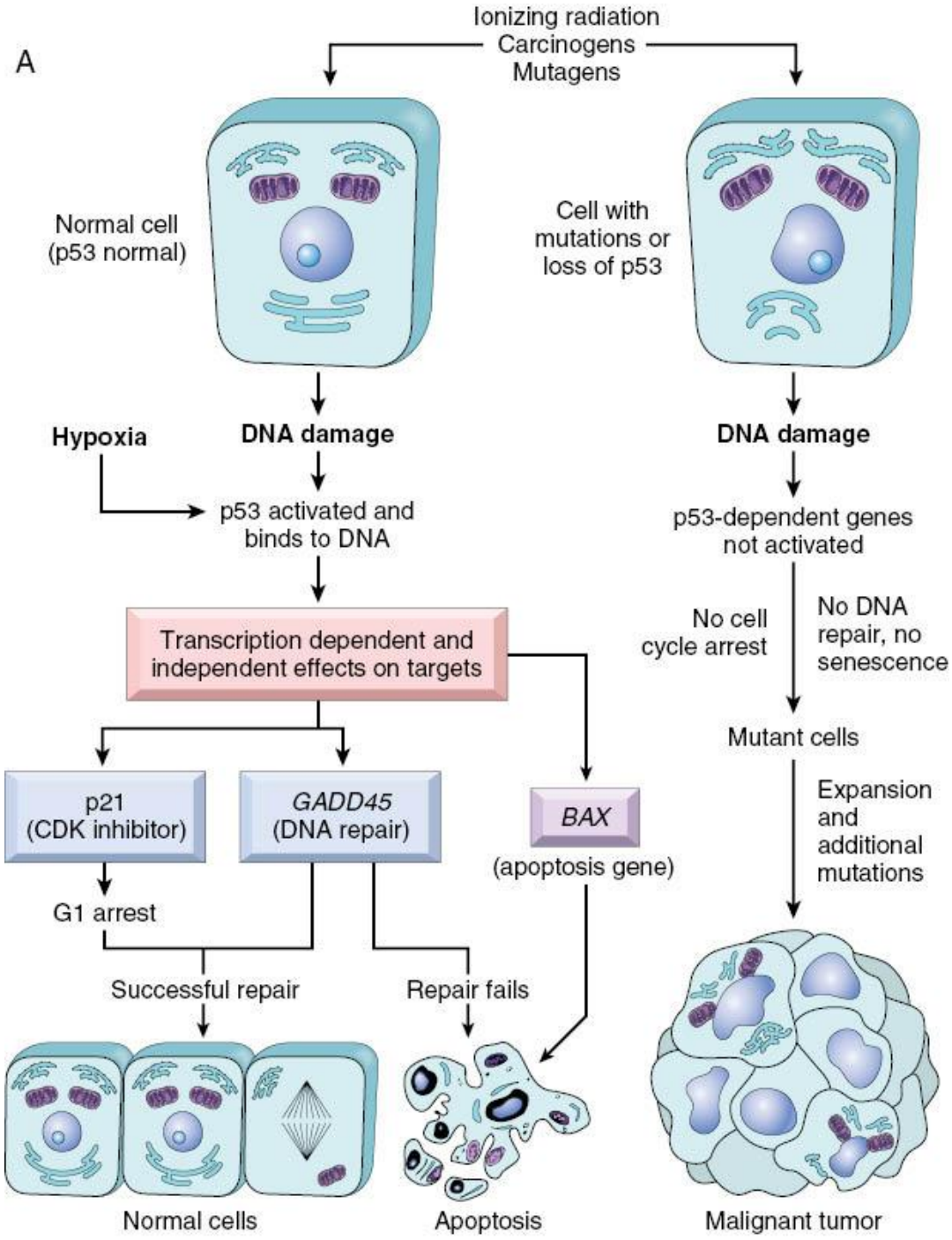
p53 guardian of genome

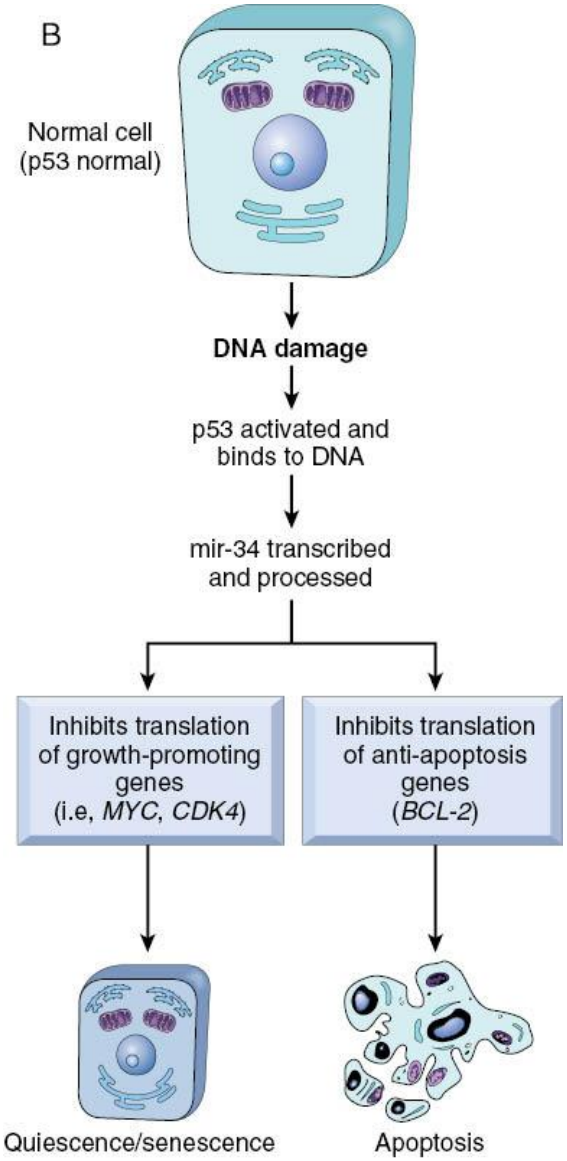
1. Fxn: Regulates cell damage
- If damage cannot be fixed, activates apoptosis

Normal p53 = TUMOR SUPPRESSOR

2. Mutated alleles = no regulation, cell cycle goes nuts!

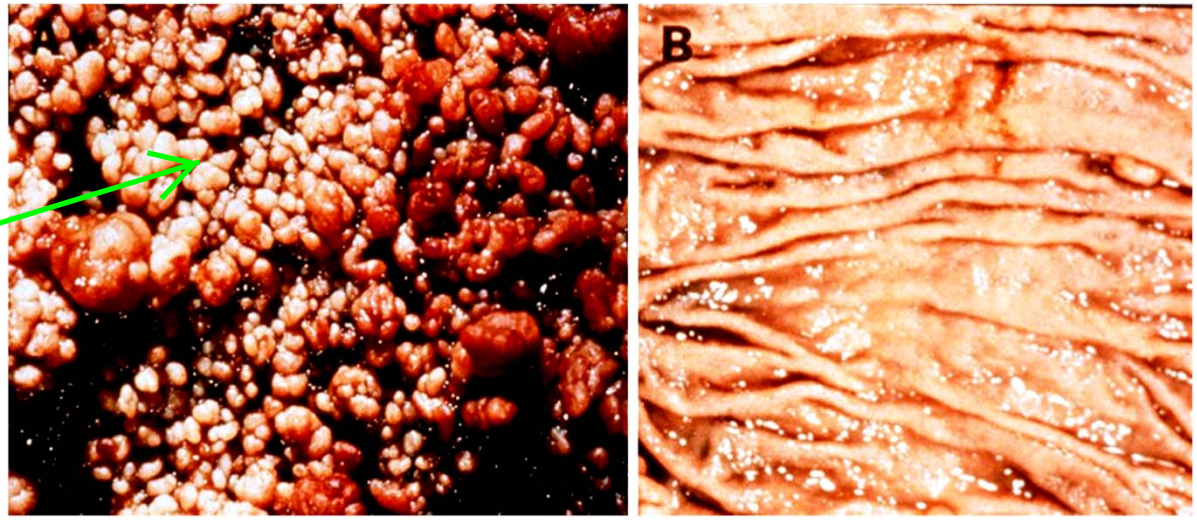






Tissue specific cancer genes:
APC mutations > colon cancers!
BRCA1 mutations> breast cancer

Gate Keeper Genes

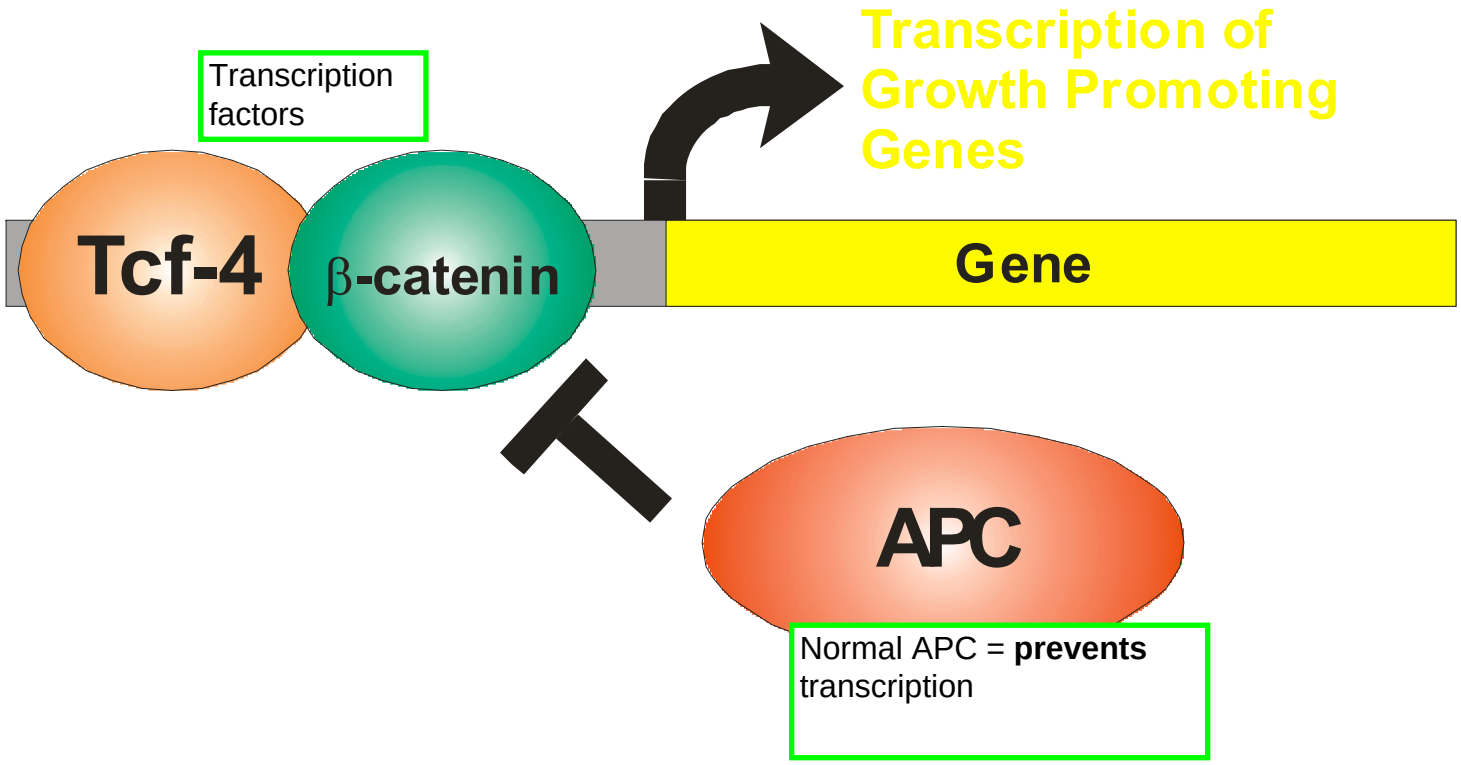


all polyps!
- Some of them benign, but some gain additional mutations!

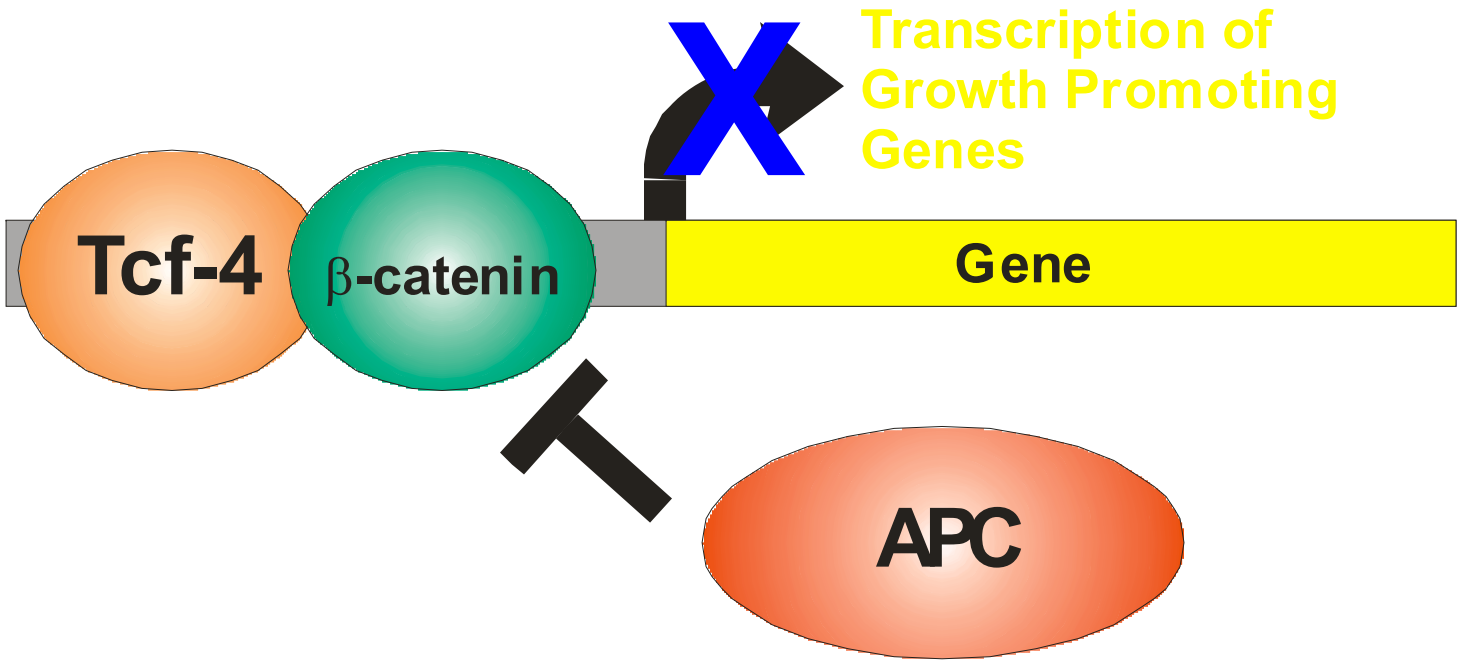
Familial Adenomatous Polyposis **Normal**

- Affects ~1 in 7,000 individuals
- Inherited in an autosomal dominant manner
- Multiple benign tumors (adenomas) of the colon and rectum
- *APC* germline mutation

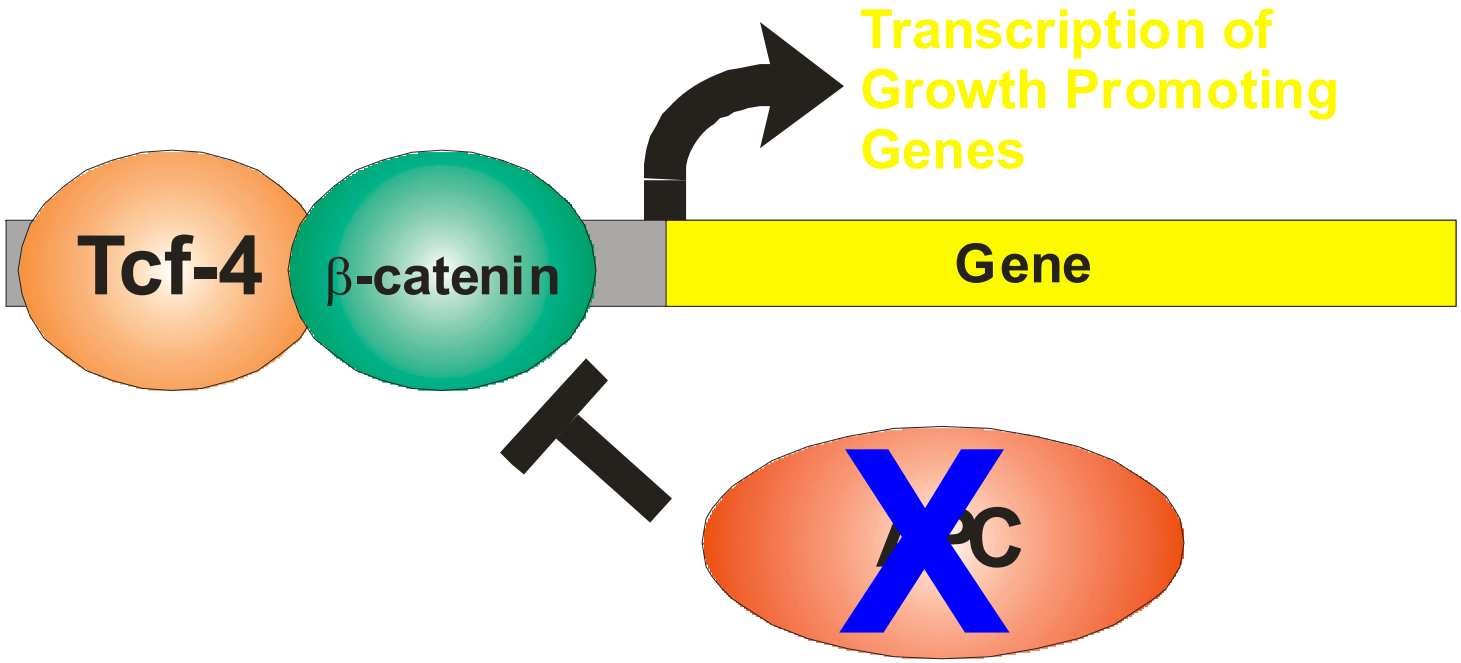
The APC/β-Catenin Pathway in Colorectal Epithelial Cells



Normal Colorectal Epithelial Cells

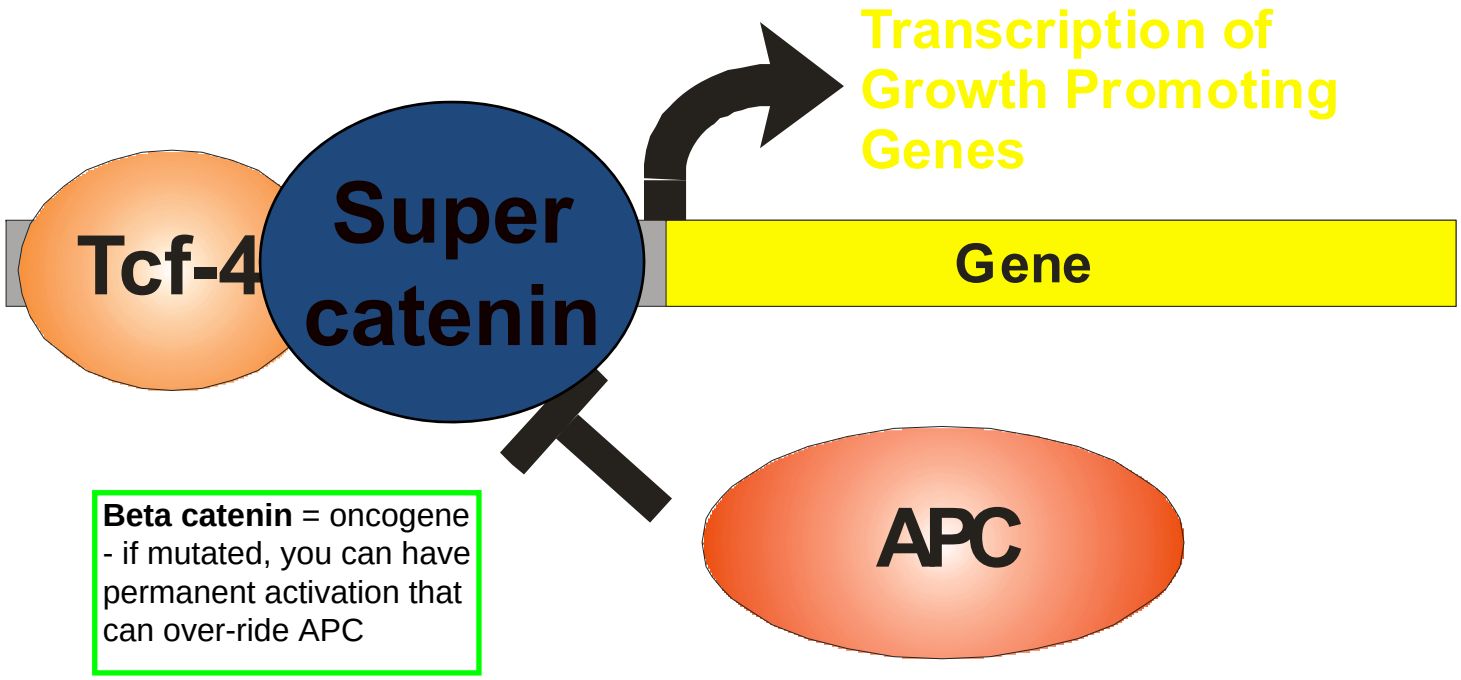


~80% of Colorectal Tumors



When mutated, "releases the brakes" and transcription continues without stopping!

~5 % of Colorectal Tumors



Beta catenin = oncogene - if mutated, you can have permanent activation that can over-ride APC

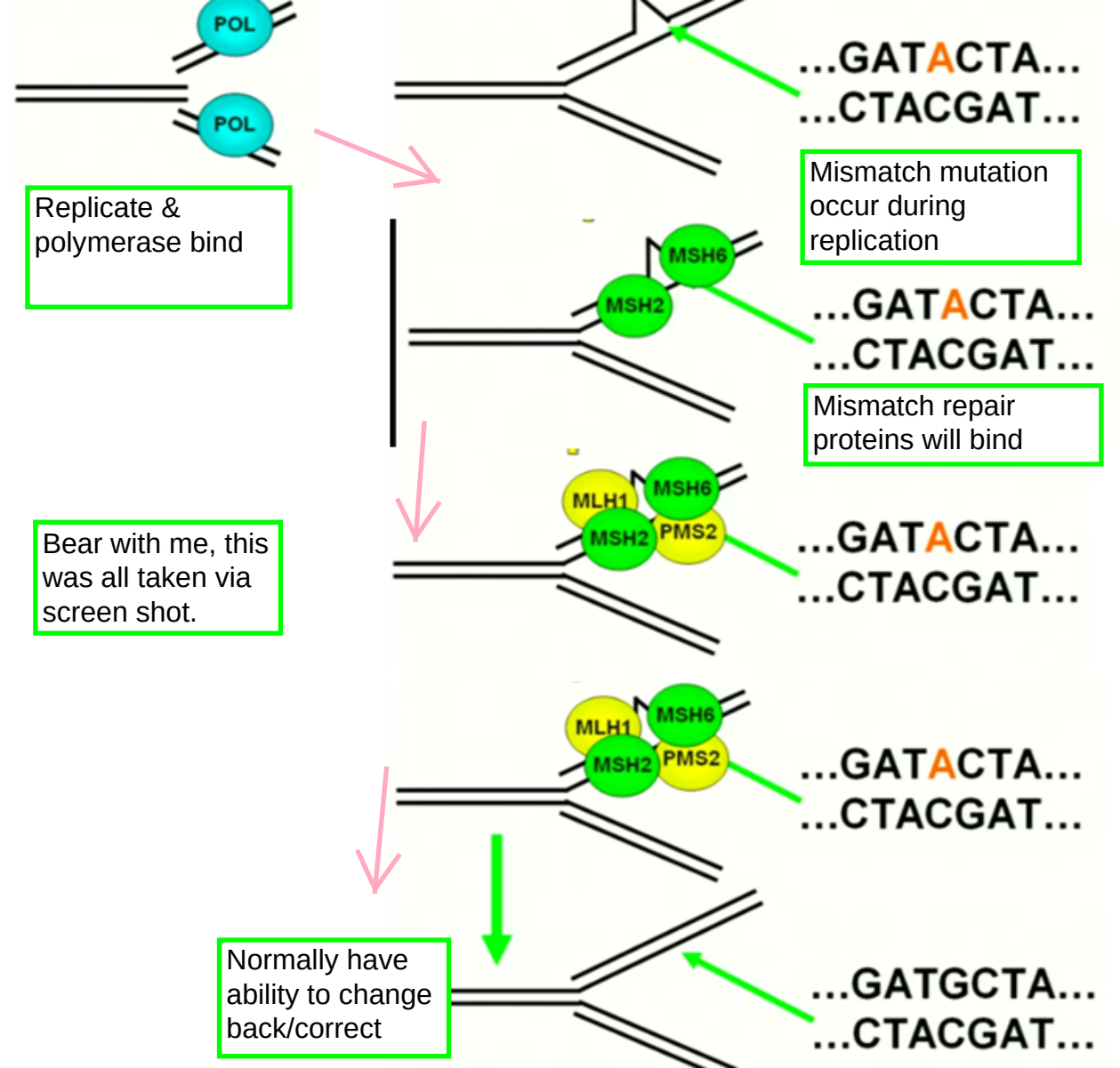
Caretaker genes

Maintain genomic stability by DNA repair or whole chromosome stability.

Caretaker genes are not oncogenic, but their loss of function increases the risk of mutations in other genes, including oncogenes and tumor suppressor genes.

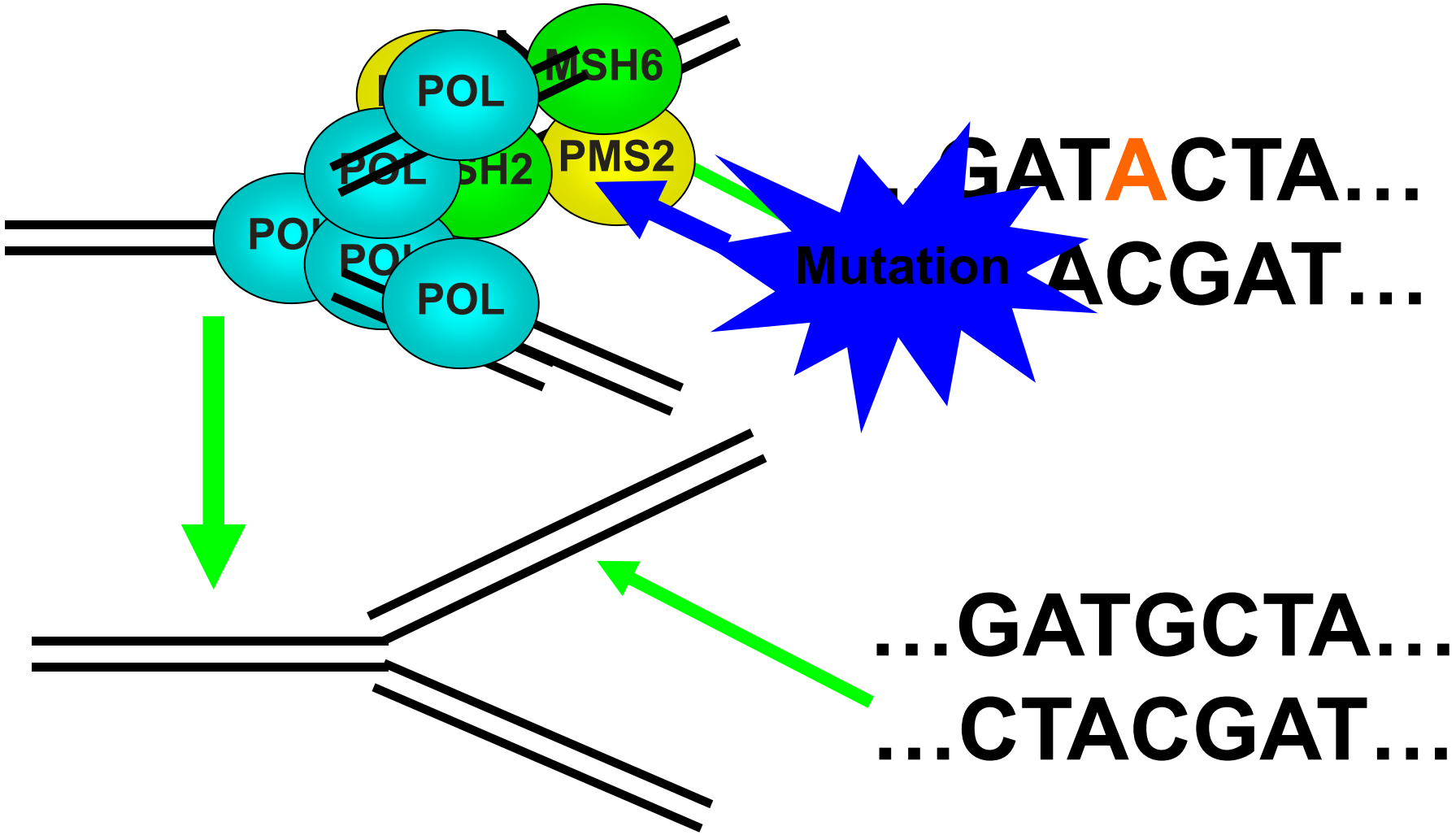
Hereditary Nonpolyposis Colon Cancer (HNPCC)

- Do not arise in polyps
- Colon cancer occurs at young age
- Multiple direct family relatives
- Microsatellite instability
- DNA mismatch repair genes mutations


POL
POL
Replicate & polymerase bind
…GATACTA…
…CTACGAT…
Mismatch mutation occur during replication
MSH6
MSH2
…GATACTA…
…CTACGAT…
Mismatch repair proteins will bind
Bear with me, this was all taken via screen shot.
MLH1
MSH6
MSH2
PMS2
…GATACTA…
…CTACGAT…
MLH1
MSH6
MSH2
PMS2
…GATACTA…
…CTACGAT…
Normally have ability to change back/correct
…GATGCTA…
…CTACGAT…

However if any of the DNA repair proteins are mutated (MLH1, PMS2, MSH2, MSH6) -- will not fix mismatch!

Mismatch Repair

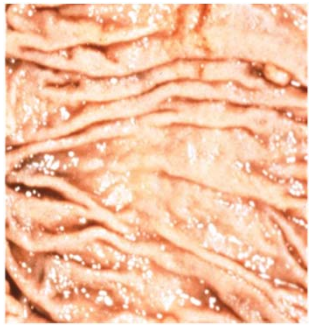


familial adenomatous polyposis

hereditary nonpolyposis colon cancer

FAP **HNPCC**

Normal Colon



APC

Tumor Initiation

Accelerated **Normal**

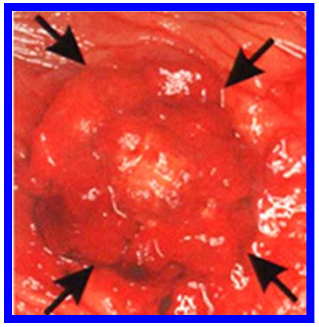
Polyp

RAS, p53,…

Tumor Progression

Normal **Accelerated**

Cancer



Intro: Because genome sequence is finished, we know a lot about it!
- Past 5 years, whole genomes of cancers have been sequences

Genetic Landscape of Cancers

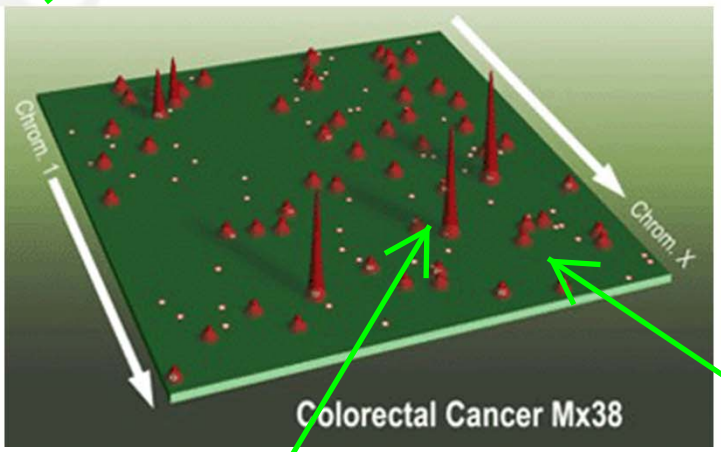
most common childhood brain tumor

most common adult brain tumor

Table 1. Summary of somatic sequence [illegible] five tumor types

	Medulloblastoma*	Pancreas#	Glioblastoma†	Colorectal‡	Breast‡
Number of samples analyzed	22	24	21	11	11
Number of mutated genes	218	1007	685	769	1026
Average number of non-silent mutations per sample	8	48	36	77	101

Why such a low number?
A: You only need to mutate the master regulators (gene transcription, protein expression, etc)



Landscape of a typical colorectal cancer. The large peaks indicate the gene mountains, small peaks indicate the hills.
Image Courtesy of Bert Vogelstein/HHMI at Johns Hopkins

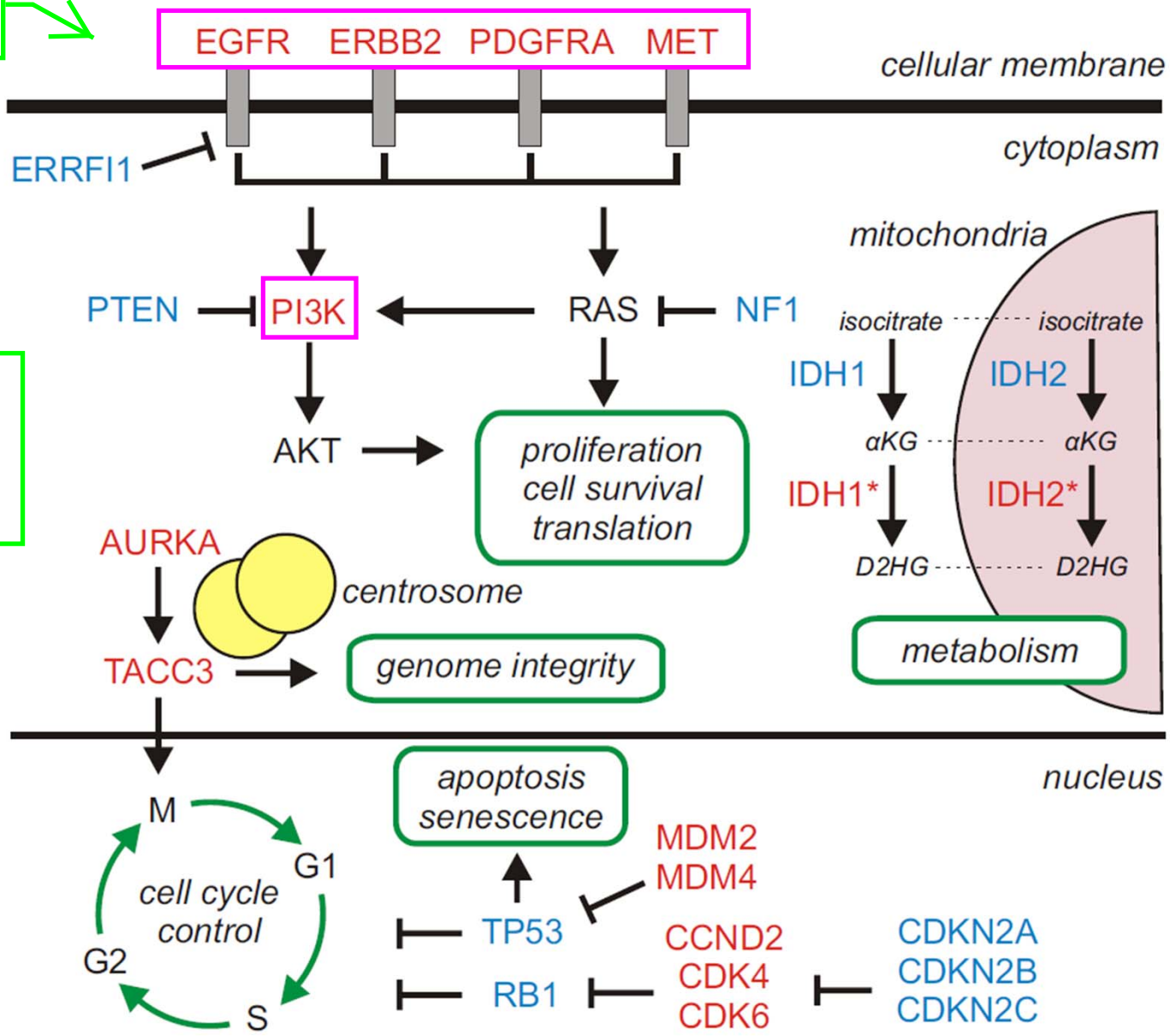
high frequency of gene mutation are the higher peaks

found many small mounds, not as many high peaks
Meaning: lots of different tumors, not all the same

Oncogenic pathways in GBMs

most drugs involved with these pathways

90% of cancer have genome aneuploidy
- multiple chromosomes



Cancer cell
- use more glycolysis/ metabolism, oxidation

Hypothesis: Primary cause of cancer hypothesized to be replacement of normal respiration of oxygen in normal body cell by the aerobic glycolysis

Warburg's hypothesis

“The prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by aerobic glycolysis.”-Warburg

Many of oncogenes and tumor suppressor genes are modulating the metabolic functioning of cancer cells.

Challenges

Diagnosis	Prognosis	Treatment
-Histology	-Karnofsky score/age	-Surgery -Radiation -Chemotherapy

Molecular solutions

-Molecular diagnosis	-Molecular prognosis	-Targeted therapy

Glioma Molecular Diagnosis at Duke

IMMUNOHISTOCHEMISTRY

	Interpretation Of IHC	% of Tumor Cells Exhibiting Staining	Score (0 – 3+)	Date of IHC Analysis dd/mm/yyyy
MGMT				
EGFR				
EGFR vIII				
PTEN				
pS6				
pMAPK				
pAKT				
Ki67 *				
CD45 (LCAg)				
CA IX				
PDGF α				
PDGF β				
VEGF				
VEGF R2				
HIF2 α				

	Interpretation	Types of Analysis*
MGMT - PCR		
EGFR vIII - PCR		
P53 Sequencing		
ICD H1 Mutation		
ICD H2 Mutation		

*** Types of Other Analysis**

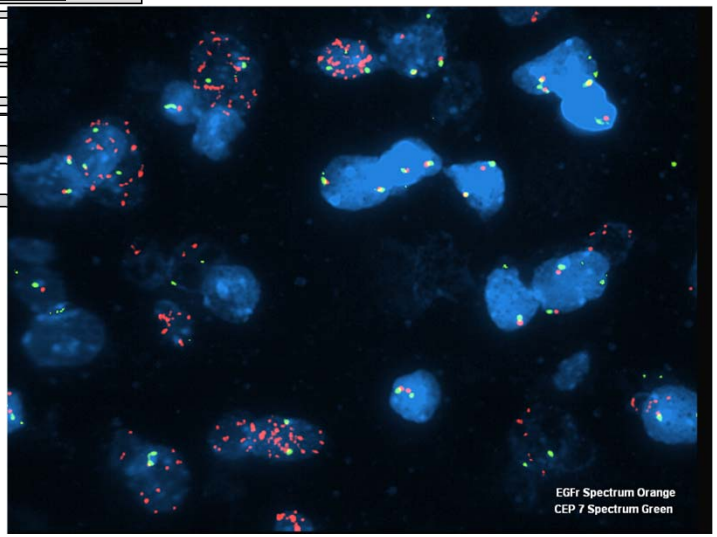
1= PCR Methylation
2= Protein Assay
3= Sequencing
4= PCR
5= Other:_____

Interpretation of other Analysis

Neg= Negative
Pos= Positive

	Interpretation Of FISH	% of Tumor Cells Exhibiting Abnormality
EGFR		
7 cep		
C-Met		
PTEN		
10 cep		
9p21		
9 cep		
C-Kit		
4 cep		
1p36		
1p32		
19 q13		

FISH and sequencing of cancer genomes does occur at Duke



EGFR amplification in a GBM sample

Oncogenes as Therapeutic Targets

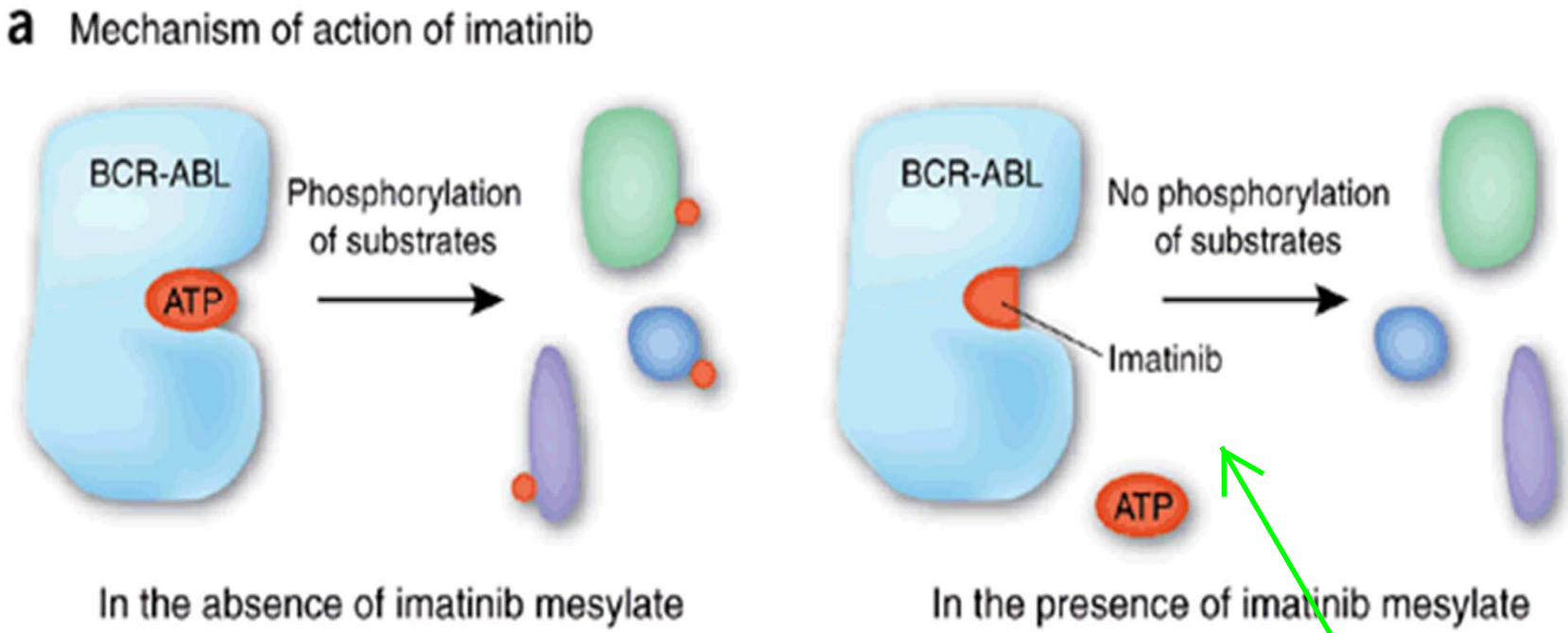
The NEW ENGLAND JOURNAL of MEDICINE

Table 1. Cancer Therapies That Target Oncogenic Proteins.*

Anticancer Drug	Target	Disease
Monoclonal antibodies		
Trastuzumab (Herceptin, Genentech)	ERBB2	Breast cancer
Cetuximab (Erbitux, ImClone)	EGFR	Colorectal cancer
Bevacizumab (Avastin, Genentech)	VEGF	Colorectal cancer, non–small-cell lung cancer
Small molecules		
Imatinib (Gleevec, Novartis)	ABL, PDGFR, KIT	Chronic myelogenous leukemia, gastrointestinal stromal tumors, chordoma
Gefitinib (Iressa, AstraZeneca)	EGFR	Non–small-cell lung cancer
Erlotinib (Tarceva, Genentech)	EGFR	Non–small-cell lung cancer
Sorafenib (Nexavar, Bayer/Onyx)	VEGFR, PDGFR, FLT3	Renal-cell carcinoma
Sunitinib (Sutent, Pfizer)	VEGFR, PDGFR, FLT3	Gastrointestinal stromal tumors, renal-cell carcinoma

* EGFR denotes epidermal growth factor receptor, FLT3 FMS-like tyrosine kinase 3, PDGFR platelet-derived growth factor receptor, and VEGF vascular endothelial growth factor.

Potential targets and mechanisms of action of mutation-targeted drugs



Magic bullets

Glivac inhibits ATP binding

Session-specific objectives

Explain the ways in which oncogenes develop from normal genes involved in growth regulation

Explain how cancer develops from modifications of tumor suppressor genes