Molecular Biology of Cancer

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September 2014

Learning outcomes

At the end of this lecture you will able to:

- Enumerate the types of cancer.
- Enumerate the main causes of cancer
- Correlate risk factors and cancer incidence
- Explain the three major cancer steps.
- Explain the role of oncogenes and tumor suppressor genes in cancer.
- Explain how oncogenes are activated
- Enumerate some oncogenes and tumor suppressor genes
- Explain the role of TP53 in the cell
- Explain how the deregulation of cell cycle and apoptosis contributes to cancer progression
- Explain the role of EMT, metastasis and tumor microenvironment contributes to cancer progression
- Explain some characteristics of cancer cells
- Explain how siRNA and miRNA-based therapies work
- Enumerate some risk factors and some ways to prevent oral cancer

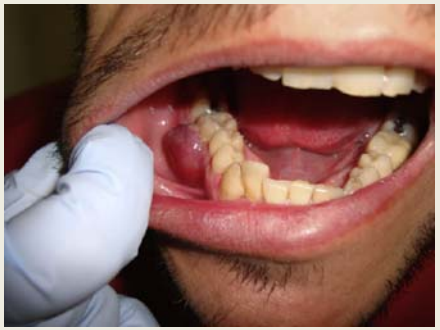
What is Cancer?

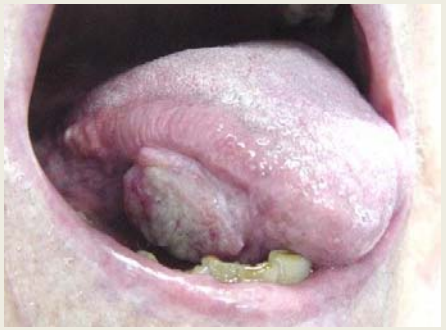
- Simplest definition

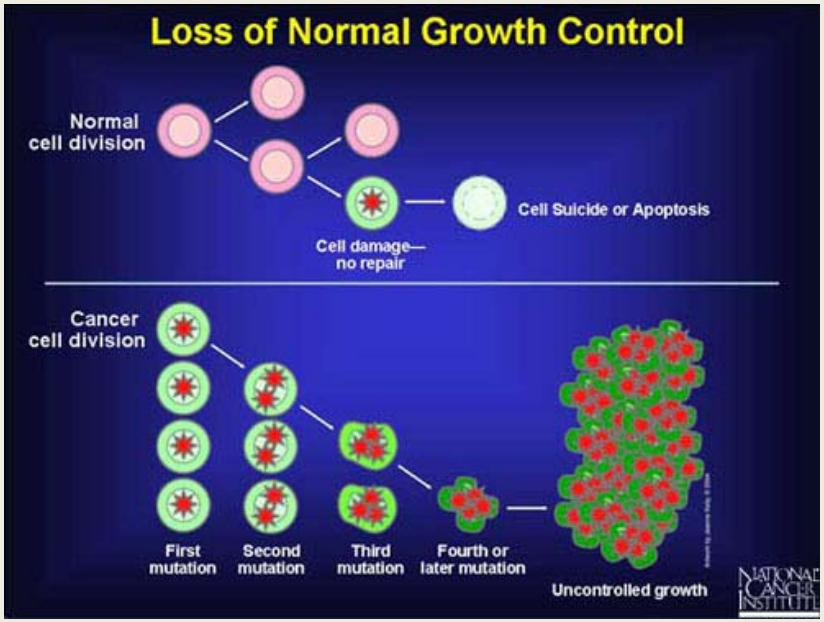
  From the American Cancer Society

  " cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death."

- Tumor
  - Two types:
    - ***Benign*** (non-cancerous) – this is *not* cancer!
      - Does not spread; it can eventually become malignant in some cases.
    - ***Malignant*** (cancerous) – this is cancer!
      - Has the potential to spread to other parts of body.



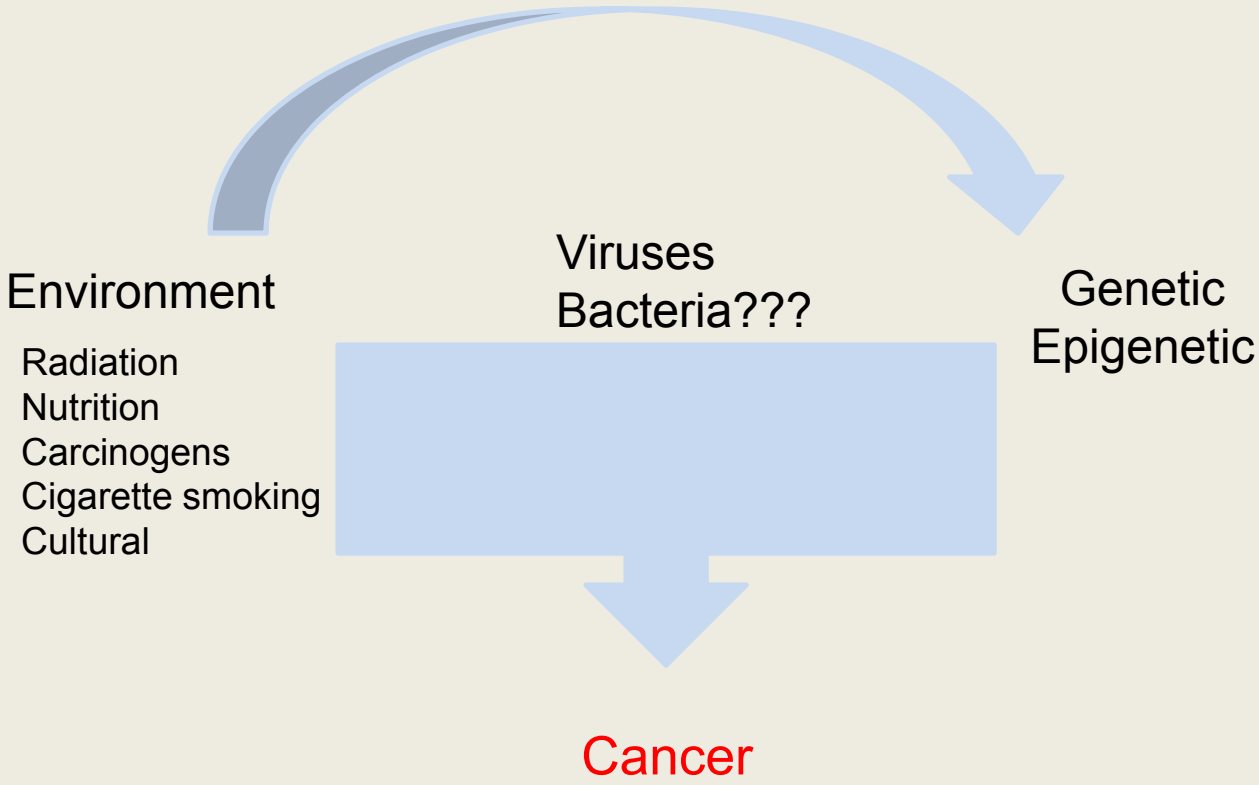




Types of Cancer

- Carcinoma
  - Cancer of epithelium tissue.
  - Skin, breast, prostate, lung, ovary, liver cancers.
- Sarcoma
  - Cancer of connective and fibrous tissue.
  - Bone, muscle, cartilage, fat cancers.
- Lymphoma
  - Cancer of lymph nodes or system.
- Leukemia
  - Cancer of blood-forming cells of marrow.

Cancer, risk factors (origin, causes)

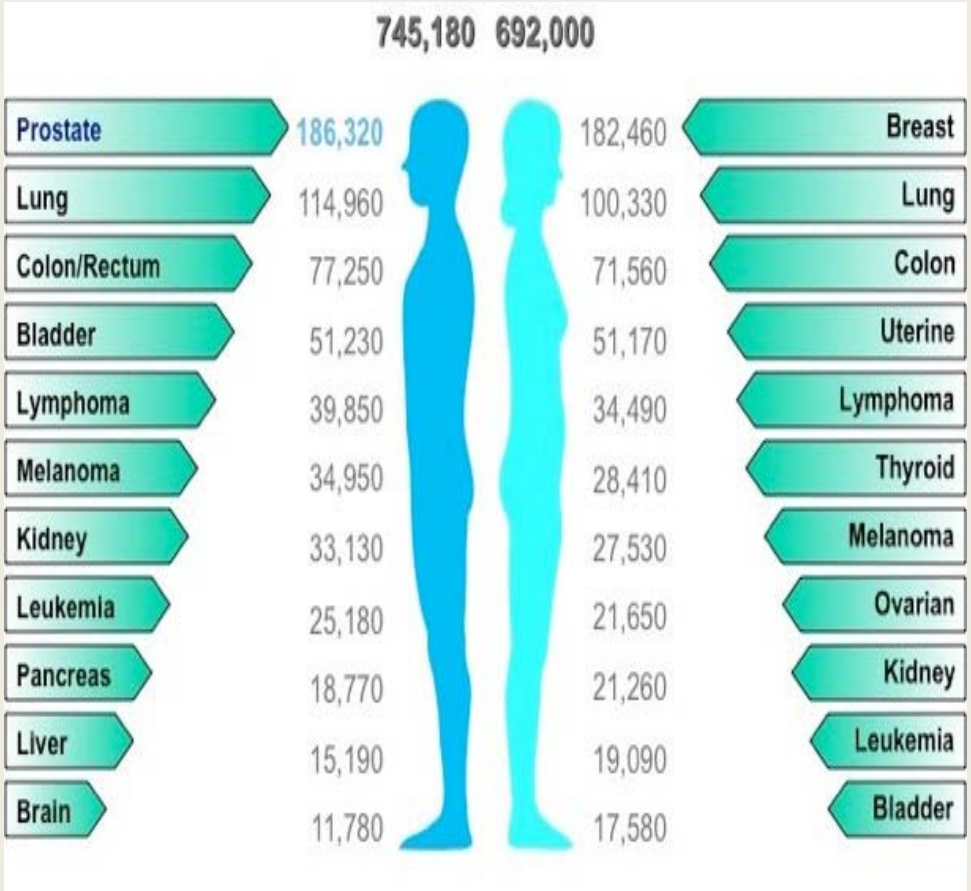


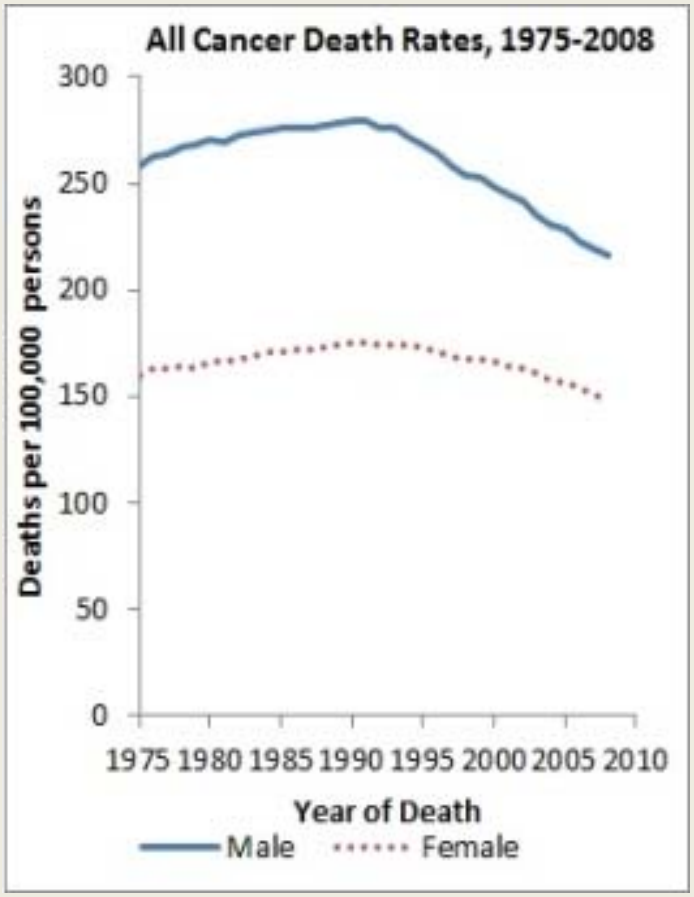
Human Cancers Known to Be Caused by Viruses

CANCER	ASSOCIATED VIRUS
Liver cancer	Hepatitis B virus
Lymphoma, nasopharyngeal cancer	Epstein–Barr virus
T cell leukemia	Human T cell leukemia virus
Anogenital cancers	Papillomavirus
Kaposi's sarcoma	Kaposi's sarcoma herpesvirus

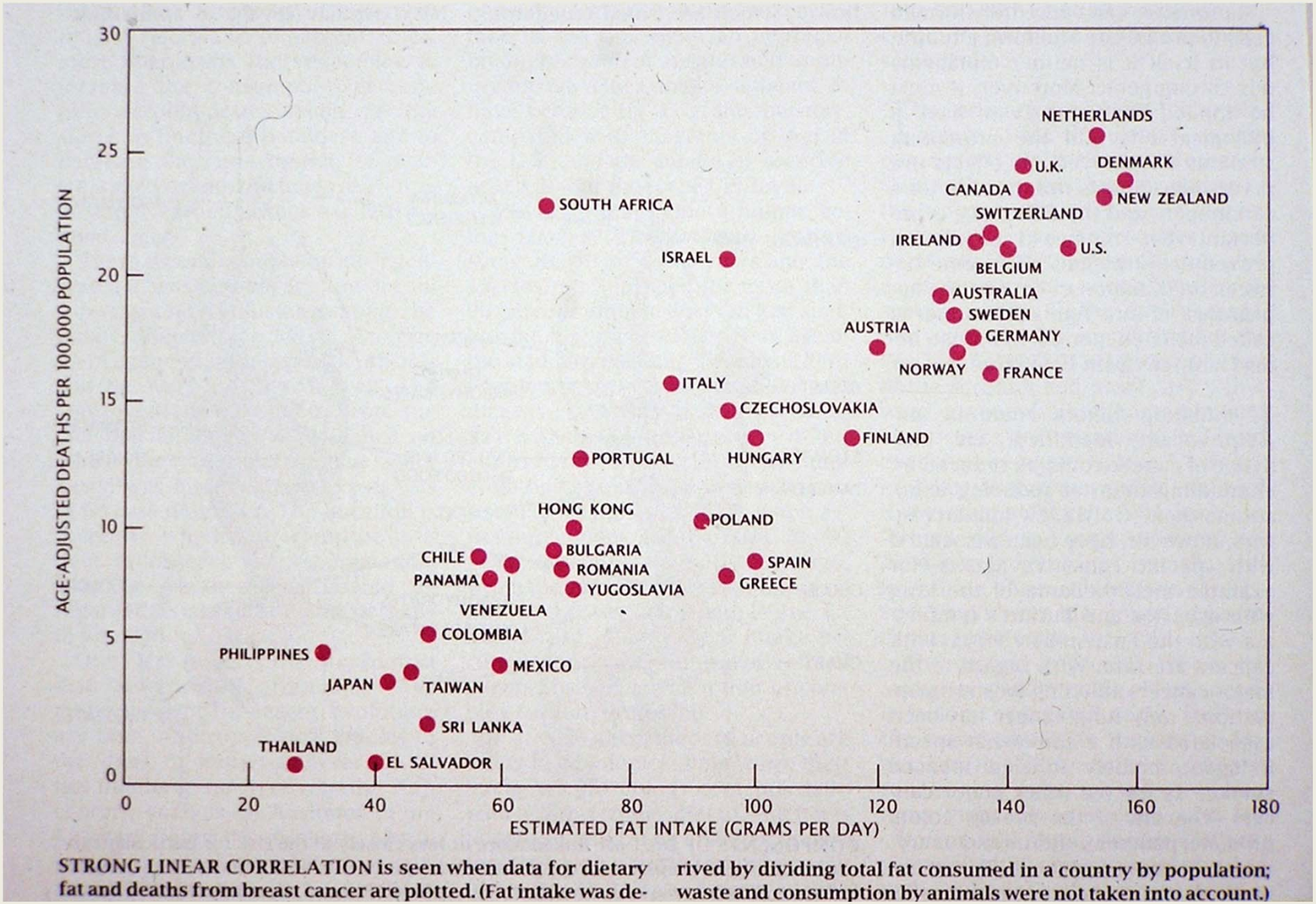
Cancer statistics

New cases, 2012



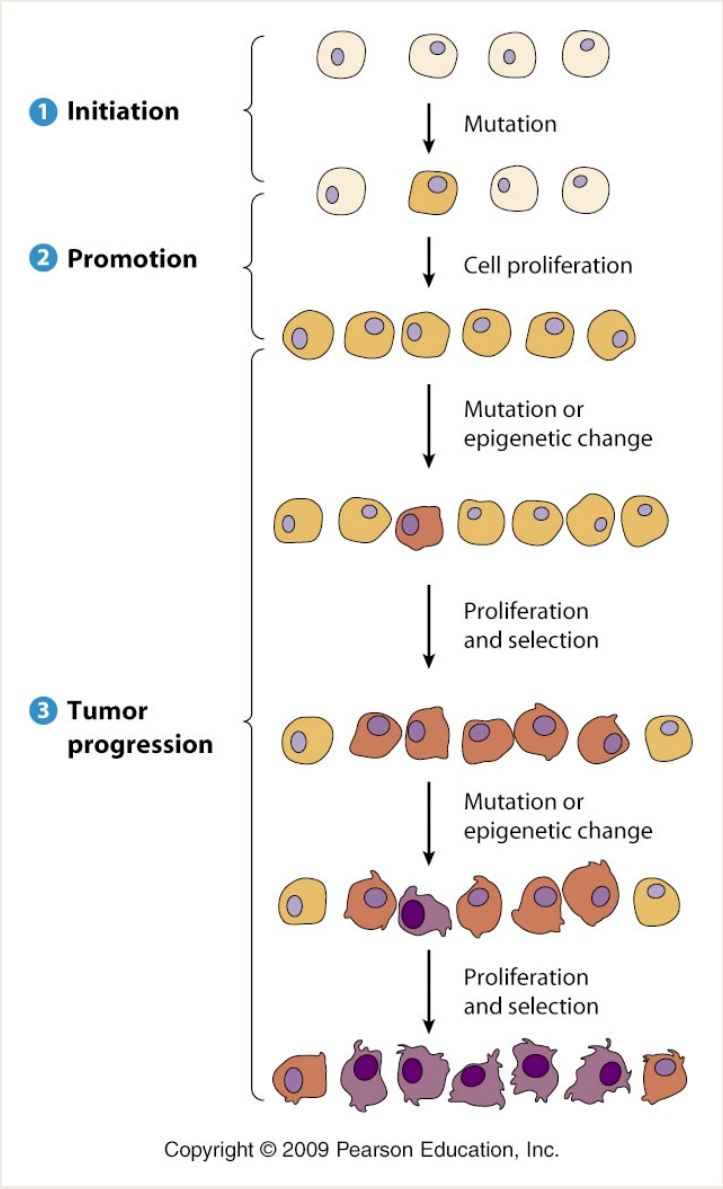


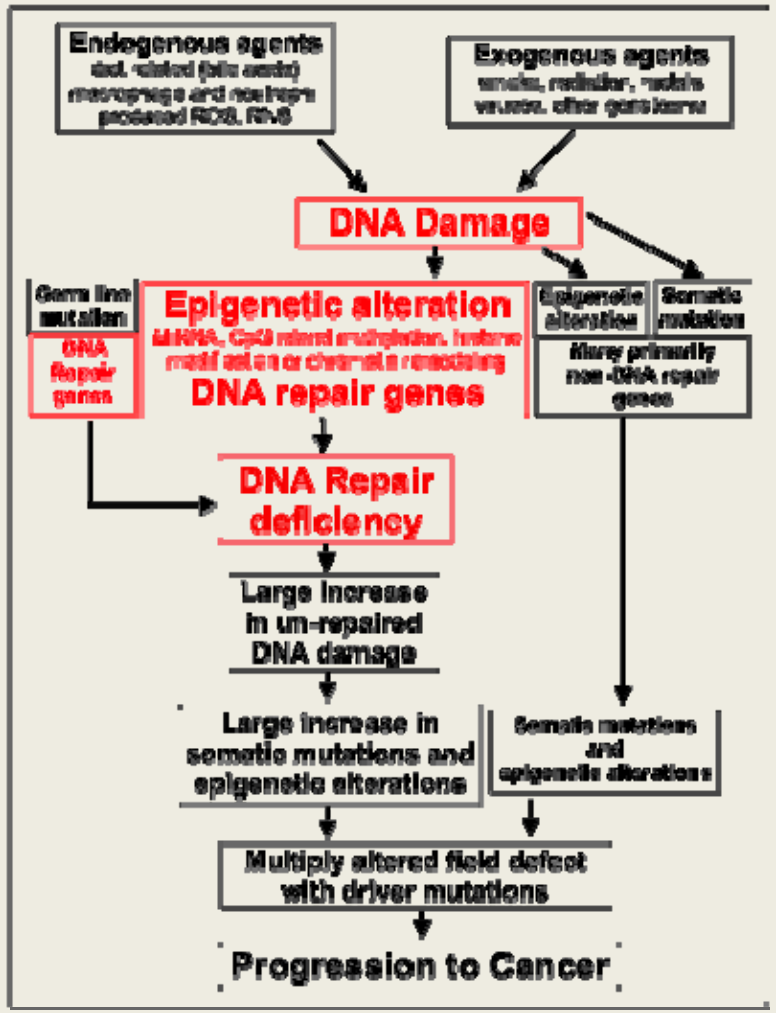
Dietary Fats and Breast Cancer Deaths



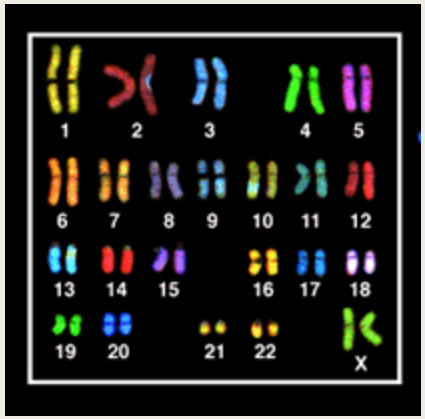
STRONG LINEAR CORRELATION is seen when data for dietary fat and deaths from breast cancer are plotted. (Fat intake was derived by dividing total fat consumed in a country by population; waste and consumption by animals were not taken into account.)

Stages of Cancer Development
Multiple mutations Are Required to Form Cancer

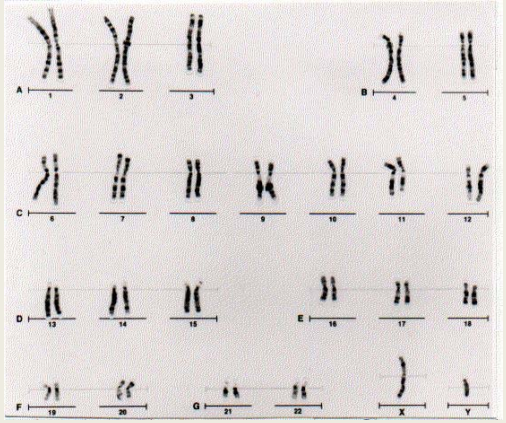




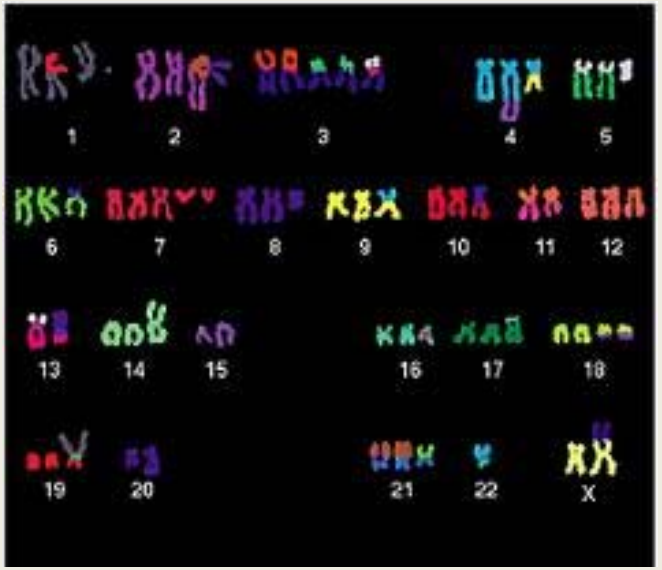
Karyotype of a Cancerous cell



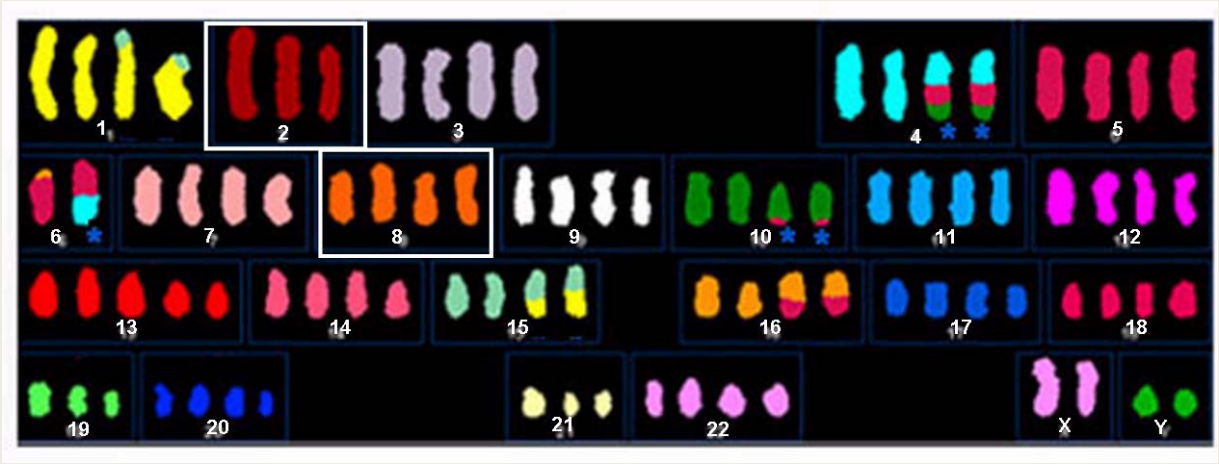
Female



Male



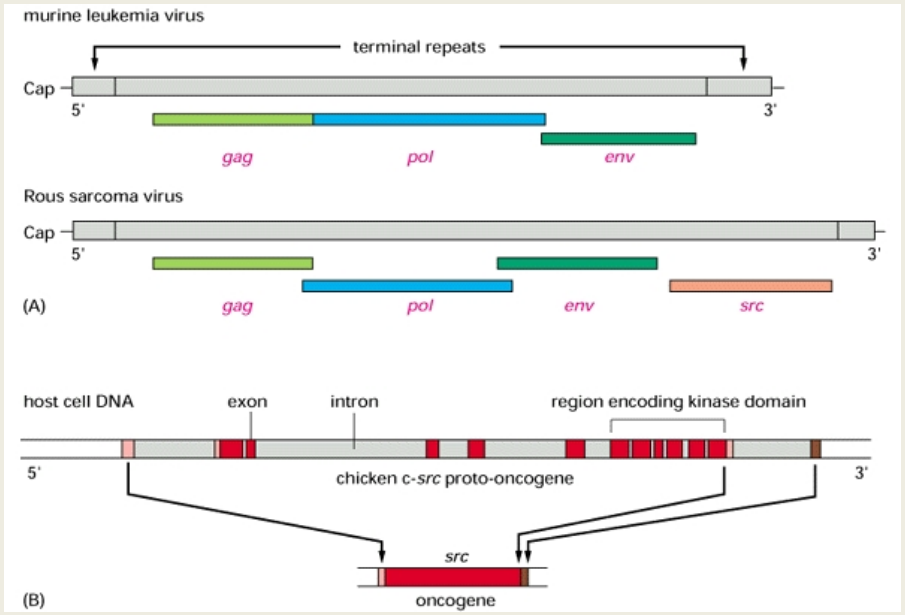
Breast cancer cells



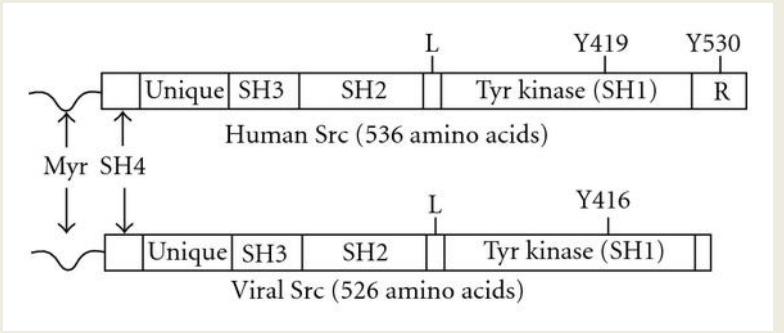
Prostate Cancer Cell

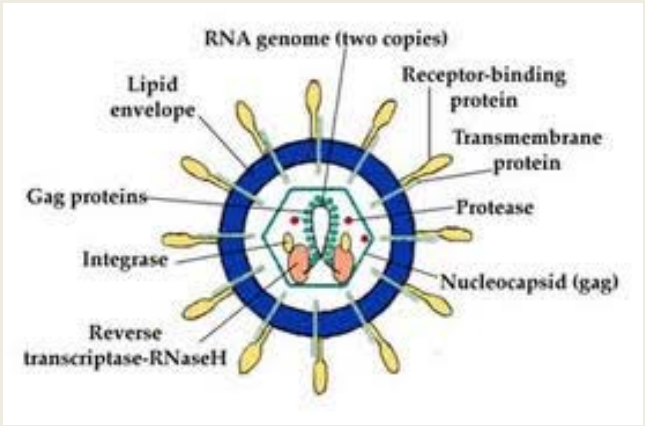
Oncogenes and Tumor Suppressor Genes

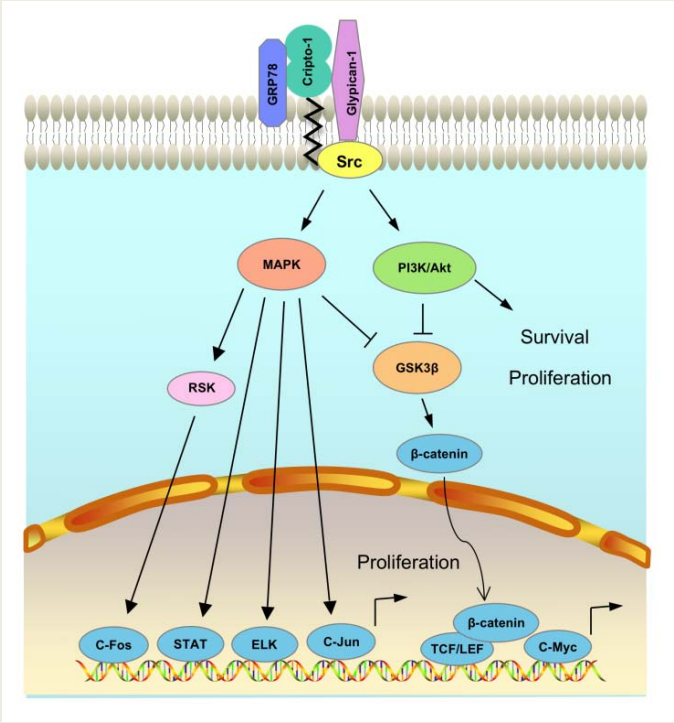
Rous sarcoma virus: In 1911 Peyton Rous discover that this retrovirus causes cancer in a chicken.



v-*src* c-*src*







Some Common Oncogenes

HER-2/*neu*

HER-2/*neu* encodes for a cell surface receptor that can stimulate cell division
The HER-2/*neu* gene is amplified in up to 30% of human breast cancers

RAS

The *Ras* gene products are involved in kinase signaling pathways that ultimately control transcription of genes, regulating cell growth and differentiation.
Overexpression and amplification of *RAS* can lead to continuous cell proliferation.

MYC

The *Myc* protein is a transcription factor and controls expression of several genes.
Myc is thought to be involved in avoiding the cell death mechanism.
MYC oncogenes may be activated by gene rearrangement or amplification.

SRC

SRC was the first oncogene ever discovered.
The *Src* protein is a tyrosine kinase which regulates cell activity.

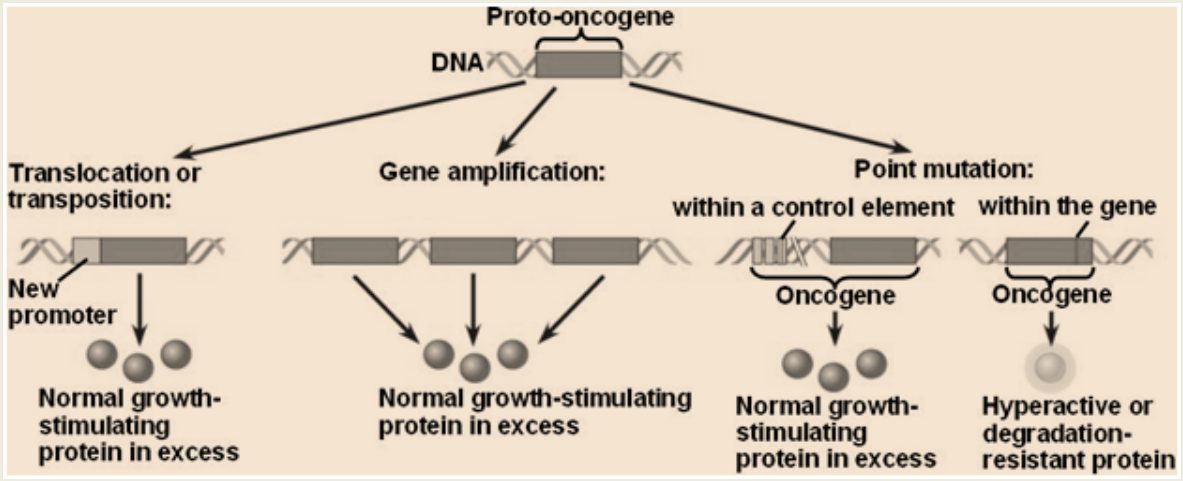
hTERT

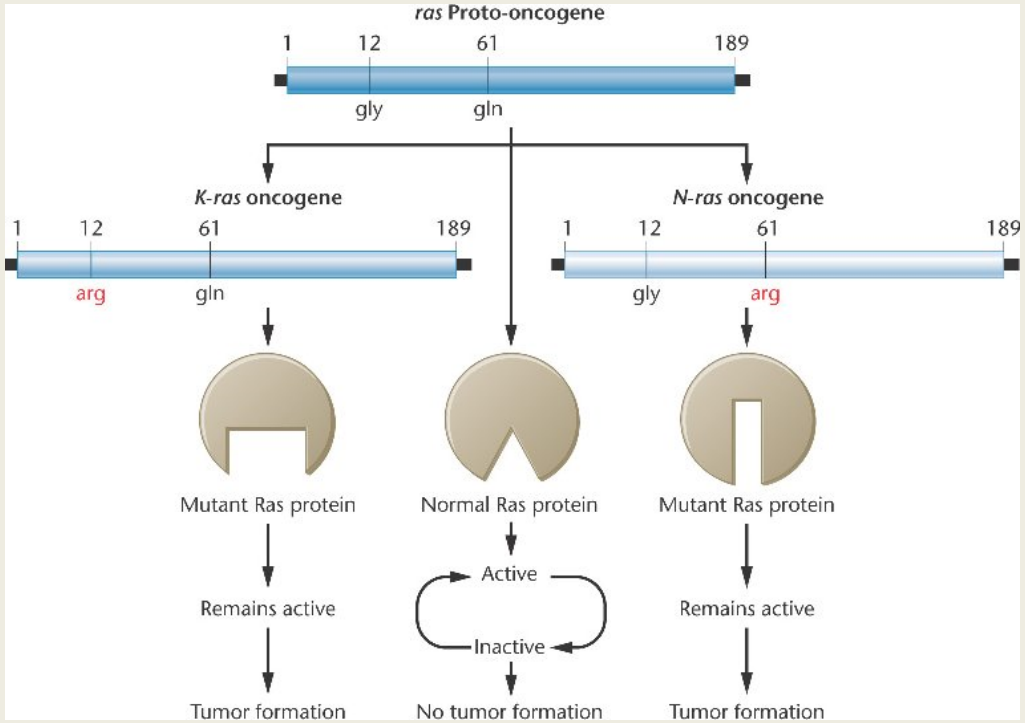
hTERT codes for an enzyme (telomerase) that maintains chromosome ends.
In most normal cells telomerase is only present during fetal development.
Activation of *hTERT* in adult cells gives them the ability to divide indefinitely.

BCL-2

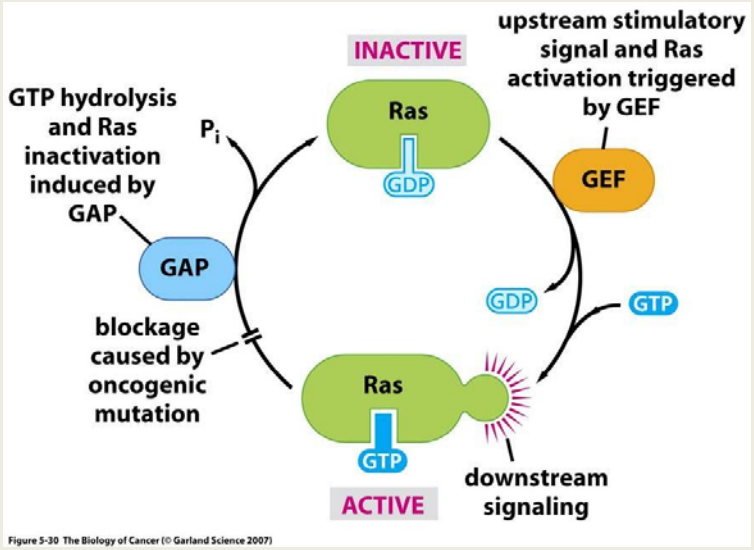
The Bcl-2 protein works to prevent cell death (apoptosis).
Overexpression of BCL-2 allows continued division of mutated cells.

Cellular proto-oncogenes



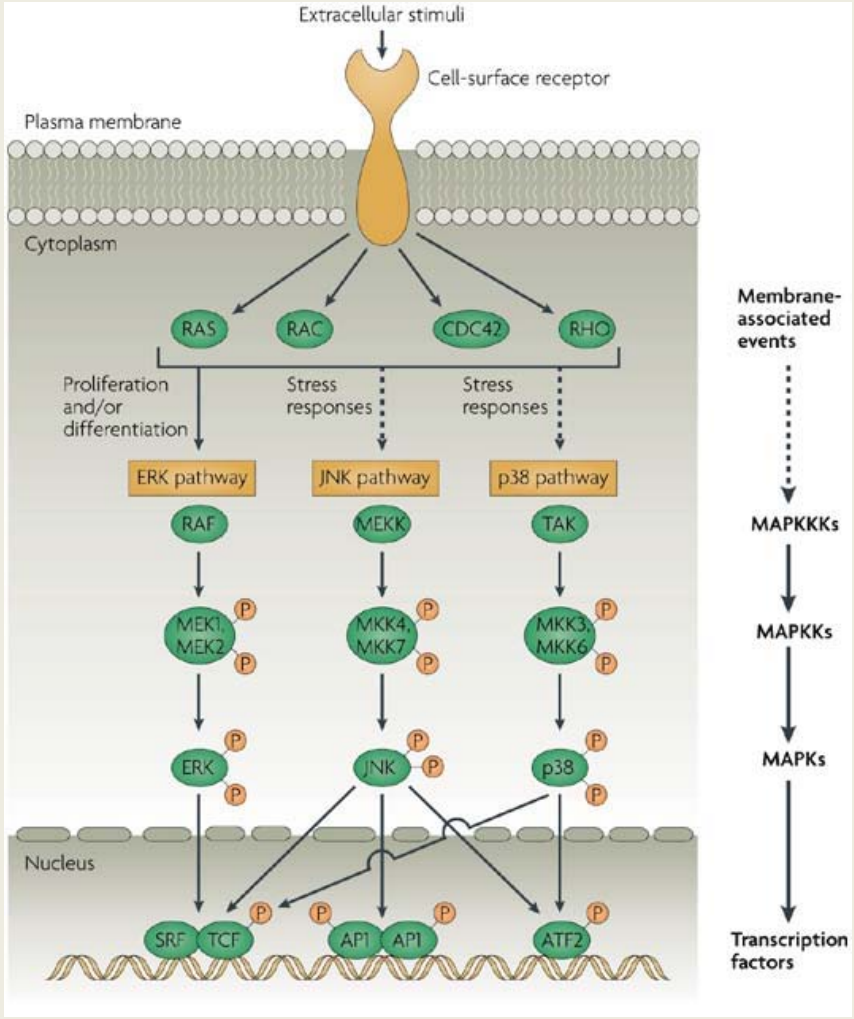


Growth Factor Signaling Transduction Pathway



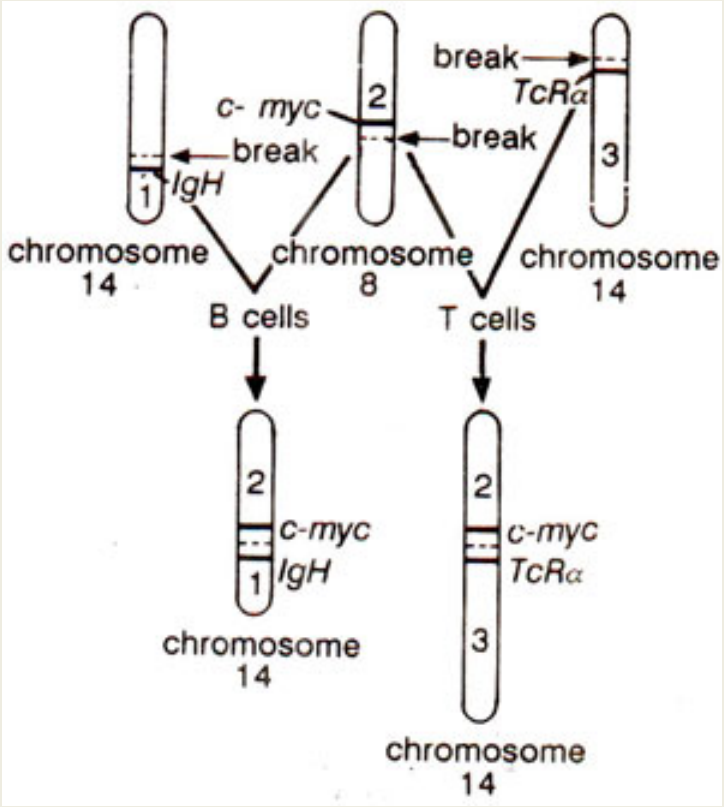
GEF: guanine nucleotide exchange factor

GTPase-Activating Proteins

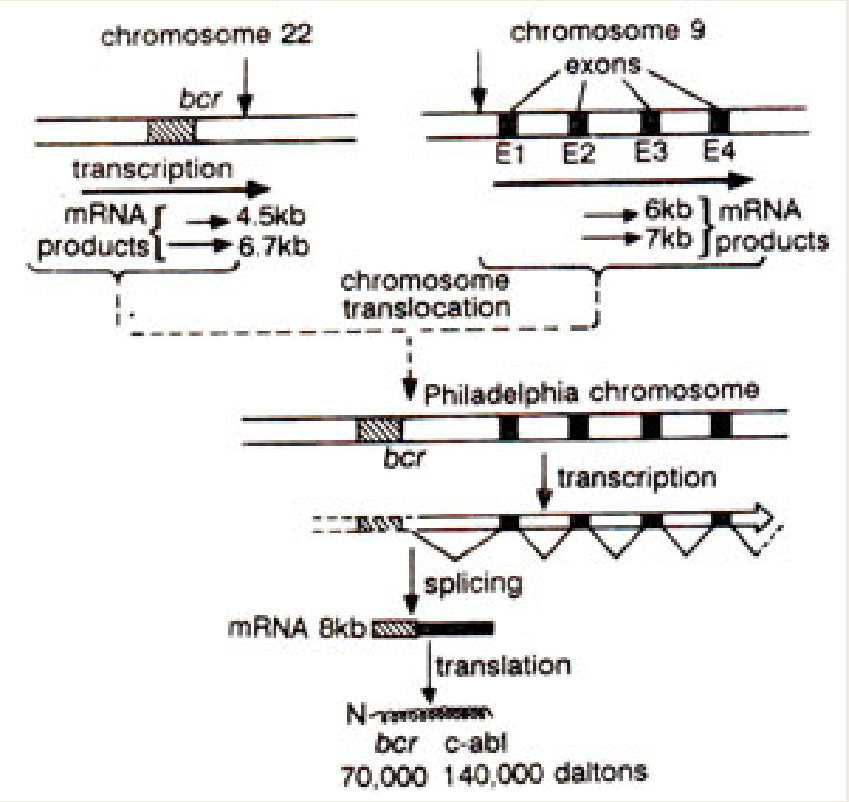


Ras is mutated in 30% of human cancers.
Mutations maintain Ras-GTP (active).
Wild-type Ras is a proto-oncogene, its mutations are oncogenes.

Activation of proto-oncogenes by cellular mechanisms



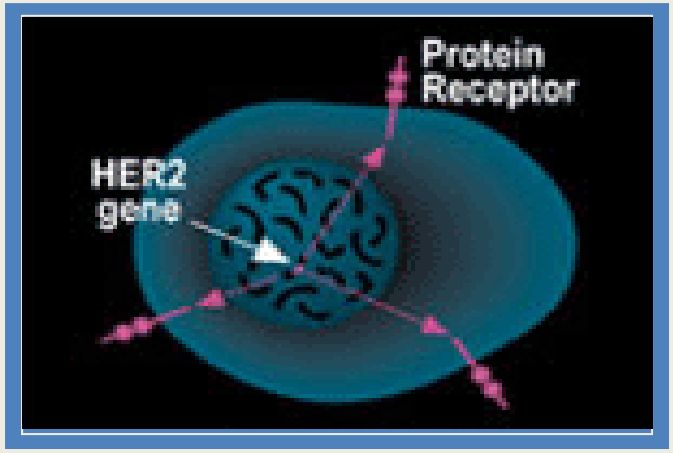
Translocations leading to activation *of c-myc* gene; in B cells *c-myc* is translocated to the proximity of Ig locus, while in T cells, it is translocated to the vicinity of TcRα locus (in both cases *c-myc* gets activated).

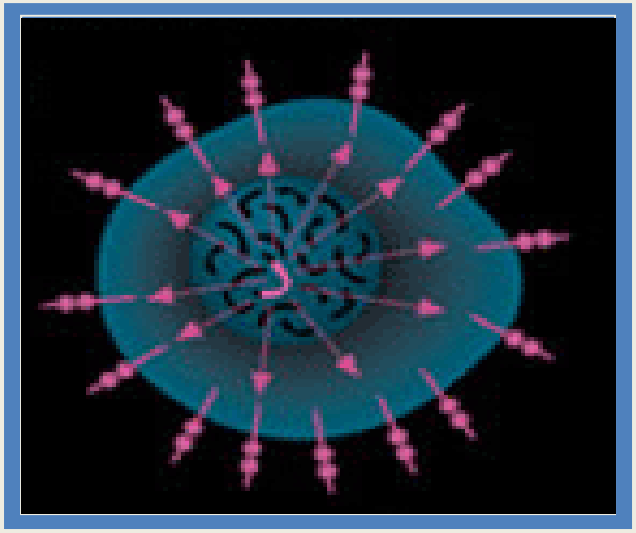


breakpoint cluster region-Abelson

A translocation between chromosome 22 and chromosome 9 generating a Philadelphia chromosome (*PH*[1]), associated with chronic myelogenous leukemia (CML).

HER2 is amplified in many breast cancers

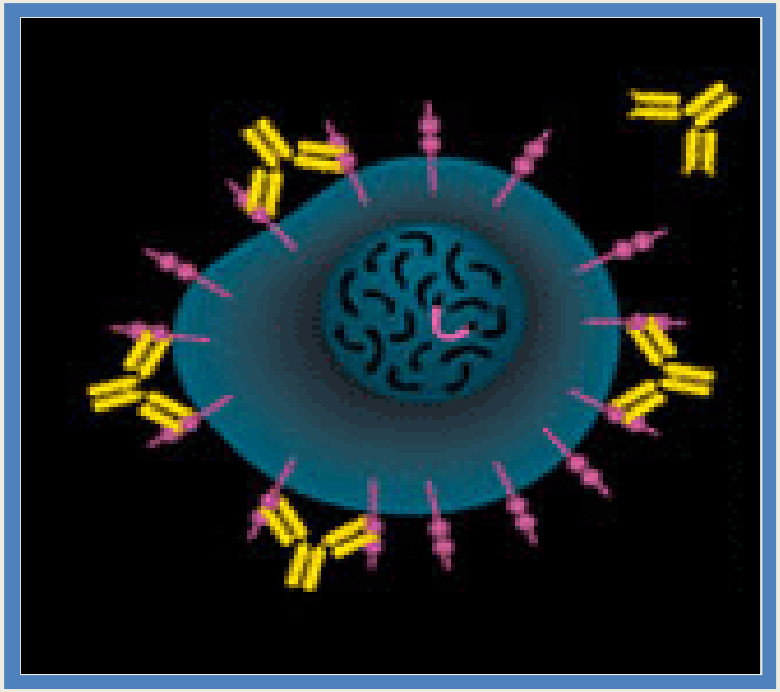




- Encodes transmembrane receptor tyrosine kinase, overexpression leads to homodimer formation → constitutively active expression
- HER2 amplification is found in 20-25% of breast cancers
- leads to increased gene expression and an increase in cell proliferation
- amplification correlated with
  - More likely lymph node metastasis
  - Shortened time to relapse
  - Reduced overall survival

Antibodies to HER2 may become part of clinical treatment

- Antibodies to erbB2
  - are able to convert rapidly dividing breast cancer cells into growth-arrested cells
  - Remove the receptor from the cell surface
  - Attract natural killer cells to the cell, targeting it for destruction
  - Commercially available as Trastuzumab (Herceptin™) from Genentech and used in conjunction with chemotherapy

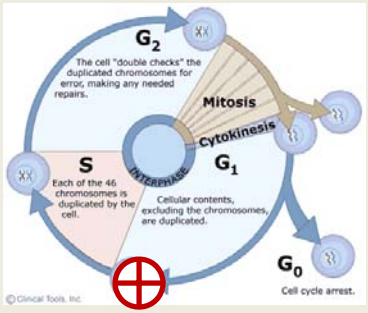


Main tumor Suppressor Genes

Tumor Suppressor	Function	Cancer
APC	Controls the function of specific transcription factors	Familial adenomatous and non-inherited colorectal carcinomas
BRCA1, 2	DNA damage repair	Inherited breast cancers; ovarian cancers
CDKN2A	Gene locus that encodes p16 and p14ARF	Brain tumors
DCC	Function is still unknown	Colorectal carcinomas
DPC4 (SMAD4)	Mediates signaling from growth factor receptors	Colorectal tumors, pancreatic neoplasia
MADR2/JV18 (SMAD2)	Mediates signaling from growth factor receptors	Colorectal cancer
MTS1	Inhibitor of cyclin-dependent kinases	Melanomas
NF1	RAS GTPase activating protein	Neurofibromatosis type 1
NF2	RAS GTPase activating protein	Neurofibromatosis type 2
p53	Encodes a transcription factor for p21 that arrests the cell cycle in G1 phase	Bladder, breast, colorectal, esophageal, liver, lung, prostate, and ovarian carcinomas; brain tumors, sarcomas, lymphomas, and leukemias
PTEN	Lipid phosphatase that regulates cell survival	Cowden syndrome; increased risk of breast and thyroid cancer

p53 Regulation of the Cdk Inhibitor p21

DNA damage

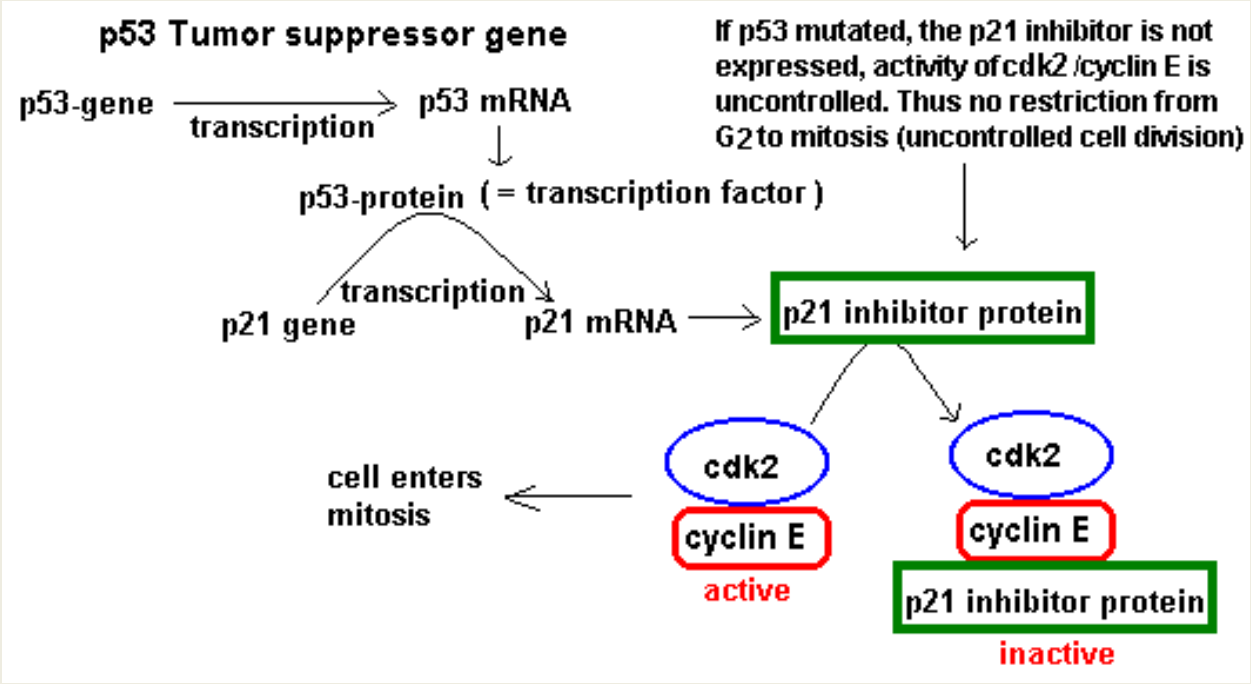


p53

p53 is a transcription factor called
"the guardian of the genome"

The loss of p53: cell division with DNA damage: Cancer

p53 is called a tumor suppressor gene



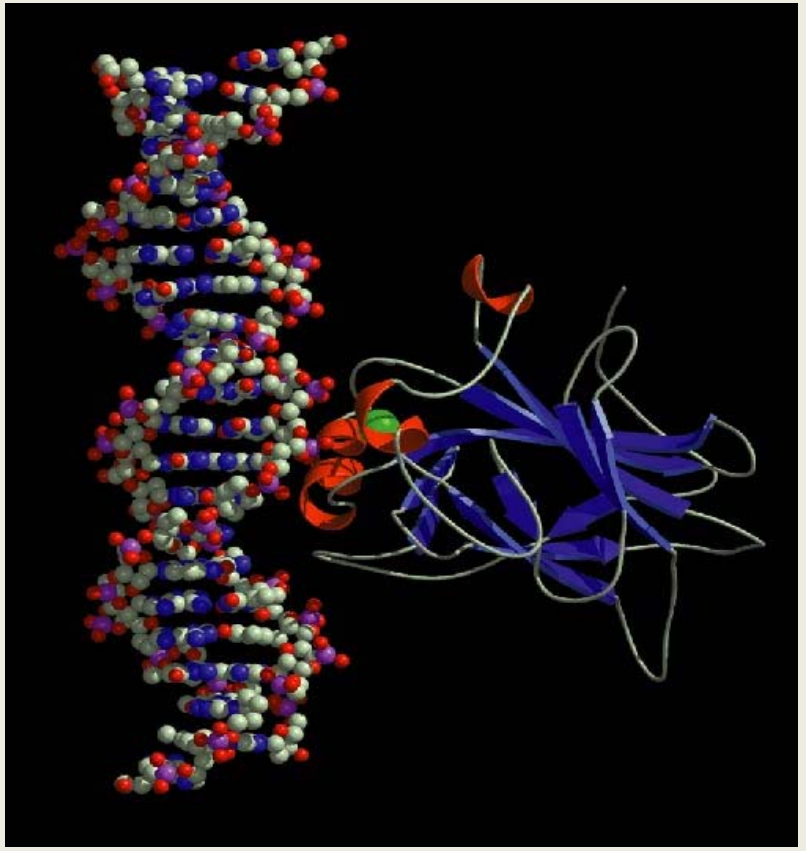
TP53 is inactivated in many forms of human cancer

One of the most commonly deleted mutated genes in human cancer

Complete loss of functional p53 occurs in over 50% of all human tumors

Loss of function is generally due to point mutation for one allele and loss of the other

Majority of mutations occur in central region of coding sequence

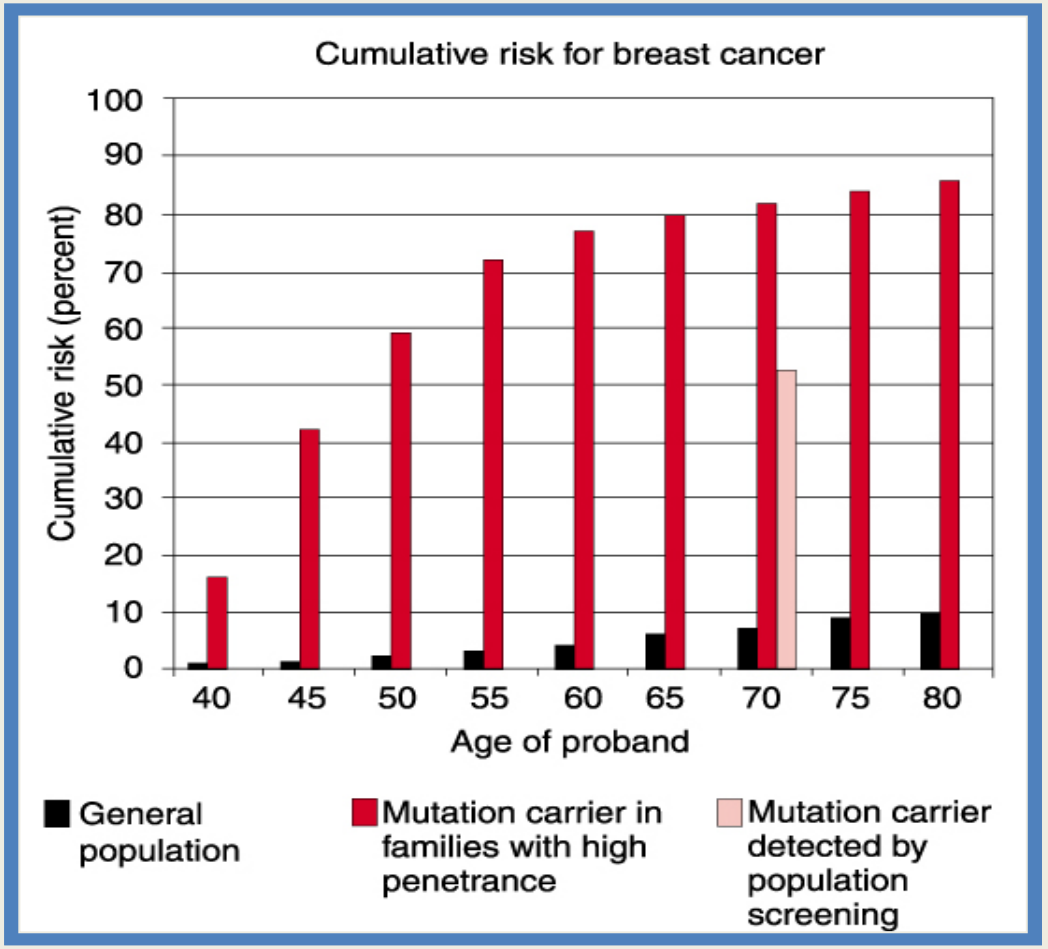


P53 binding DNA

BRCA1 & BRCA2 mutations and cancer predisposition

What is the lifetime risk for developing breast cancer for women carrying mutations in BRCA1 and BRCA2?

Originally thought to be 80%, however, when risk was estimated from pop.studies, 45-60%



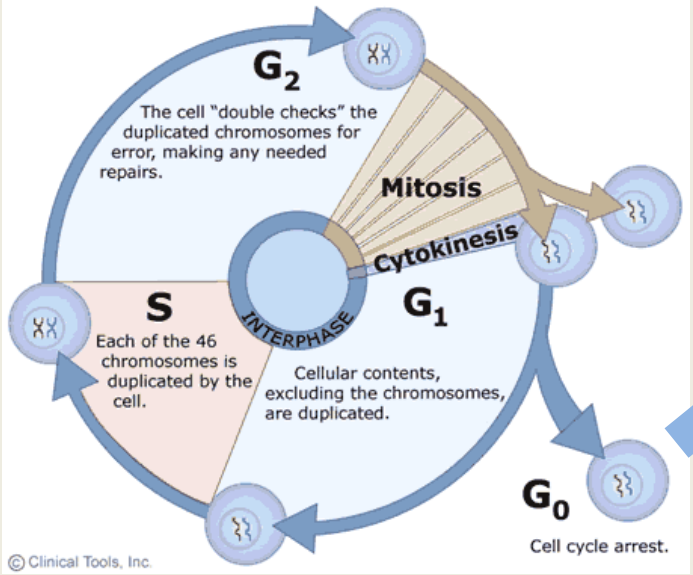
BRCA1

- Accounts for 1/2 of the autosomal dominant familial breast cancers
- Confers high risk for ovarian cancer as well
- May also predispose to prostate and colon cancer
- Encodes 1863 a.a. nuclear protein
- Most identified mutations result in a truncated protein

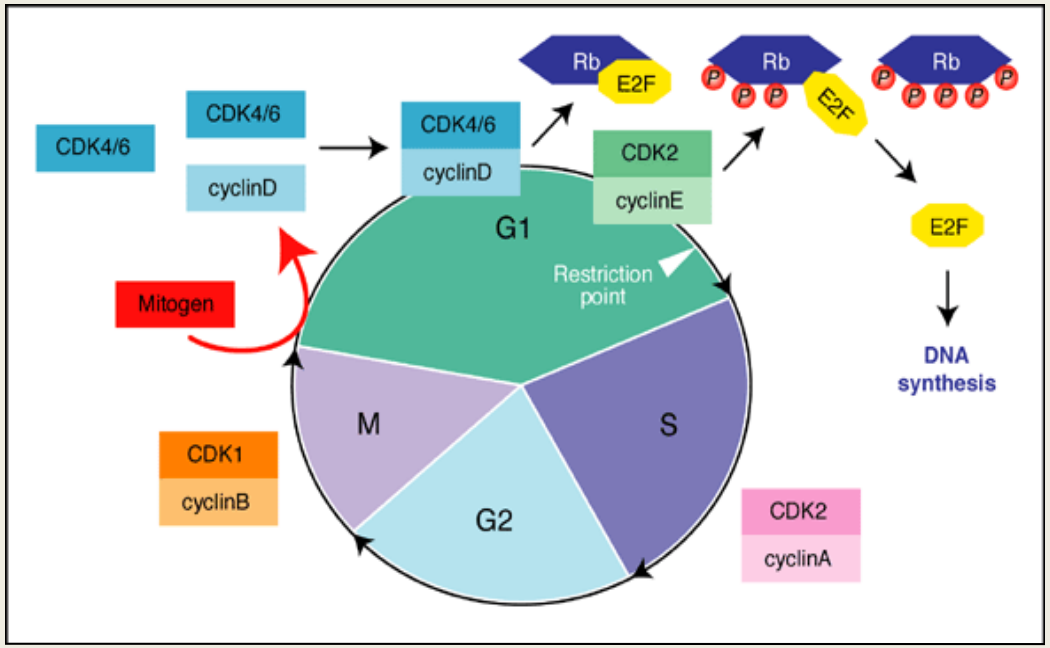
BRCA2

- Accounts for 1/3 of the autosomal dominant familial breast cancers
- Confers high risk for ovarian cancer as well (but not as high as BRCA1)
- Confers high risk for male breast cancer (10-20% of all cases have BRCA2 mutations)
- May also predispose to malignant melanoma, prostate, pancreatic, gall bladder, bile duct and stomach cancer
- Encodes 3418 a.a. nuclear protein

Cancer: uncontrolled cell cycle progression



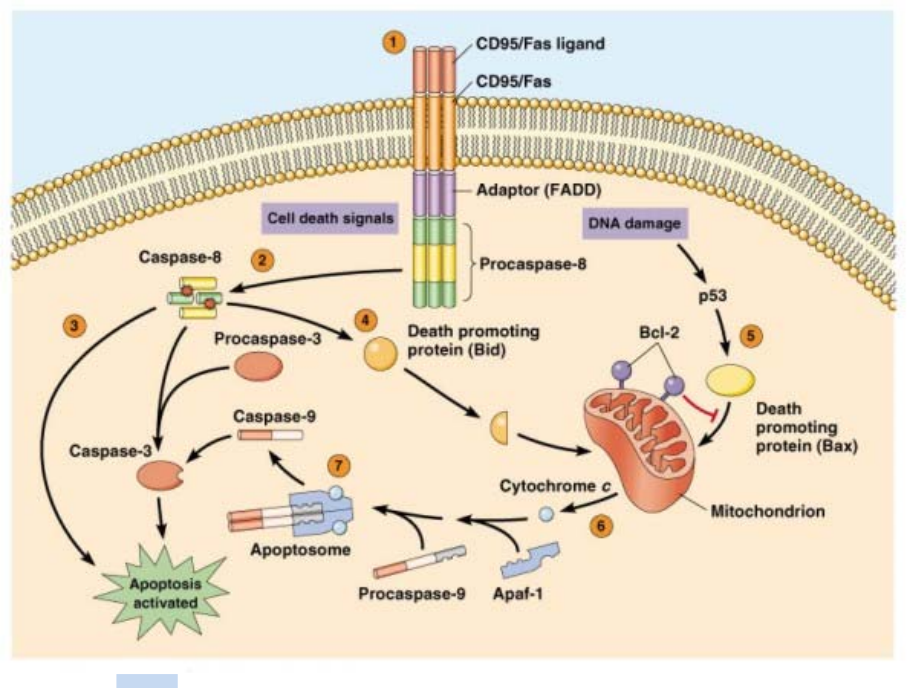
Quiescent: can re-enter to the cell cycle
Senescent: cannot re-enter to the cell cycle



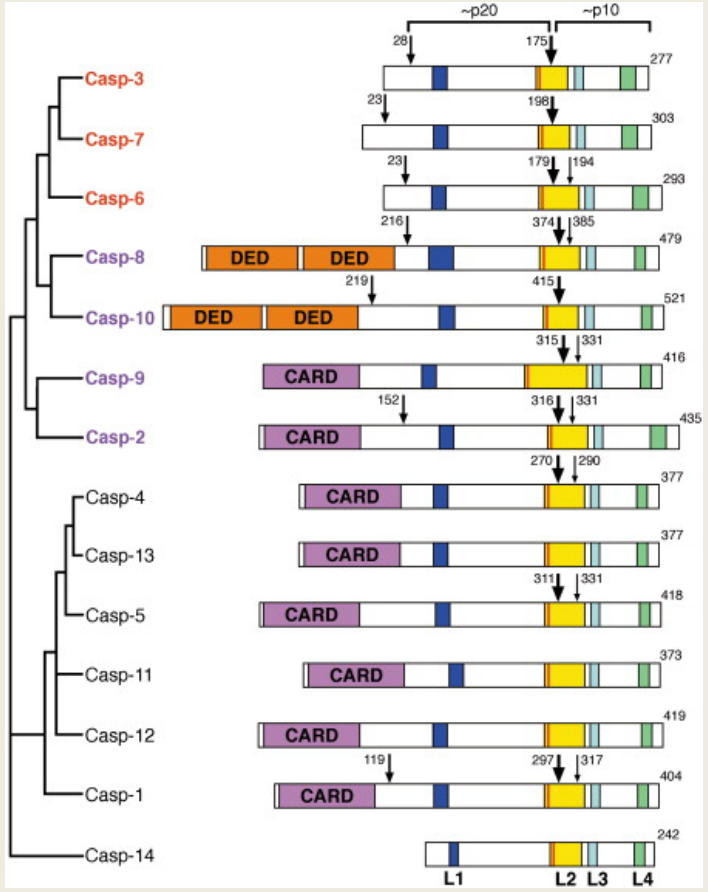
CDKs: cyclin-dependent Kinases

Rb: Retinoblastoma sensitive protein

Cancer: Cell death resistance



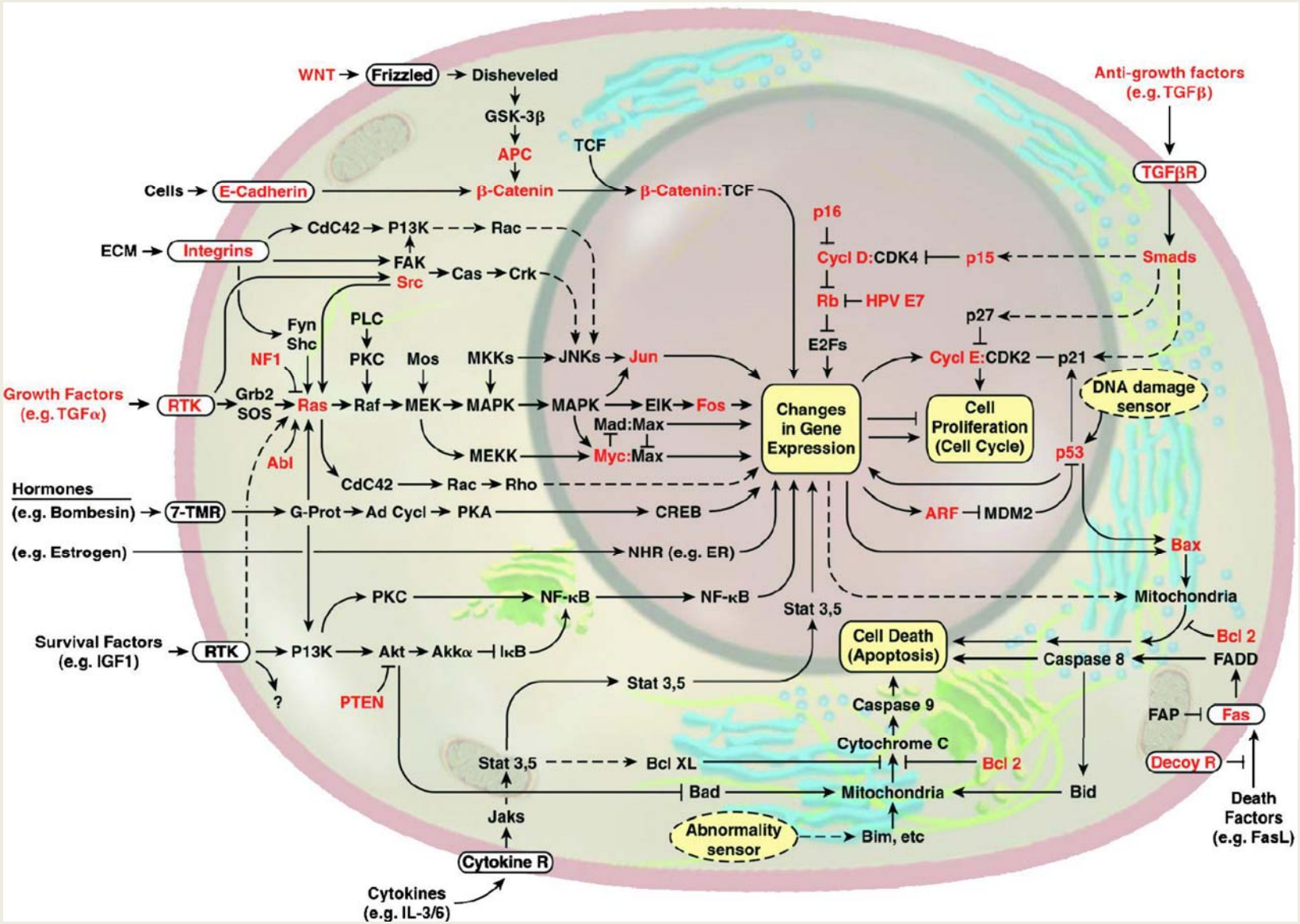
Nucleus



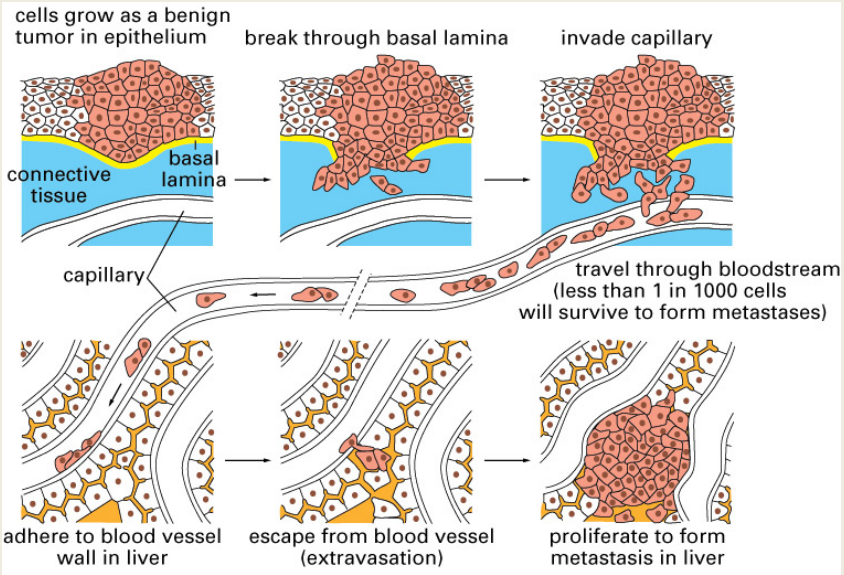
cysteine-dependent **asp**artate-directed prote**ases**

Cancer cells are resistant to apoptosis

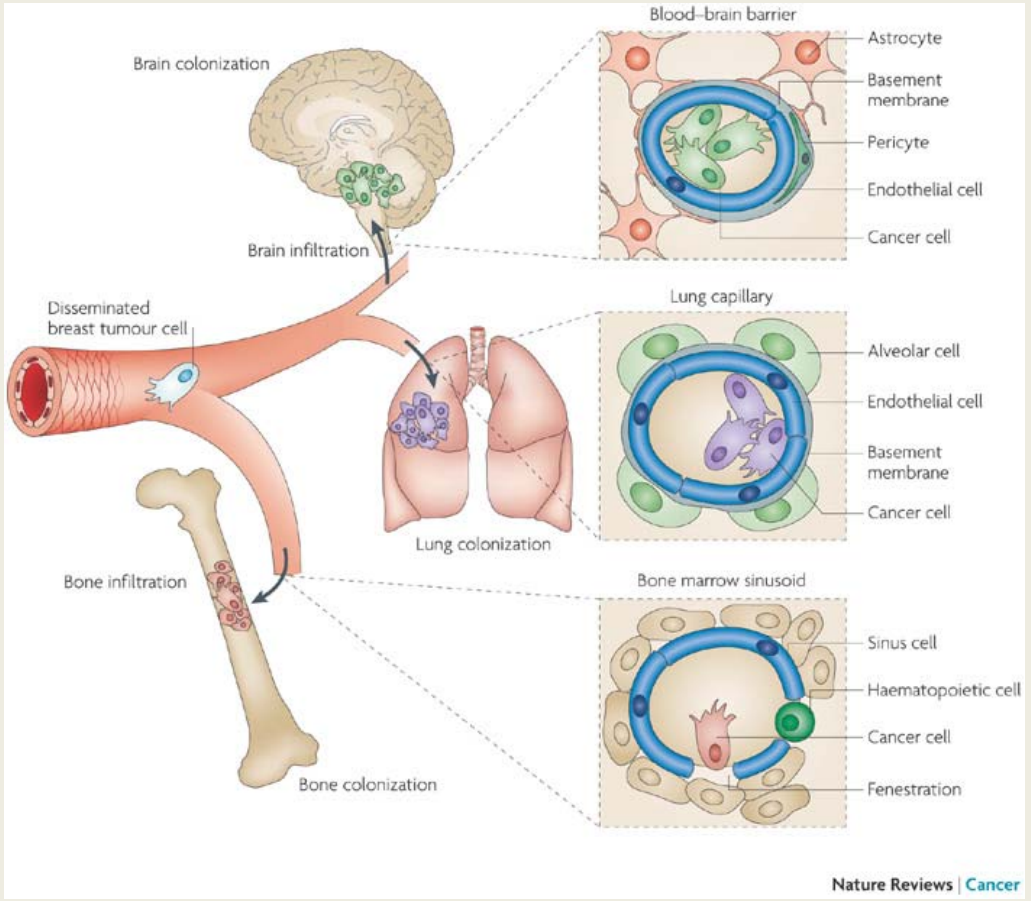
Cellular overview of the main pathways deregulated in cancer



An Overview of Metastasis

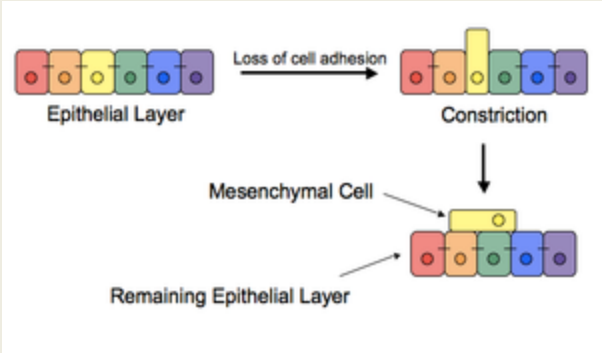


Tumour type	Principal sites of metastasis
Breast	Bone, lungs, liver and brain
Lung adenocarcinoma	Brain, bones, adrenal gland and liver
Skin melanoma	Lungs, brain, skin and liver
Colorectal	Liver and lungs
Pancreatic	Liver and lungs
Prostate	Bones
Sarcoma	Lungs
Uveal melanoma	Liver

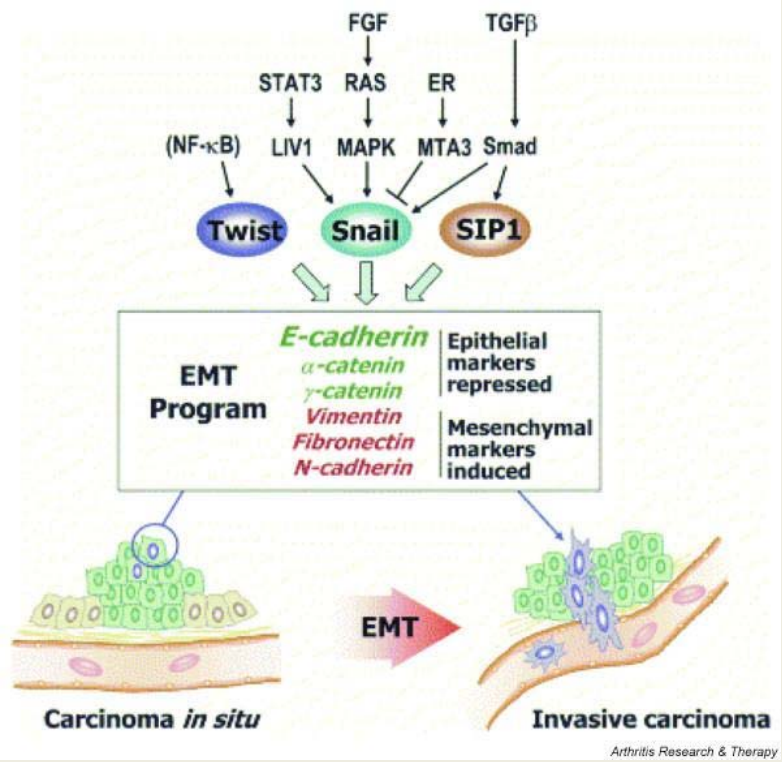


Epithelial–mesenchymal transition

Epithelial–mesenchymal transition or **transformation** (**EMT**). Process that produces complete loss of epithelial traits by the former epithelial cells accompanied by total acquisition of mesenchymal characteristics, such as vimentin, myosin, and invasive motility.

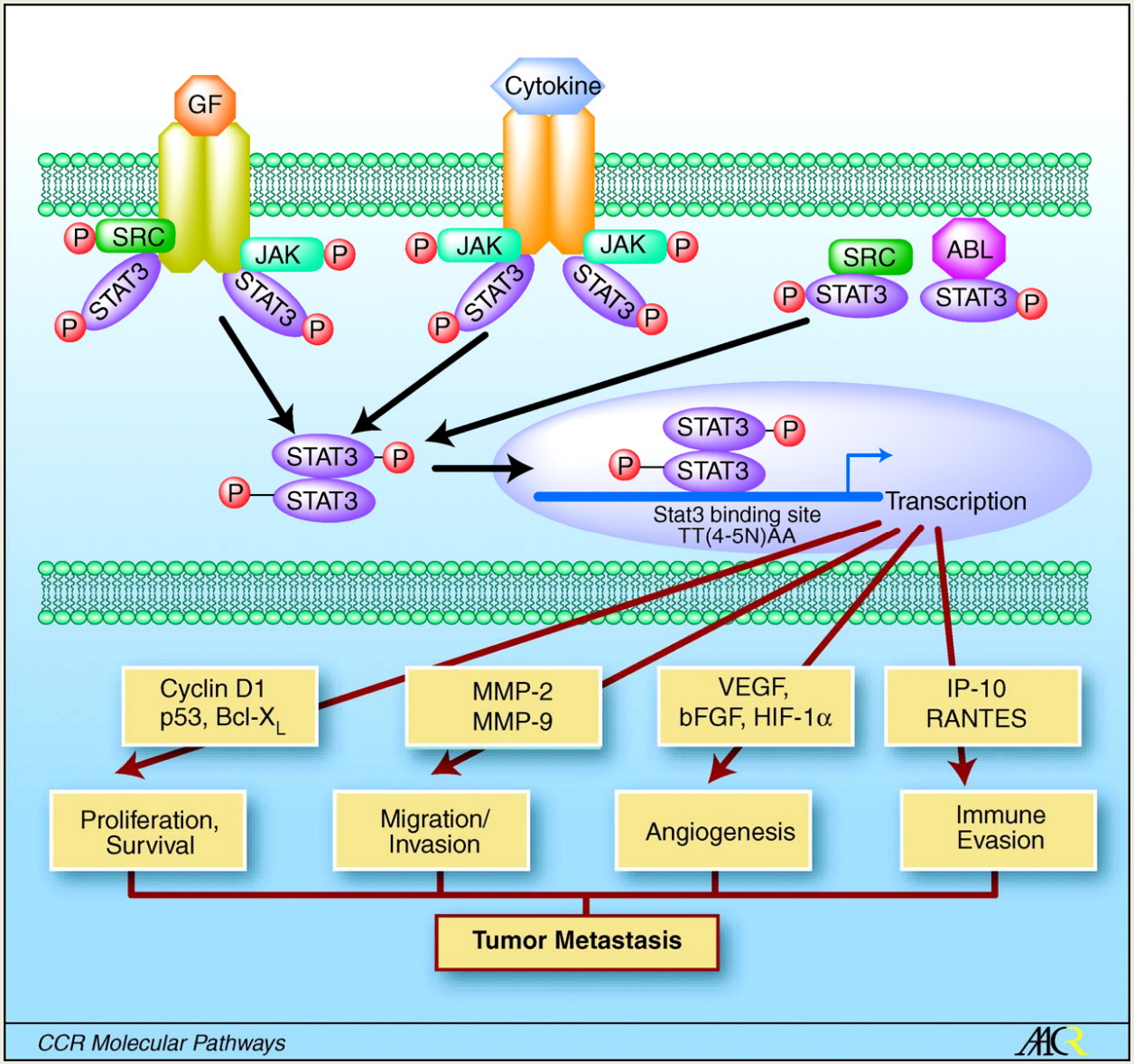


Epithelial to Mesenchmyal Cell Transition – loss of cell adhesion leads to constriction and extrusion of newly mesenchymal cell.

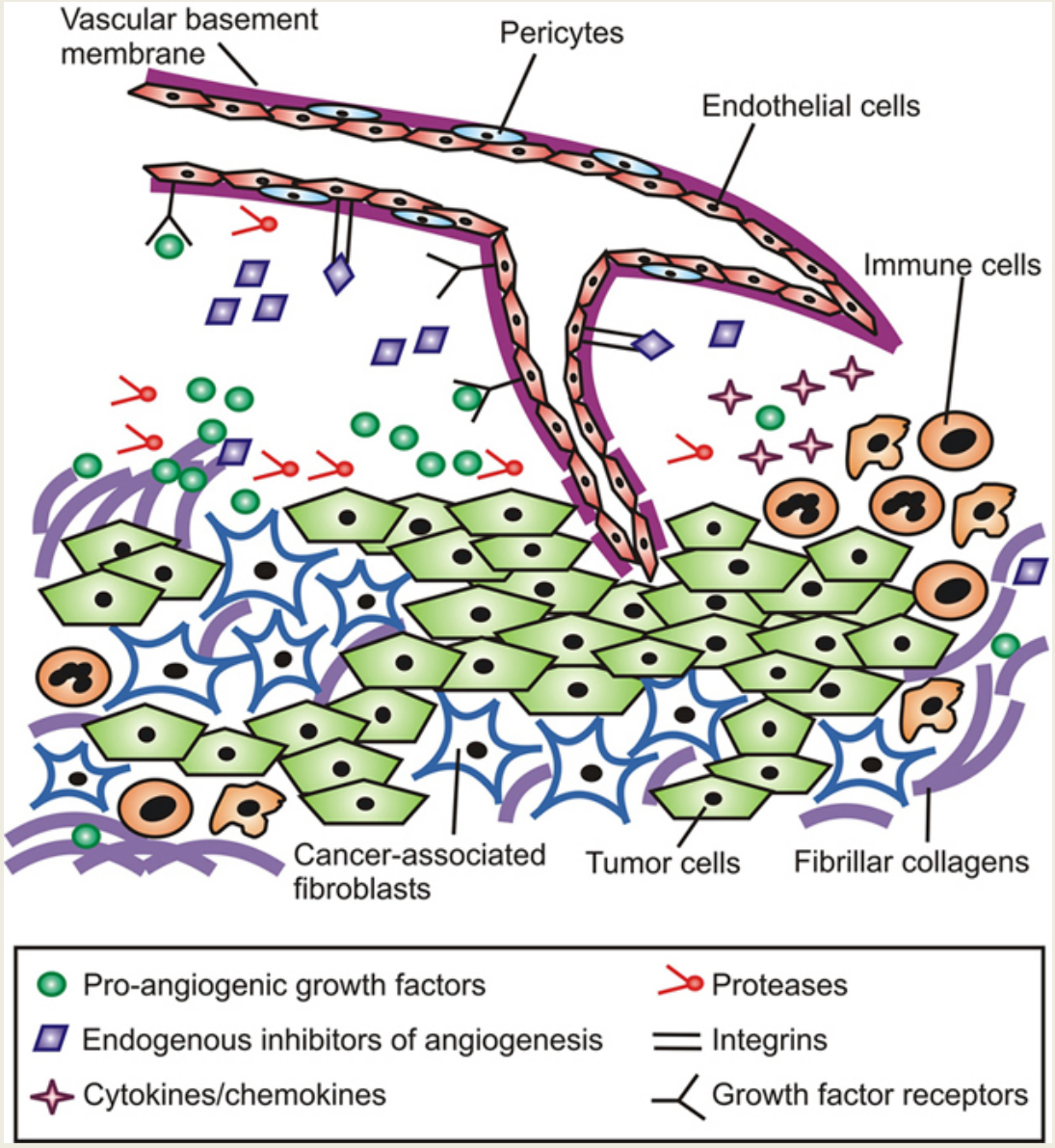


Initiation of metastasis involves invasion, which has many phenotypic similarities to EMT, including a loss of cell-cell adhesion mediated by E-cadherin repression and an increase in cell mobility. Loss of certain genes (e.g. Hedgehog family) has been shown to activate integrin, Wnt, and possibly other signaling pathways, leading to alterations in cell-cell adhesion.

Regulation of Metastases



Tumor Microenvironment



Properties of Cancer Cells

❑Acquisition of self-sufficiency in growth signals, leading to unchecked growth (cell cycle division).

❑Loss of capacity for apoptosis, in order to allow growth despite genetic errors and external anti-growth signals.

❑Loss of capacity for senescence, leading to limitless replicative potential (immortality): telomerase activity.

❑Acquisition of sustained angiogenesis, allowing the tumor to grow beyond the limitations of passive nutrient diffusion.

❑Acquisition of ability to invade neighboring tissues (metastases).

❑Acquisition of ability to build metastases at distant sites.

❑Loss of capacity to repair genetic errors, leading to an increased mutation rate (genomic instability), thus accelerating all the other changes.

❑Anchorage-independent growth: most cell require a supporting surface and will grow in a monolayer in culture. Cancer cells can grow suspended in liquid or semi-solid media.

❑Reduced sensitivity to density dependent inhibition of growth in culture. Most normal cells divide until a monolayer is formed. Cancer cells "pile up".

Treating Cancer

- Surgery
  - The physical removal of cancerous growth.
  - Most successful type of treatment.
- Chemotherapy
  - Use of cell-killing drugs.
  - Tends to kill fast-growing cells (including hair, skin).
- Radiation
  - Gamma or X-rays directed at tumors.
  - Kills or stuns growth of cancer cells.
  - Radiation “seeds” can be placed onto tissue.

Current Areas in Cancer Research

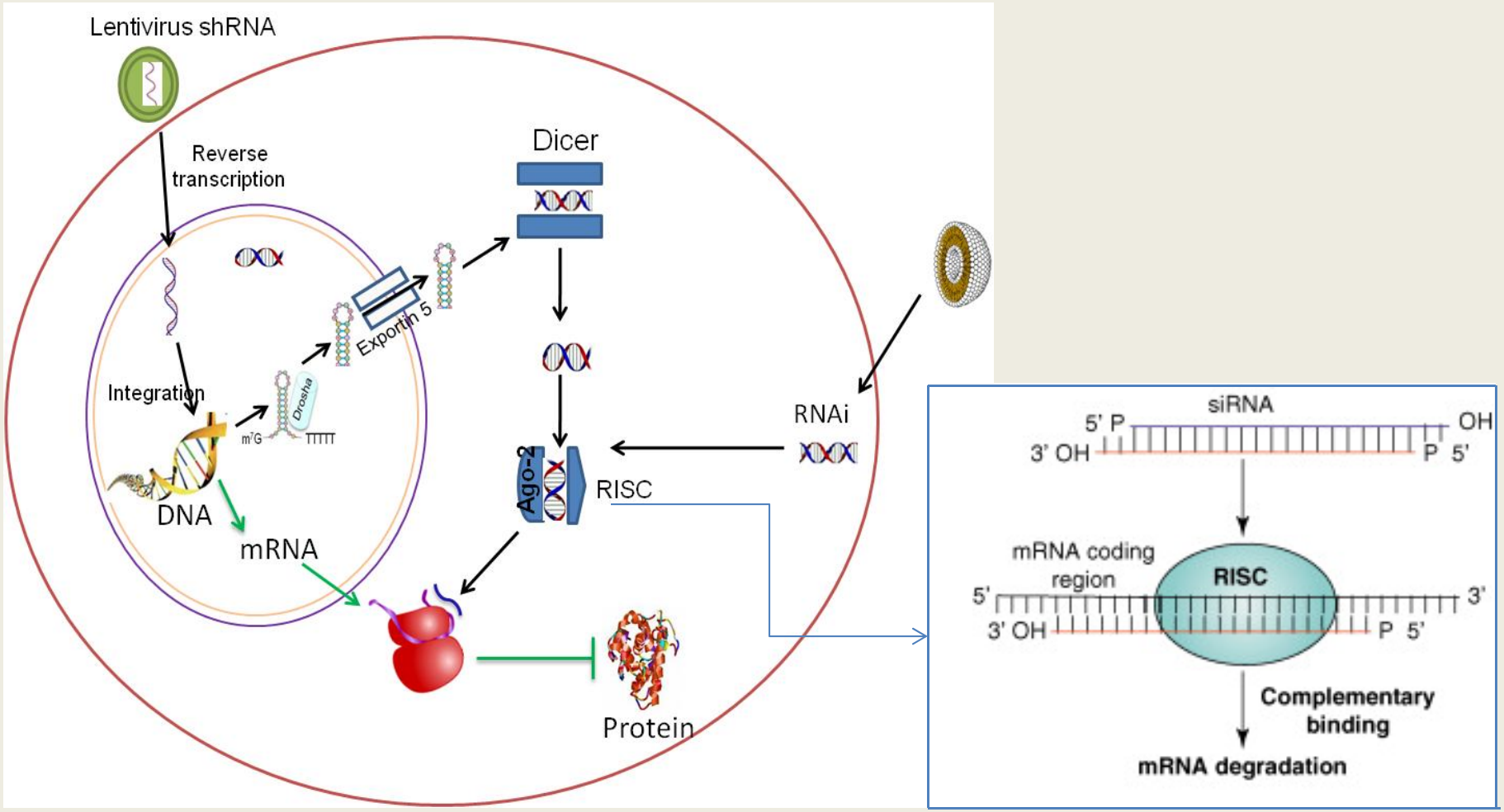
- Cause: genetic, environmental factors, died, etc. Epigenetic, microRNA
- Type of cells involved in cancer growth (i.e. cancer stem cells)
- Oncogenomics/genes involved in cancer
- New drugs, new treatments
- Mechanisms of drug resistance
- Early diagnosis/Technology
- Prevention

Current Cancer Chemotherapies

- Gene therapy.
  - Reprogramming genes to fight off cancer.
- Bone marrow/stem cell transplants.
  - Restore healthy bone marrow.
  - Stem cells are not “rejected” as much.
- Biological therapies
  - Enhancement the immune system.
  - Cytokines.
- Protease inhibitors.
  - Interfere with the growth of cancer cells.
- Anti-angiogenesis drugs.
  - Keep cancer cells from attracting blood vessels.
- Telomerase inhibitors
  - Normal cells die in a normal time frame, cancer cells don’t.
- Small interference RNA (siRNA or miRNA-based molecules)

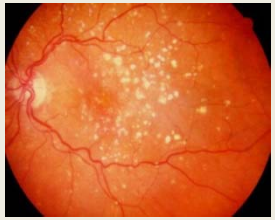
Small interference RNA-based therapies

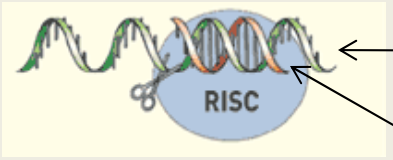
siRNAs are 21-bp double strand RNAs. siRNAs are designed against messenger RNAs that abnormally overexpressed in several diseases.



Examples of siRNA-based therapies

VEGF are highly abundant in
age-related macular degeneration





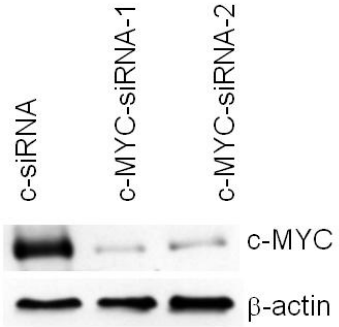
mVEGF

siRNA against mVEGF binds to the mRNA

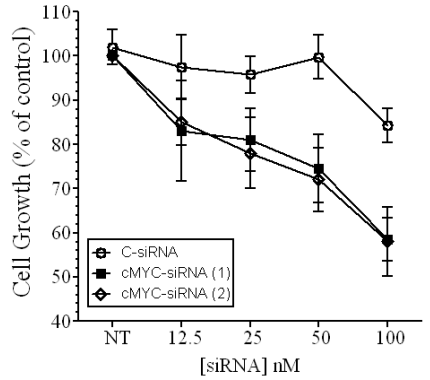
mRNA degradation

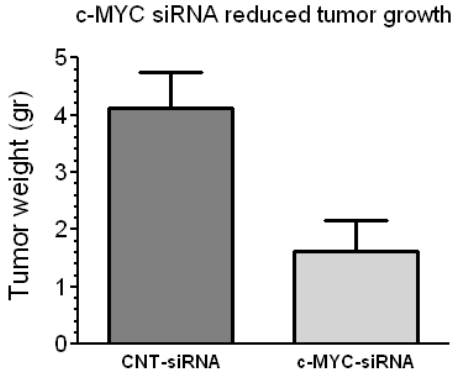
c-MYC is up-regulated in
cisplatin resistant ovarian cancer

Western blot analysis demonstrated that both siRNAs can reduce the amount of c-MYC protein levels



c-MYC siRNAs reduced cell growth of ovarian cancer cells



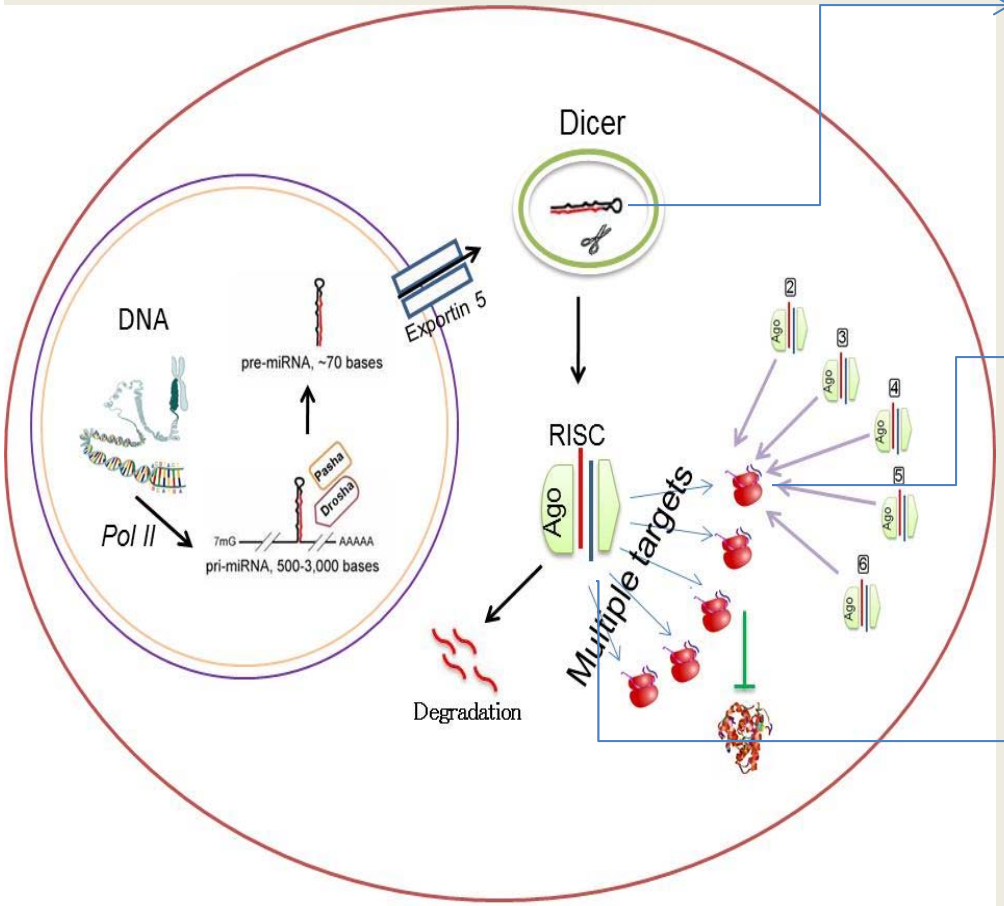


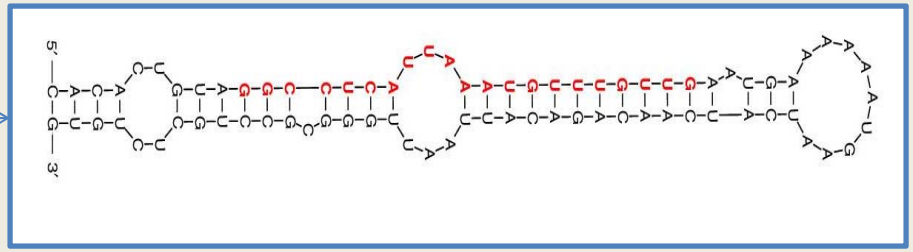
Clinical trials using siRNA technology: cancers, viral respiratory infections, hypercholesterolemia, macular degeneration, diabetic retinopathy and glaucoma.

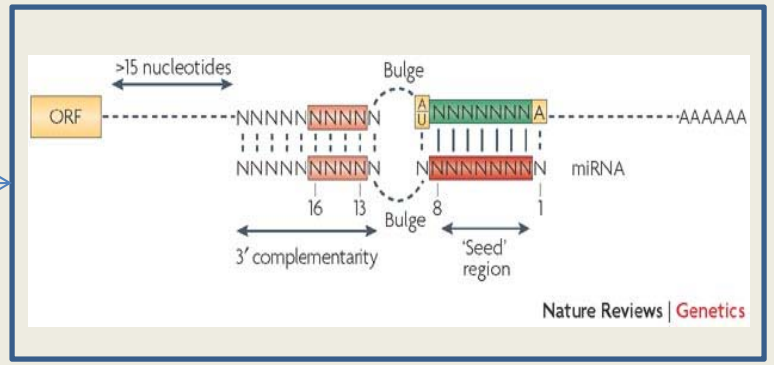
Drug	Target	Delivery system	Disease	Phase	Status	Company	ClinicalTrials.gov identifier
DPC, dynamic polyconjugate; LNP, lipid nanoparticle; NP, nanoparticle; shRNA, short hairpin RNA.							
ALN–VSP02	KSP and VEGF	LNP	Solid tumours	I	Completed	Alnylam Pharmaceuticals	NCT01158079
siRNA–EphA2–DOPC	EphA2	LNP	Advanced cancers	I	Recruiting	MD Anderson Cancer Center	NCT01591356
Atu027	PKN3	LNP	Solid tumours	I	Completed	Silence Therapeutics	NCT00938574
TKM–080301	PLK1	LNP	Cancer	I	Recruiting	Tekmira Pharmaceutical	NCT01262235
TKM–100201	VP24, VP35, Zaire Ebola L-polymerase	LNP	Ebola-virus infection	I	Recruiting	Tekmira Pharmaceutical	NCT01518881
ALN–RSV01	RSV nucleocapsid	Naked siRNA	Respiratory syncytial virus infections	II	Completed	Alnylam Pharmaceuticals	NCT00658086
PRO-040201	ApoB	LNP	Hypercholesterolaemia	I	Terminated	Tekmira Pharmaceutical	NCT00927459
ALN–PCS02	PCSK9	LNP	Hypercholesterolaemia	I	Completed	Alnylam Pharmaceuticals	NCT01437059
ALN–TTR02	TTR	LNP	Transthyretin-mediated amyloidosis	II	Recruiting	Alnylam Pharmaceuticals	NCT01617967
CALAA-01	RRM2	Cyclodextrin NP	Solid tumours	I	Active	Calando Pharmaceuticals	NCT00689065
TD101	K6a (N171K mutation)	Naked siRNA	Pachyonychia congenita	I	Completed	Pachyonychia Congenita Project	NCT00716014
AGN211745	VEGFR1	Naked siRNA	Age-related macular degeneration, choroidal neovascularization	II	Terminated	Allergan	NCT00395057
QPI-1007	CASP2	Naked siRNA	Optic atrophy, non-arteritic anterior ischaemic optic neuropathy	I	Completed	Quark Pharmaceuticals	NCT01064505
I5NP	p53	Naked siRNA	Kidney injury, acute renal failure	I	Completed	Quark Pharmaceuticals	NCT00554359
siG12D LODER	KRAS	LODER polymer	Pancreatic cancer	II	Recruiting	Silenseed	NCT01676259
Bevasiranib	VEGF	Naked siRNA	Diabetic macular oedema, macular degeneration	II	Completed	Opko Health	NCT00306904
SYL1001	TRPV1	Naked siRNA	Ocular pain, dry-eye syndrome	I, II	Recruiting	Sylentis	NCT01776658
SYL040012	ADRB2	Naked siRNA	Ocular hypertension, open-angle glaucoma	II	Recruiting	Sylentis	NCT01739244
CEQ508	CTNNB1	*Escherichia coli*-carrying shRNA	Familial adenomatous polyposis	I, II	Recruiting	Marina Biotech	Unknown
RXi-109	CTGF	Self-delivering RNAi compound	Cicatrix scar prevention	I	Recruiting	RXi Pharmaceuticals	NCT01780077
ALN–TTRsc	TTR	siRNA–GalNAc conjugate	Transthyretin-mediated amyloidosis	I	Recruiting	Alnylam Pharmaceuticals	NCT01814839
ARC-520	Conserved regions of HBV	DPC	HBV	I	Recruiting	Arrowhead Research	NCT01872065

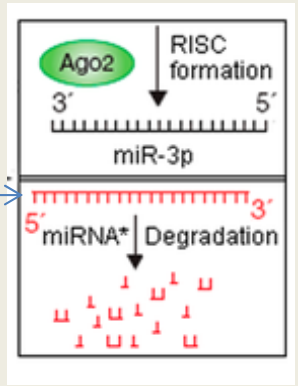
MicroRNA-based therapies

MicroRNAs are small non-coding RNAs that regulates gene expression at the post-transcriptional level.







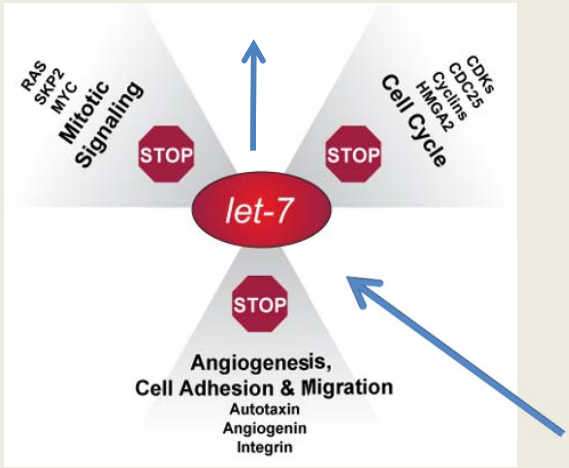


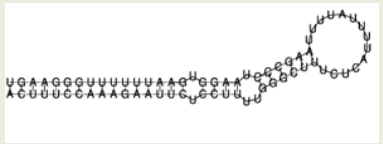
Each miRNA can potentially target 800-1000 mRNAs

MicroRNA-based therapies

MiRNAs are deregulated (increased or decreased) in human diseases.

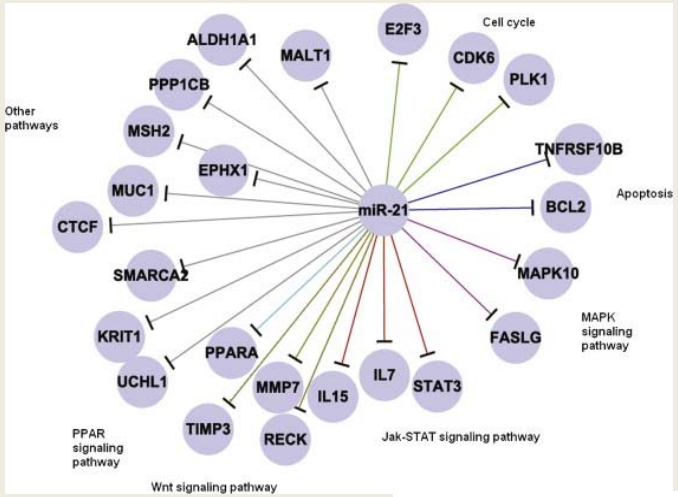
Let-7 is abnormally decreased in several cancer types

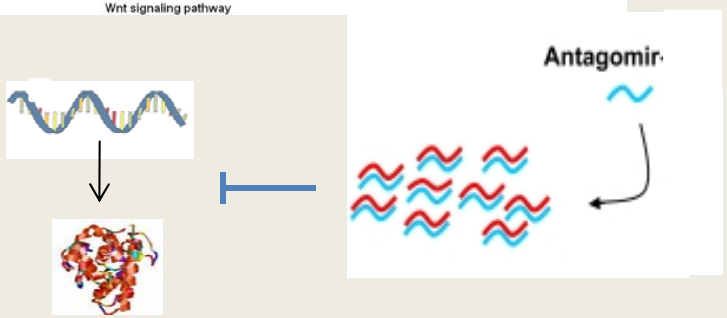




A Let-7 mimic will increase the amount of let-7 and will degrade Let-7-regulated mRNAs.

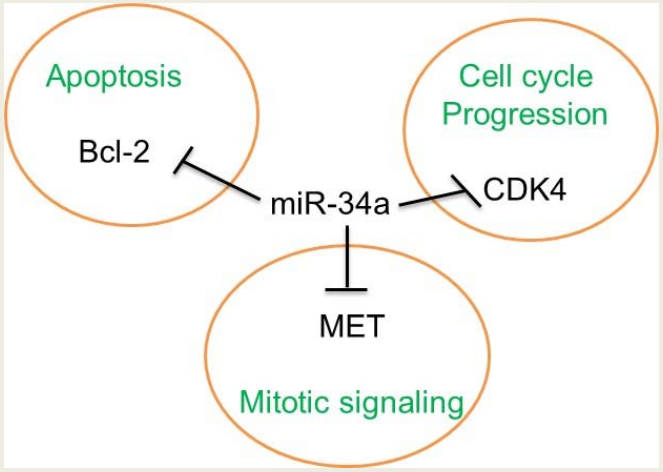
miR-21 is abnormally increased in cardiovascular diseases and cancer

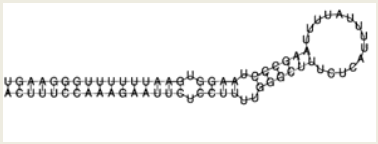




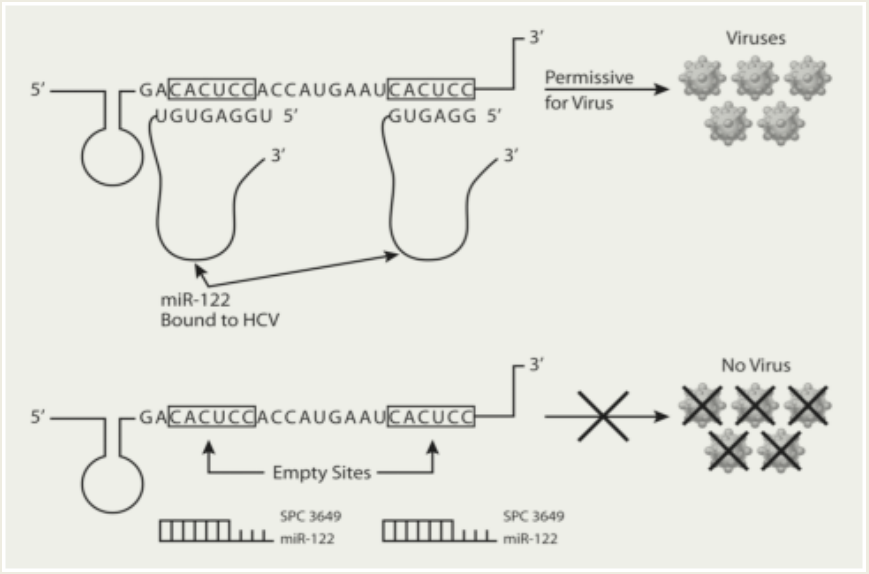
A miRNA-21 inhibitor will reduce miR-21 levels and will increase miR-21 target mRNAs

MiRNA therapies: mechanism of action





RRX34 (miR-34 mimic)



In healthy individuals, miR-122 is an abundant, liver specific microRNA that regulates a host of messenger RNAs in the liver, many of which encode proteins involved in lipid and cholesterol metabolism. When normal liver cells are infected by the Hepatitis C virus, the virus co-opts miR-122 to two binding sites located close to the 5'end of its linear RNA genome. This binding enables the virus to replicate and create new copies of itself, which can further spread the infection throughout the liver. SPC3649 is designed to specifically recognize and sequester miR-122 thus making it unavailable to the Hepatitis C virus. As a result, the replication of the virus is effectively inhibited.

Current MiRNA Clinical Trials

miRNA	Indication	Status of development
miRNA antagonists		
miR-122	Hepatitis C virus	Phase 2 clinical trials
miR-208/499	Chronic heart failure	Preclinical development
miR-195	Post-myocardial infarction remodelling	Preclinical development
miRNA replacement		
miR-34	Cancer	Preclinical development
let-7	Cancer	Preclinical development

ORAL CANCER

Basic description

❑Cancer can affect any part of the oral cavity, including the lips, tongue, mouth, and throat.

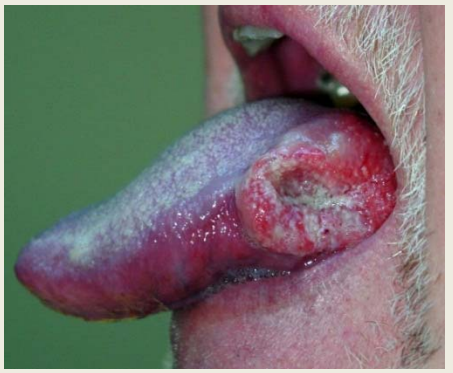
❑There are 2 kinds of oral cancer: oral cavity cancer and oropharyngeal cancer.

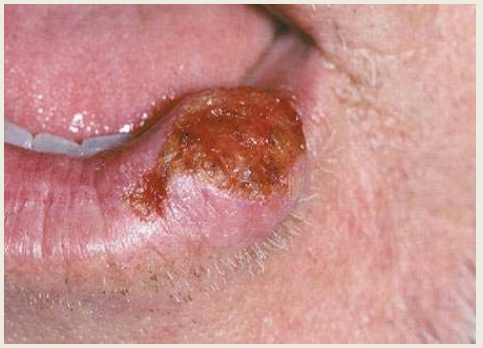
❑The most common symptom of oral cancer is a sore in the mouth that bleeds easily and does not heal.

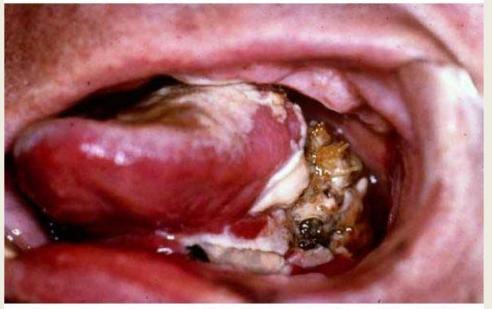
❑Another common sign of oral cancer is pain in the mouth that does not go away.

Other signs and symptoms include:

- A lump or thickening in the cheek
- A white or red patch on the gums, tongue, tonsil, or lining of the mouth.
- A sore throat or a feeling that something is caught in the throat.
- Trouble chewing, swallowing, or moving the tongue or jaw.







Types of oral cancer

squamous cell carcinoma	▪ Also known as squamous cell cancer, this type of cancer originates in the squamous cell layer in the lining of the oral cavity and oropharynx. ▪ In the early stages, this cancer is present only in the lining layer of cells (called carcinoma in situ). ▪ When the cancer spreads beyond the lining, it is called invasive squamous cell cancer.
verrucous carcinoma	▪ Although also considered a type of squamous cell carcinoma, this low-grade cancer rarely metastasizes (spreads to distant sites). ▪ Comprising less than 5 percent of all diagnosed oral cancers, verrucous carcinoma can spread deeply into surrounding tissue, requiring surgical removal with a wide margin of surrounding tissue.
minor salivary gland cancers	▪ The lining of the oral cavity and oropharynx contains numerous salivary glands. ▪ Sometimes cancer will originate in a salivary gland. ▪ Treatment depends on the type and location of the salivary gland cancer, as well as the extent of spreading

RISK FACTORS/PREVENTION

- Oral cancer in the United States: 2012 estimates:
  New cases: 40,250 Deaths: 7,850

- Gender: Oral cancers are about twice as common in men as in women.

- Age: The likelihood of developing oral cancer increases with age, but about 1 out of every 3 people diagnosed are younger than 55.

- Tobacco use and alcohol About 80% of patients with oral cancers use tobacco. The risk of developing these cancers increases with the amount smoked or chewed and the duration of the habit. About 70% of all patients with oral cancer drink alcohol frequently. The combination of smoking and drinking increases a person's risk much more than either by itself.

- HPV infection Human papilloma virus may contribute to the development of about 25% of oral cancer cases.

- Sun exposure Many patients with cancers of the lip have outdoor jobs associated with prolonged exposure to sunlight.

ORAL CANCER: PREVENTION

- Quitting tobacco and limiting alcohol use significantly lower the risk of developing these cancers, even after many years of use.

- In addition, eating a healthy, balanced diet with at least 2½ cups of vegetables and fruits every day may provide some protection against oral cancer.

- The American Cancer Society recommends that primary care clinicians and dentists examine the mouth and throat as part of a routine cancer-related check-up.

- Dentists and primary care clinicians have the opportunity, during regular check-ups, to see abnormal tissue changes and detect cancer at an early stage.