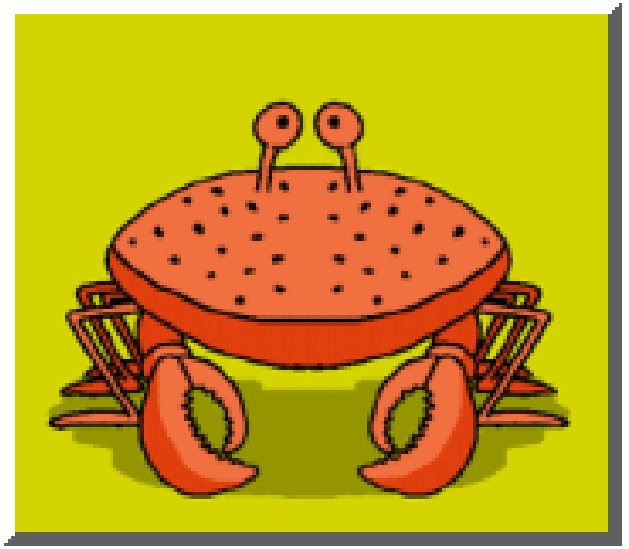
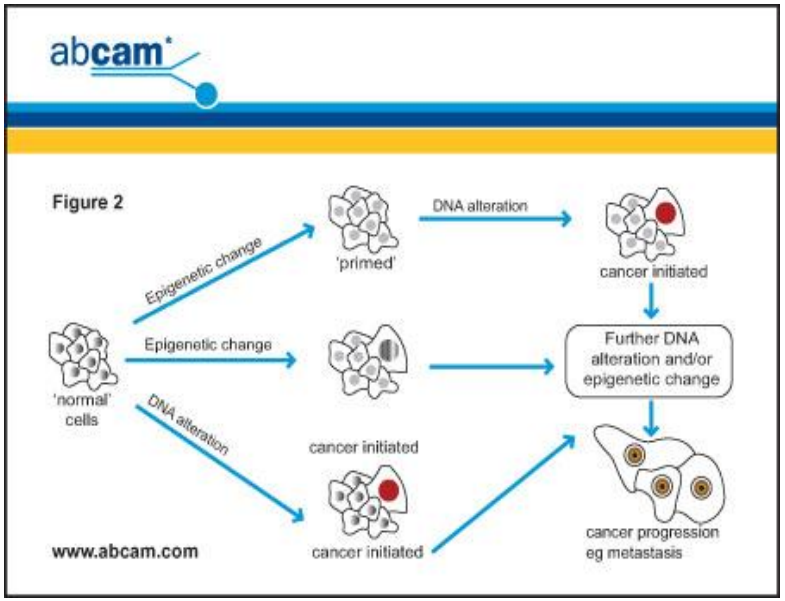
Malignant transformation of the cell - cancer

Cell cycle control
Oncogene × suppressor genes/proteins
Principle of malignant transformation
Interaction of tumor and organism
Metastases



Malignant transformation

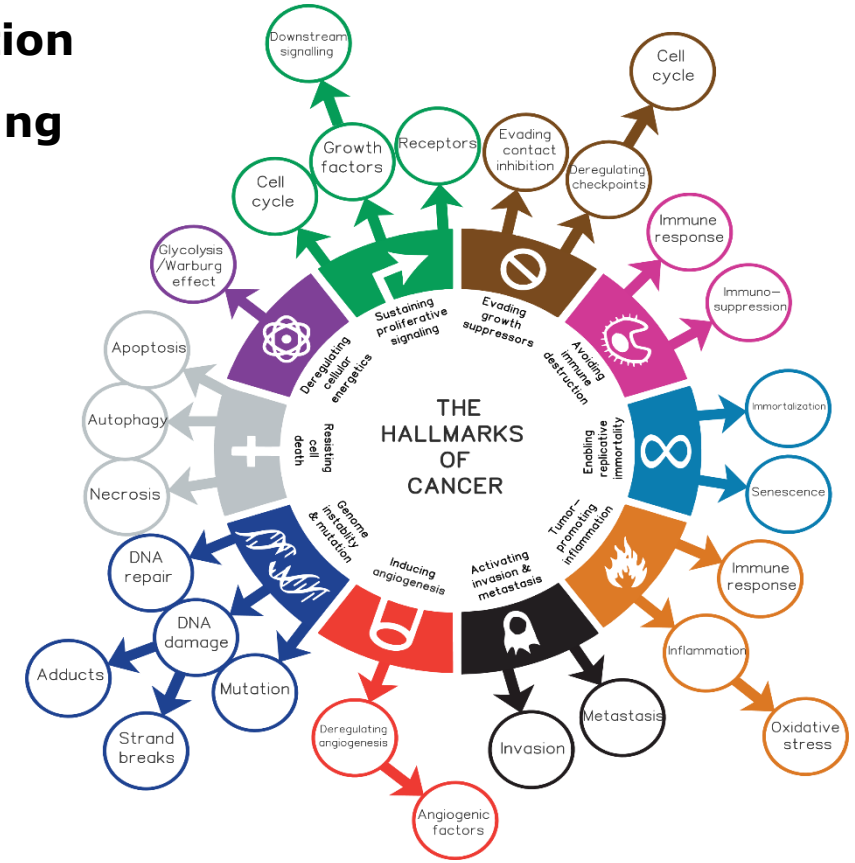
- The process of tumor formation is a **complex** involving multiple alterations of cells and their physiologic control mechanisms.
- The complexity of this process is reflected in the **long time periods** required for most human cancers to develop.
- Multi-step tumor progression can be depicted as a form of **Darwinian evolution** occurring within tissues.
- Some of the critical changes occurring during tumorigenesis are **epigenetic** and the rate of genetic diversification can occur very rapidly.



Genetic alteration can appear
– (1) due to internal errors during DNA replication and cell division
– (2) as a consequence of exposure to the external factors (**carcinogens**)
physical – e.g. UV and ionising light
chemical – organic substances, toxins, heavy metals
biologic – some RNA and DNA viruses

Hallmarks of cancer

- **Continual unregulated proliferation of cancer cells (sustaining proliferative signaling and evading growth suppressors)**
- **Replicative immortality**
- **Genome instability**
- **Resisting cell death and senescence**
- **Inducing angiogenesis**
- **Inflammation**
- **Avoiding immune destruction**
- **Altered metabolism**
- **Invasion and metastasis**



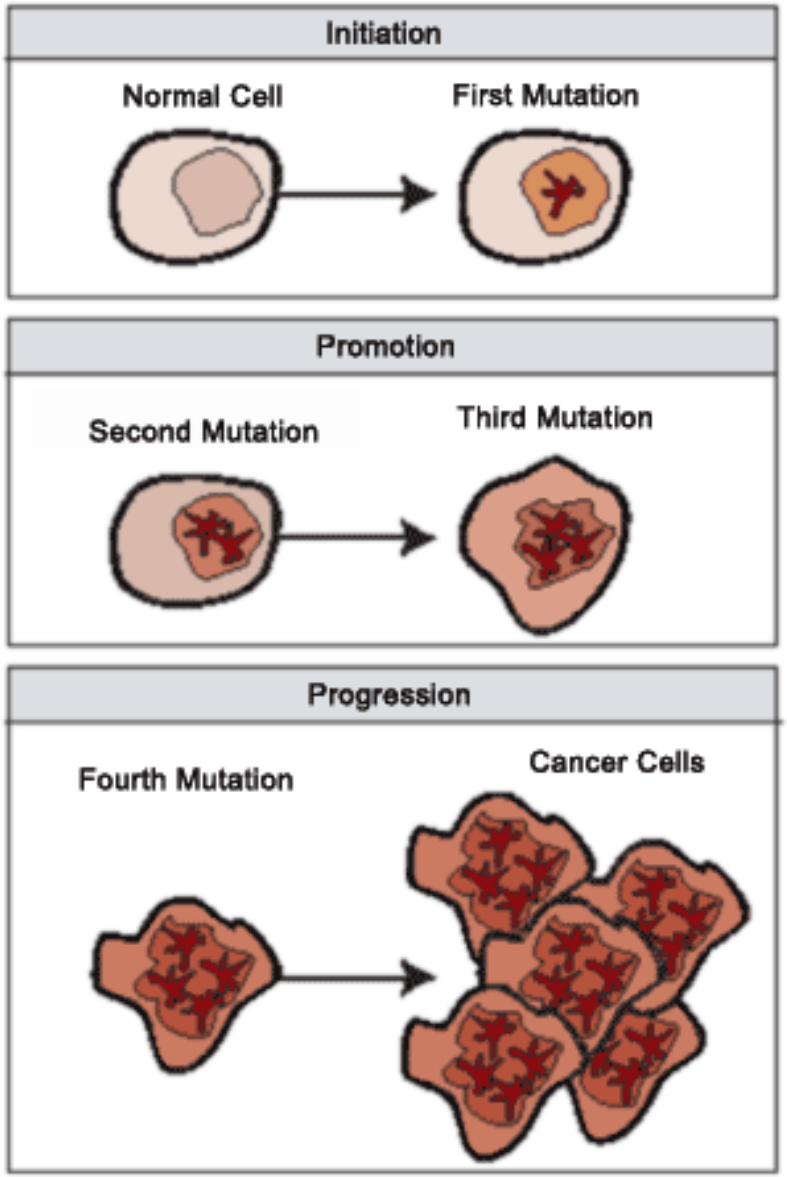
All these features do not have to be newly evolved, because they are part of physiological processes such as embryogenesis and wound healing. Cancer cells only use these processes in wrong intensity, time, and place. Cancer is a disease of regulation.

Cancer – basic facts

- Pathological process (disease) due to **impaired control of cell cycle and thus cell division**
  - if genes that control the orderly cell replication become damaged it allows the cell to reproduce without restraint
    - ☛ our genome is constantly attacked by mutagens, but fortunately most of the alterations are harmless, i.e. become repaired or affects genes not critical for cell division
  - it might eventually spread into neighbouring tissues and set up secondary growths throughout the body (metastases)
  - the reason for this dys-regulation is genetic – mutations of originally just 1 somatic cell (but also germline in some cases)
    - tumor classification according to the growth rapidity
      - ☛ **benign** – grow only in the site of origin, not aggressive, maintain differentiation
      - ☛ **malignant** – rapid growth, invasive, spreading to other places, undifferentiated

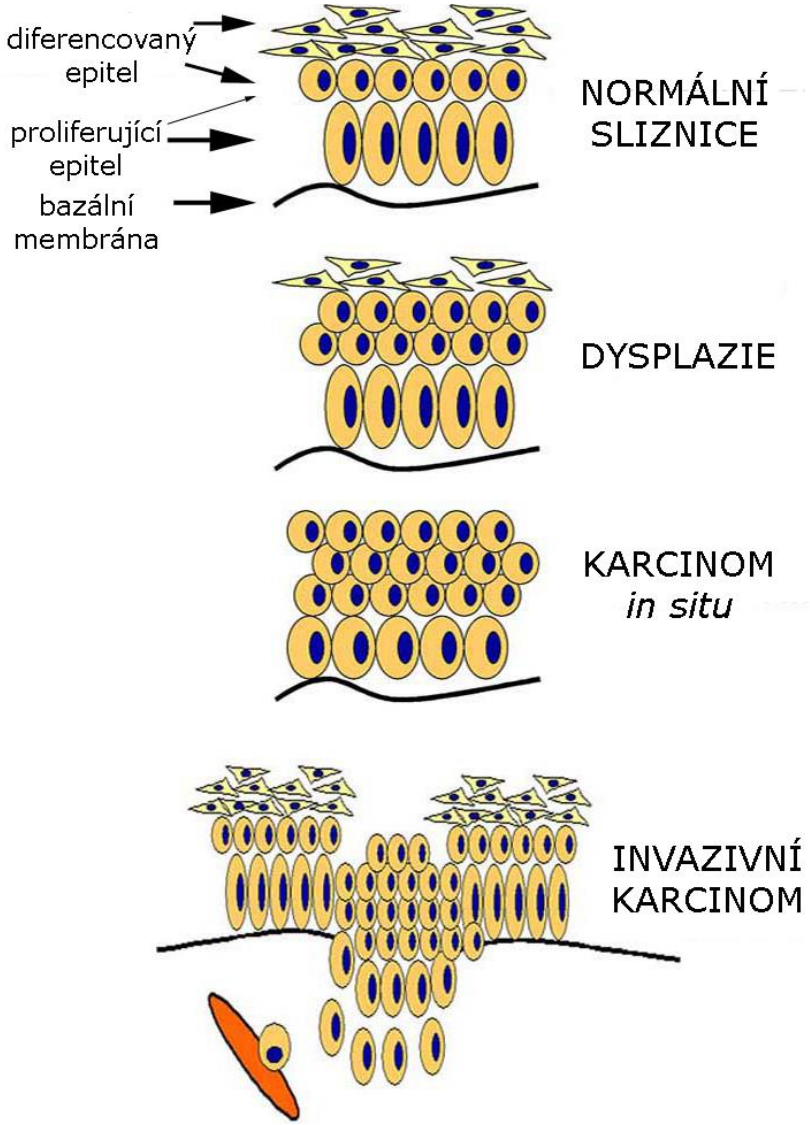
Cancer – basic facts

- All types of cancers are due to **genetic alteration** of key genes controlling cell cycle
  - however, only a few are inherited (i.e. **familiar**) = due to the mutation in germ line cell
  - majority of cancers are due to the acquired genetic changes during the life (i.e. **sporadic**) = due to the mutation in somatic cell
- Key genes controlling cell cycle
  - **(proto)oncogens** – genes that normally potentiate cell division and growth under the physiologic stimuli, if mutated process becomes uncontrolled and spontaneous
  - **suppressor genes** – genes that normally inhibit cell division, initiate DNA repair or apoptosis, if mutated growth becomes uncontrolled and resistant to apoptosis
  - **DNA repair genes** – genes encoding enzymes that can repair reparable DNA damage occurring due to the environmental or endogenous agents (e.g. UV light, oxygen radicals), if mutated unrepaired alteration can be transmitted into daughter cells

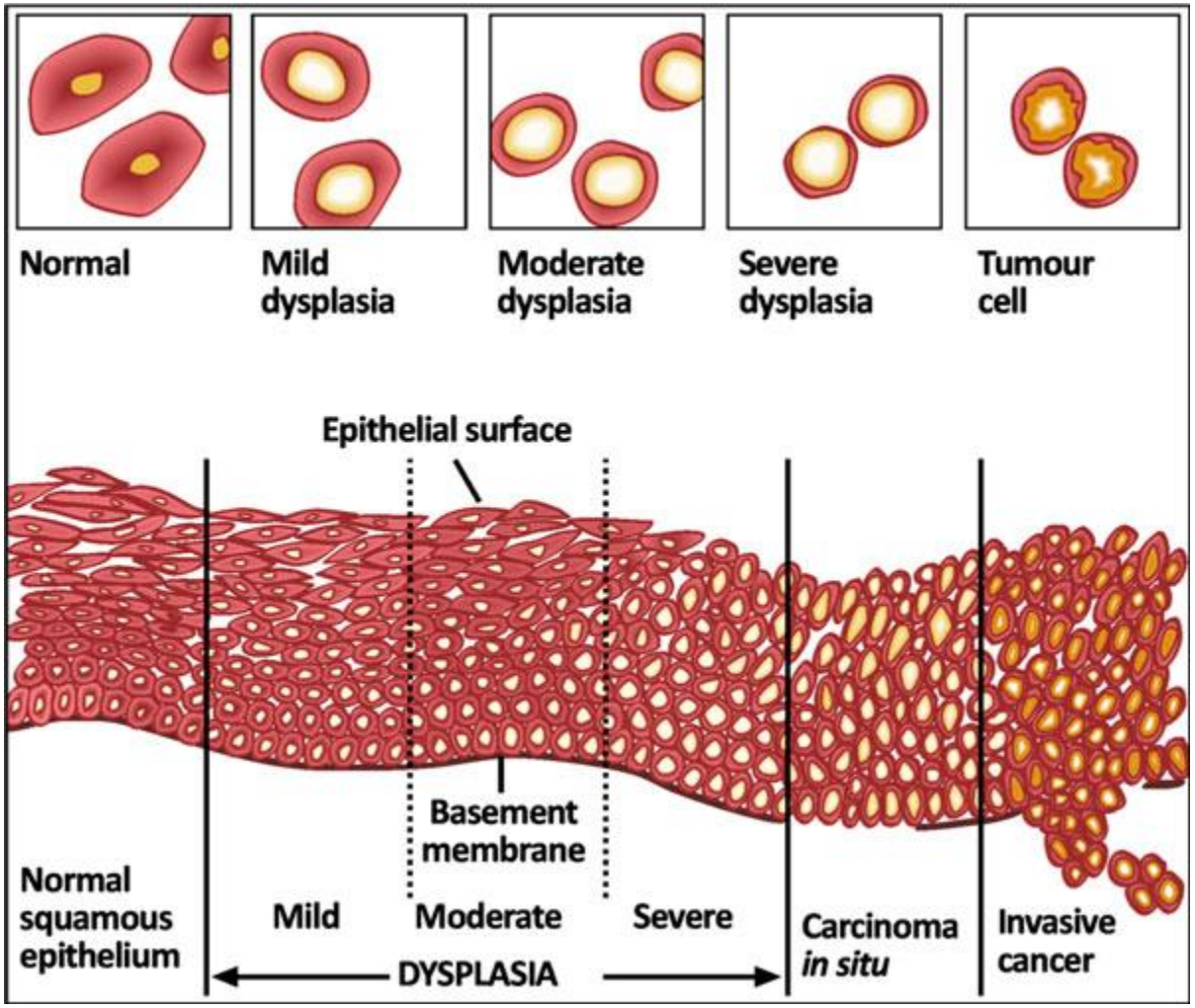


Cancer – basic facts

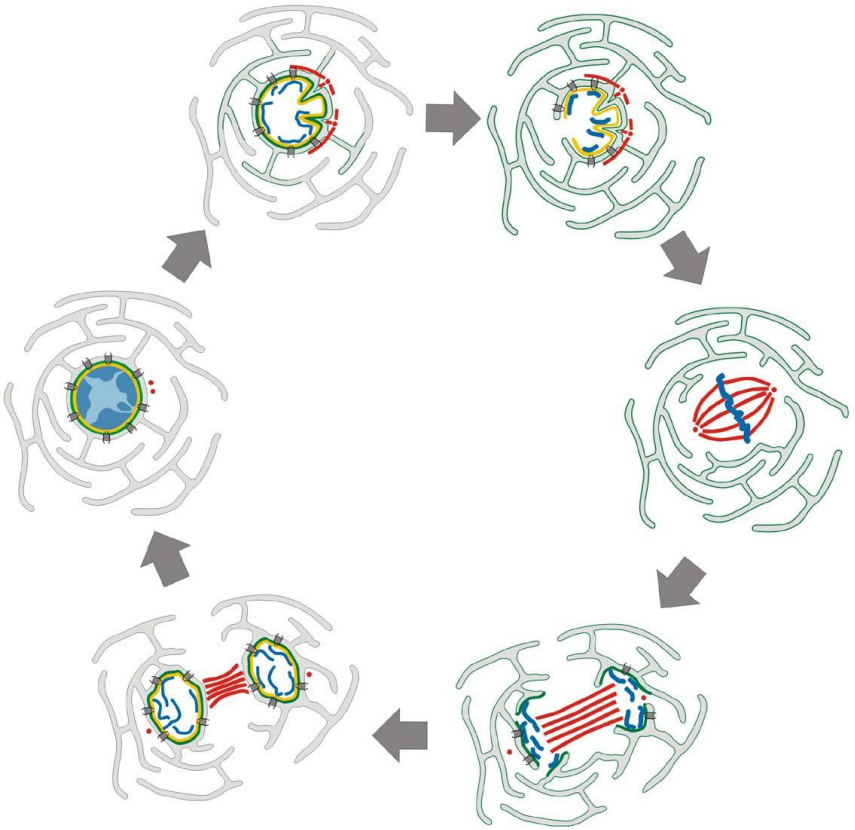
- Genetic alteration can appear
  - (1) due to internal errors during DNA replication and cell division
  - (2) as a consequence of exposure to the external factors (**carcinogens**)
    - physical – e.g. UV and ionising light
    - chemical – organic substances, toxins, heavy metals
    - biologic – some RNA and DNA viruses
- Tumor usually stems from a mutation in a single cell (**monoclonal**)
  - tumor cell transformation is a **multistep process** (i.e. subsequent accumulation of mutations), therefore, tumor becomes genetically **heterogeneous**
    - tumor goes from the stage of precancerosis (metaplasia, dysplasia) through benign to malignant
- Histologicaly – based on the original tissue –3 groups can be distinguished
  - epithelial
    - skin, mucose membranes, ductal lining
      - papilloma, adenoma (b.), carcinoma (m.)
  - mesenchnymal
    - connective tissue, endothelia, muscle. hematopoietic and lymphatic tissue, bone
      - fibroma, haemangioma, myoma (b.), sarcoma, lymphoma, leukaemia (m.), …….
  - neuroectodermal
    - CNS and peripheral nerves, pigment nevi
      - astrocytoma, glioma, blastoma, neurinoma, melanoma



Gradual change of cellular phenotype



Cell cycle (CC)

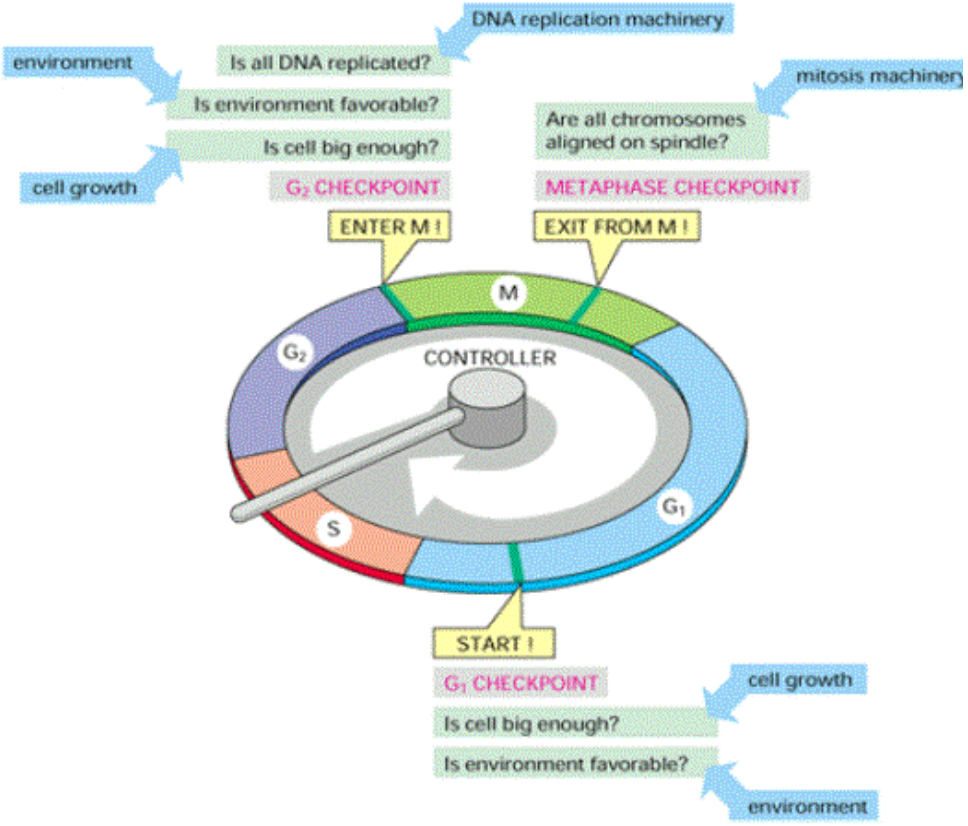


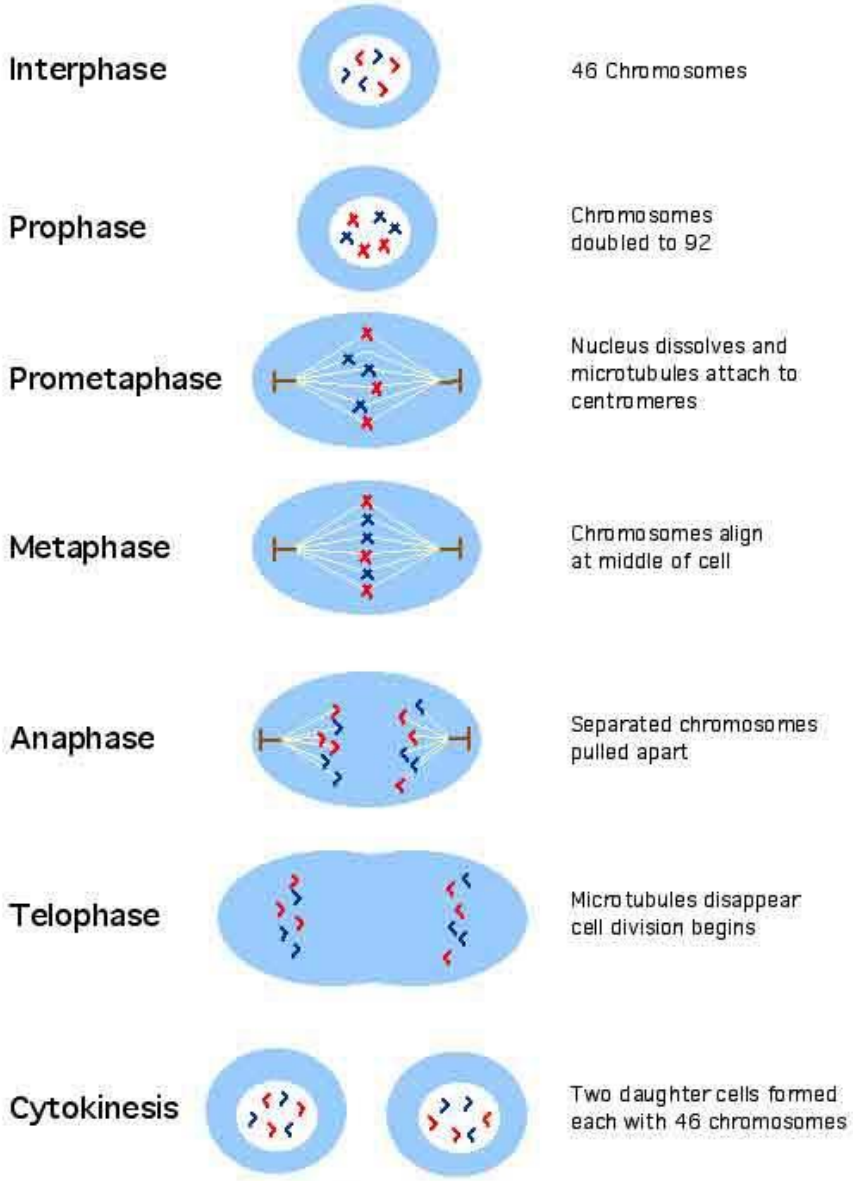
- most of the somatic cells grows, double the amount of cell organelles and divide = **cell cycle**
- CC (4 phases)
  - interphasis
    - cell growth (G1-phase)
    - DNA replication (S-phase)
    - additional growth (G2-phase)
  - mitosis (M-phase)
- duration of CC is very variable in different cell types
  - hours in enterocytes
  - months in liver cells
  - life time in neurons (in G0-phase)
- G1-phase has the most variable length
  - CC is naturally inhibited in G1 (**growth arrest**)
    - by contact inhibition
    - by products of suppressor genes
- CC is highly regulated by very often counteracting
  - internal factors – e.g. inhibition by suppressor proteins
  - external factors – e.g. stimulation by growth factors
- cancer = dysregulation of CC
- cell cycle carries on only if
  - all phases proceed without errors
    - 3 check-points
  - energy is available
  - external stimuli (growth factors) are acting

CC phases

G0	Resting phase. Cell perform its function, maintain basal metabolism and does not divide.
G1	Interval between previous Mitosis and subsequent Synthesis (= DNA replication). Intensive synthesis of all sorts of RNAs in the nucleus. Proteosynthesis in the cytoplasm and overall cell growth. G1 duration basically determines the CC length. **G1/S check point** (= favourable conditions and energy supply, intact DNA)
S	DNA replication in the nucleus and histone synthesis in the cytoplasm. At the and the cell contains doubled the amount of DNA.
G2	Interval between previous Synthesis and subsequent Mitosis. Cell further grows and synthesises protein (mainly tubulin and other proteins necessary for mitotic apparatus). **G2 check point** (= completeness and correctness of DNA replication).
M	Mitosis (6 phases) – first 5 (prophasis, prometaphasis, metaphasis, anaphasis, telophasis) represent division of nucleus, the last one (cytokinesis) division of the whole cell. **M (spindle) check point** (= correct formation of mitotic spindle in metaphasis/anaphasis transition)

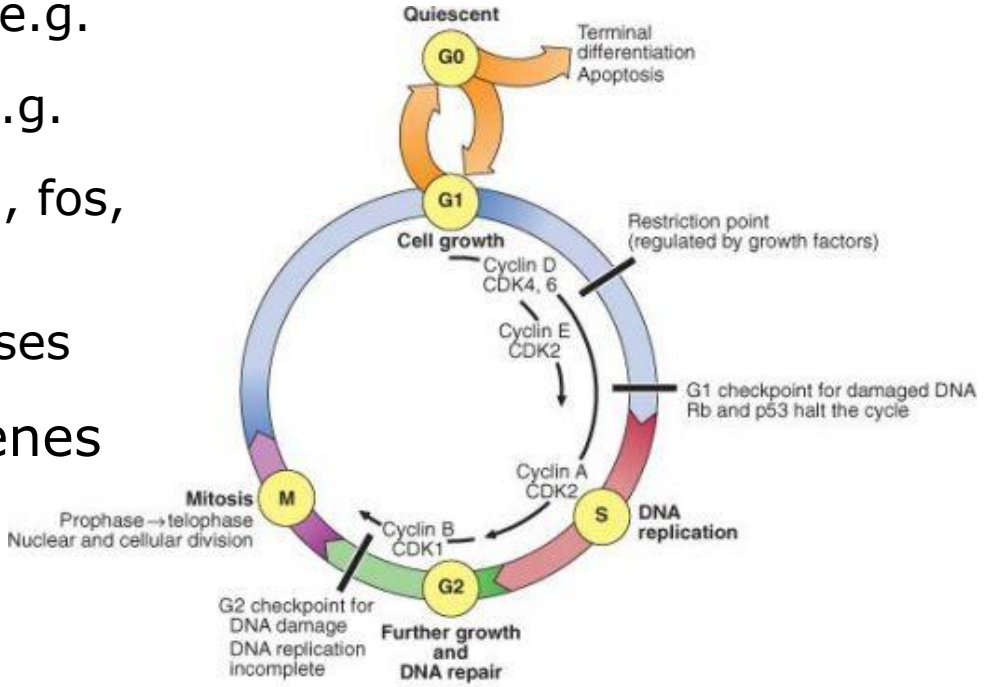
CC check points and mitosis





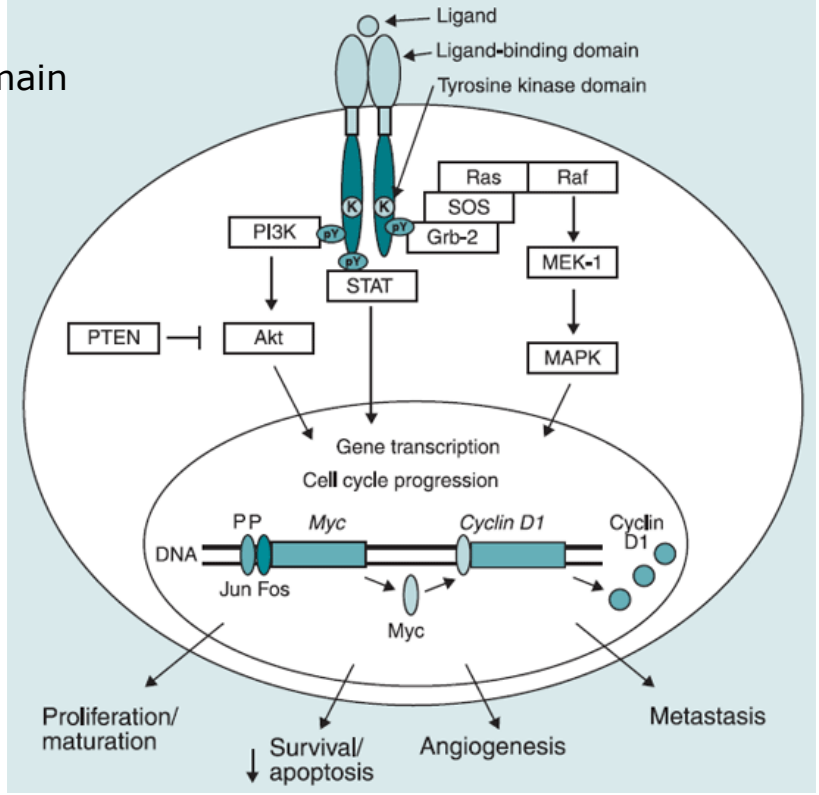
CC regulatory proteins

- (A) products of (proto)oncogenes
  - growth factors
  - receptors for growth factors
  - G-proteins (e.g. Ras)
  - membrane tyrosine kinases (e.g. abl)
  - other cytoplasmic proteins (e.g. Raf)
  - transcription factors (e.g. jun, fos, myc)
  - cyclins
  - cyclin-dependent proteinkinases (cdk)
- (B) products of suppressor genes
  - Rb
  - p53
  - p21
  - …
- (C) products of genes encoding DNA repair enzymes
  - mismatch repair
  - excision repair
  - homologous recombination

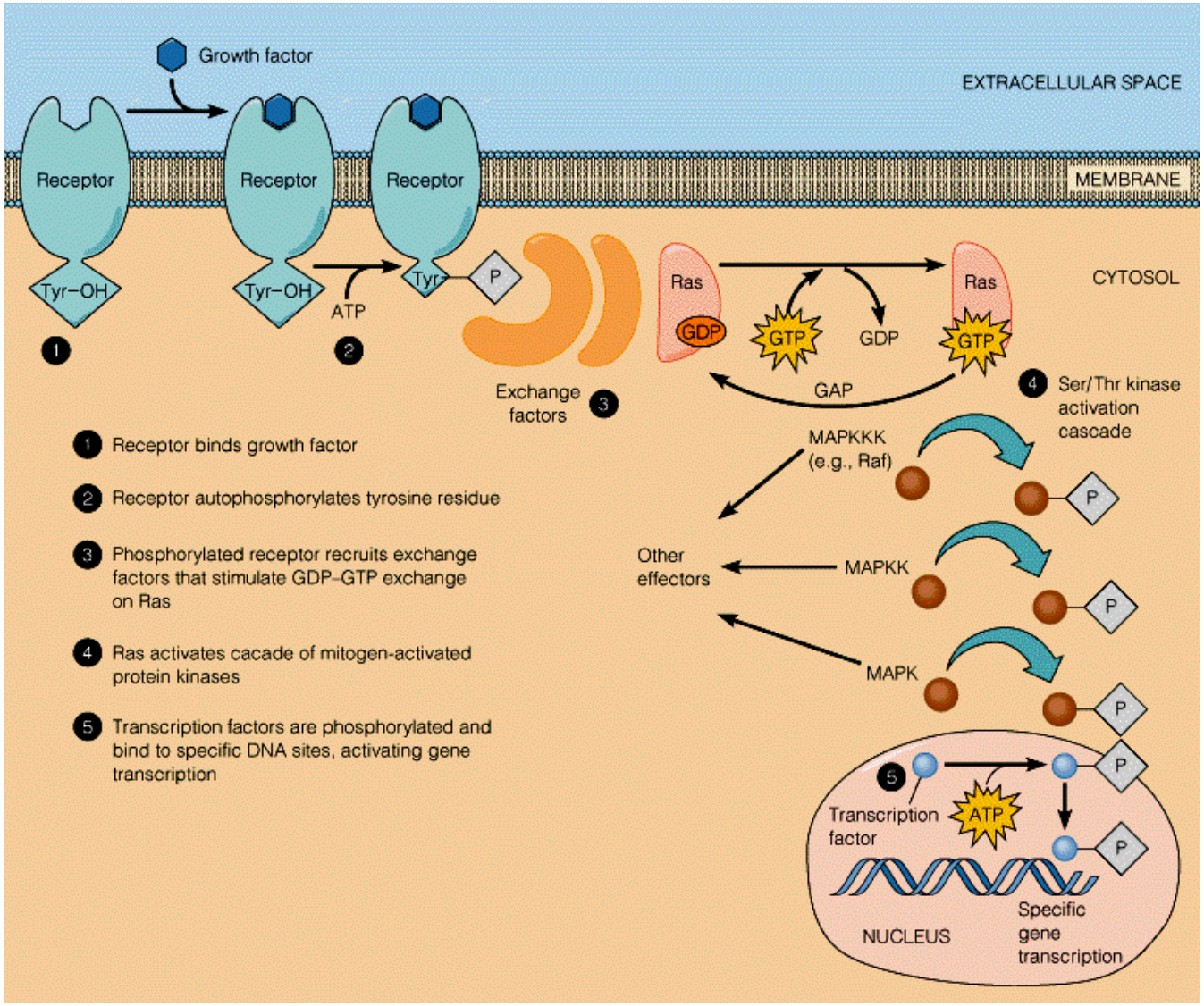


(A) Protooncogenes

- **(1) growth factors (GF)**
  - GF acts in extremely low concentrations in a paracrine fashion
    - e.g. TGF-β, PDGF, EGF, FGF, VEGF, erythropoetin, …
  - different target cells according to the expression of specific receptors
- **(2) GF receptors**
  - extracellular, transmembrane and cytoplamatic domain
    - usually with tyrosinkinase activity
- **(3) G proteins (= GTP-ases, e.g. Ras)**
- **(4) other cytoplasmic factors**
  - Tyr-kinases (Src, Abl, …)
  - Ser/Thr-kinases (Raf)
    - upstream to downstream kinases (MAPK – Mitogen Activated Protein Kinase)
    - transcription of early-response genes (~15 min)
- **(5) transcription factors/ early-response genes**
  - e.g. fos, jun a myc (= products of protooncogens fos, jun and myc)
  - regulate transcription of late-response genes
- (6) late response genes (~1 hrs) = **cyclins**
  - expression of cyclins and cdk under the stimulation with fos, jun, myc regulatory proteins
- **(7) cyclin-dependent kinases** (cdk)
- **(8) anti-apoptotic factors**
  - e.g. Bcl-2, Bcl-X
- **(9)** others (e.g. **β-catenin**)

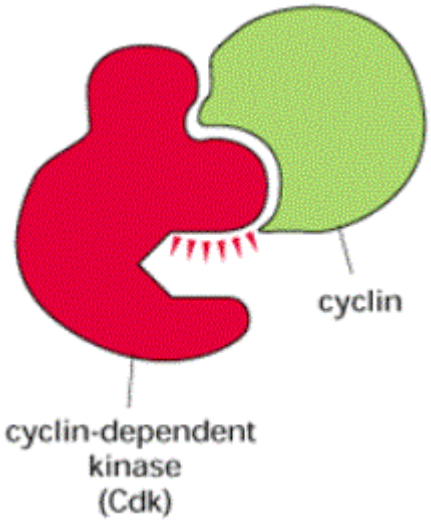


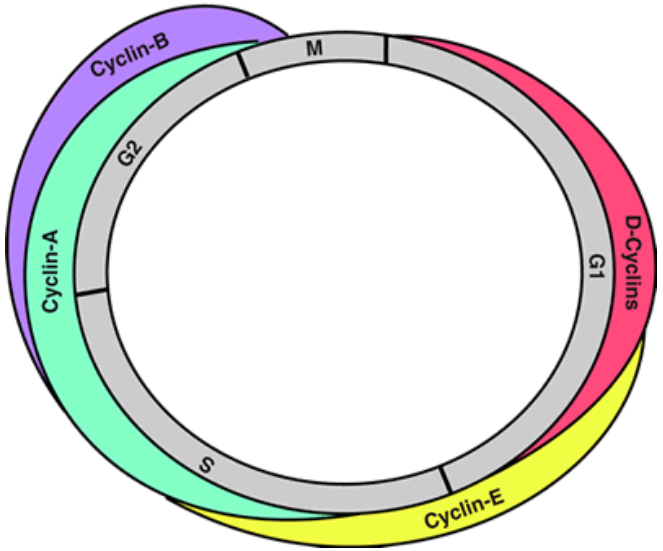
(A) Protooncogenes - summary



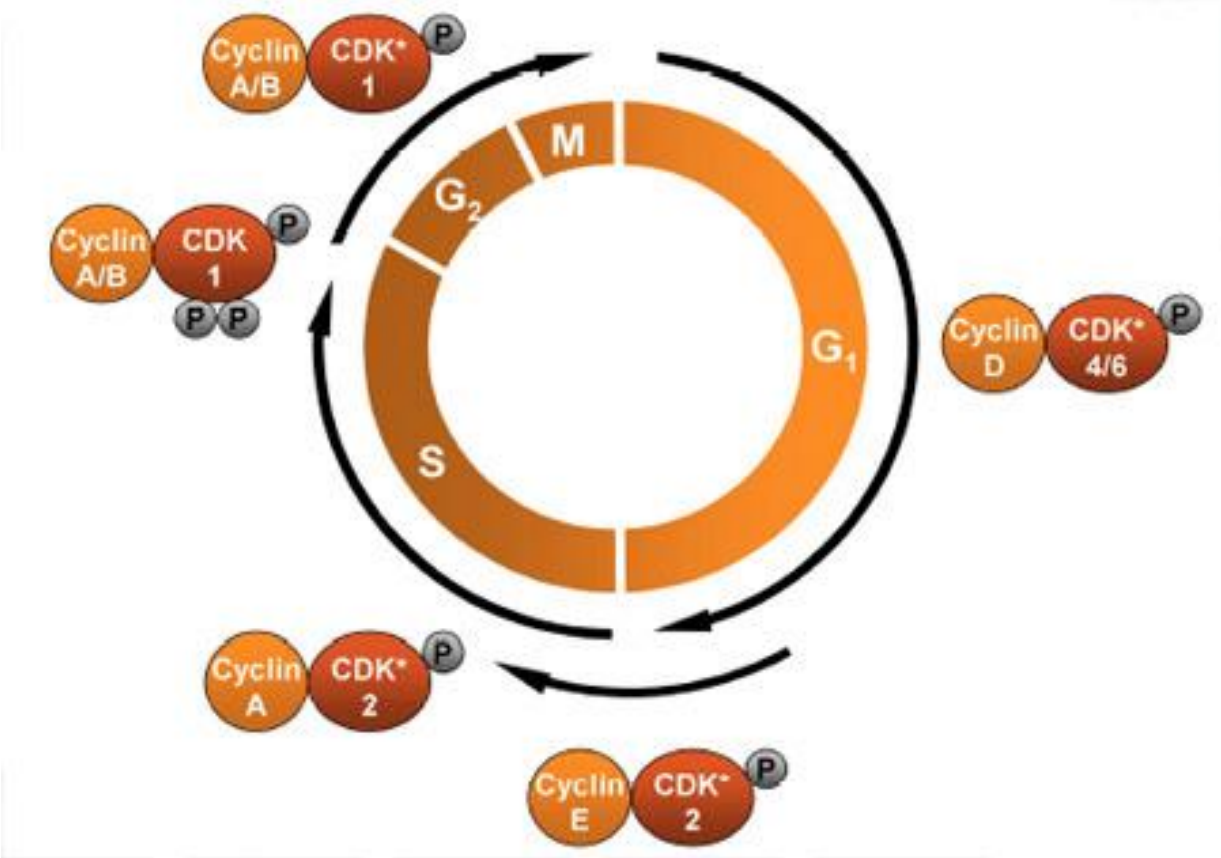
(A) Protooncogenes - continued

- **(6) cyclins**
  - 8 types – A, B, C, D, E, F, G, H
  - specific for particular CC phases
- **(7) cyclin-dependent kinases** (cdk)
  - 9 types – cdk1 to cdk9
    - only complex of cdk with cyclin is active
    - activate target proteins by phosphorylation of Ser and Thr
      - » e.g. Rb-protein
    - normally present in the complex
      - » cyclin
      - » cdk
      - » PCNA (Proliferating Cell Nuclear Antigen)
      - » cdk inhibitor (e.g. p21, p27, …)
    - proteolysis of the inhibitors allows the complex being active
  - levels of cdk maintain relatively stable throughout the CC while expression of cyclins differs
    - expressed under the stimulation with growth factors
    - degraded by ubiquitin-proteasome proteolysis

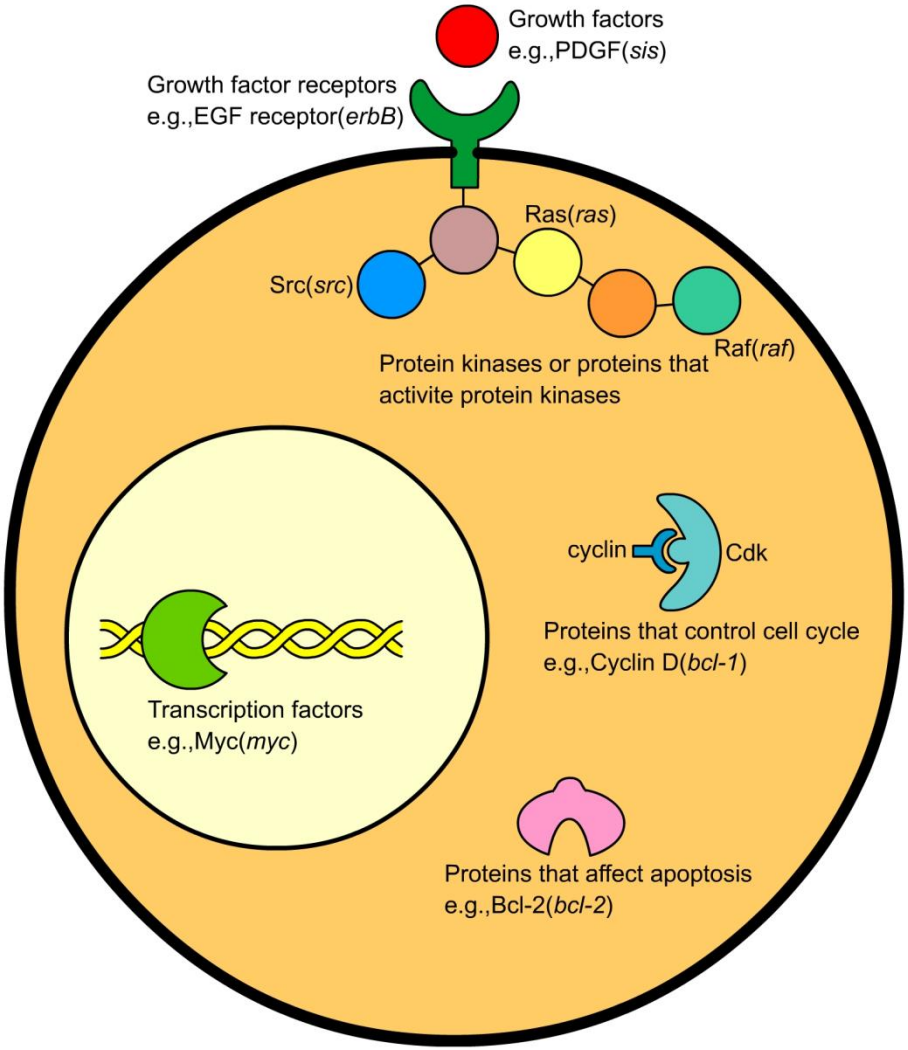




Cyclin / cdk interplay



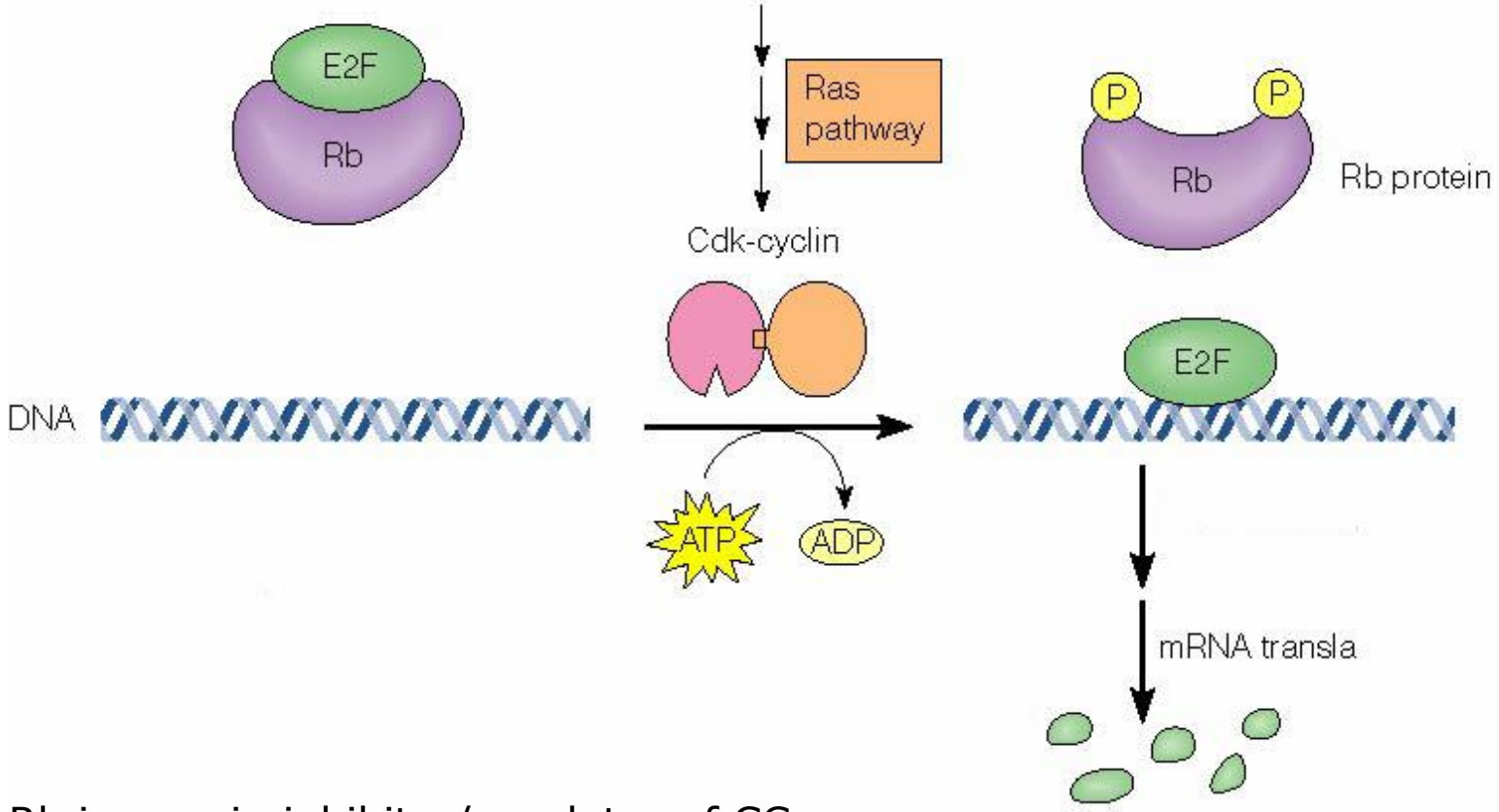
Summary of the protooncogenes



(B) Suppressor genes

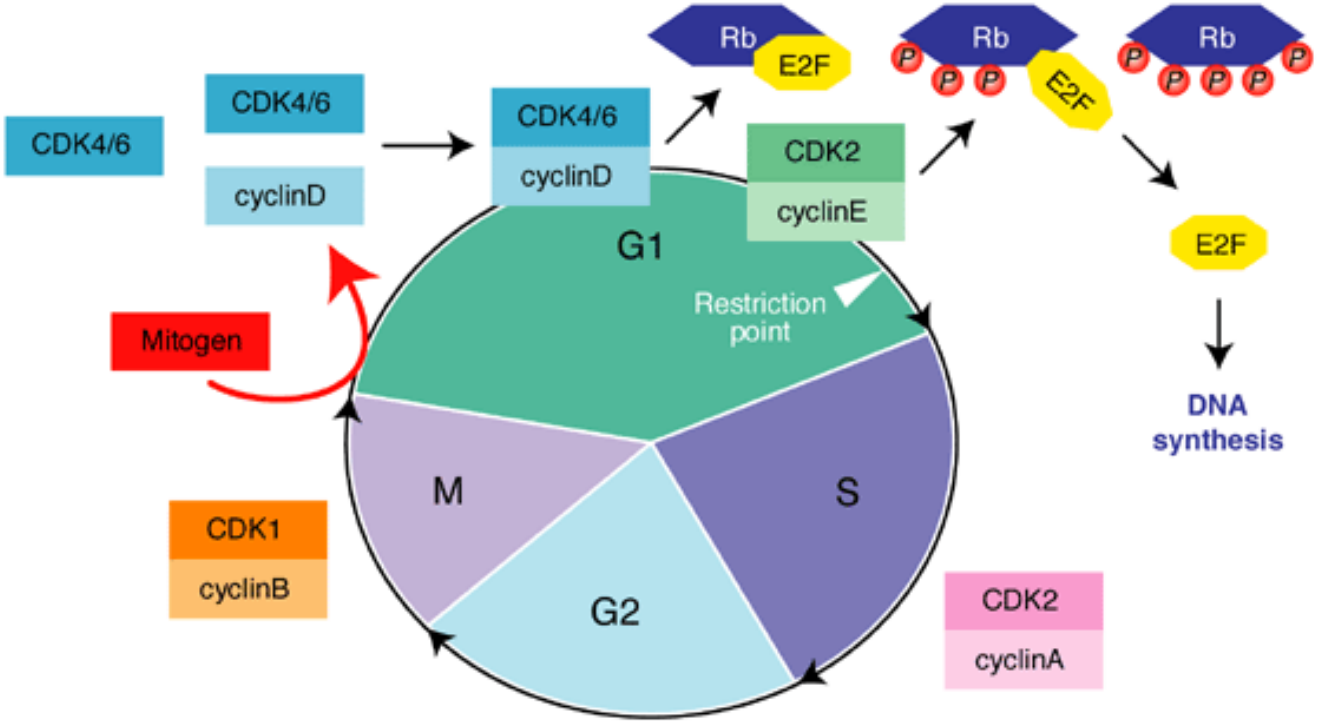
- encode proteins arresting CC, activating DNA repair and apoptosis
  - **(1) Rb protein** (ch. 13q14)
    - pocket protein family member
    - principal negative regulator of CC, controls the G1-S-phase transition, activity modulated by de-/phosphorylation (by cdk4/6 + cyclin D complex)
    - mutations in Rb (microdeletions) predispose to the retinoblastoma
  - **(2) p53 protein** (ch. 17p13)
    - "guardian of the genome" – active in G1 and G2 checkpoints
    - DNA damage increases expression of p53
    - act as a transcription factor for DNA repair and apoptosis genes
  - **(3) inhibitors of cyclin-dependent kinases (e.g. p21, p27, p16, …)**
    - p21 is the main target of p53 = inhibitor of Cdk – CC arrest in G1 phase by inhibition of Cdk2/cyclin E complex
  - **(4) pro-apoptotic genes**
    - familiy of Bcl genes (Bax, Bak, Bad, …)
  - **(5) other**, e.g. inhibitory transcription factors, M-spindle checkpoint, Wnt pathway etc.
    - FOXO, SMAD, APC, …
- inherited mutations in suppressor genes can confer susceptibility to the inherited (familiar) forms of cancer
  - very often named according to the type of tumor developing due tot their mutation
    - Rb (= retinoblastoma)
    - WT (= Wilm's tumor)
    - NF1 and NF2 (= neurofibromatosis)
    - APC (= Adenomatous Polyposis Coli)
    - DCC (= Deleted in Colon Cancer)
    - VHL (= von Hippel-Lindau syndrome)

Rb protein (Rb/E2F G_1 checkpoint)



- Rb is a main inhibitor/regulator of CC
  - binding to the transcription factor E2F, which upon release from Rb ↑ expression of S-phase genes (e.g. DNA replication enzymes, PCNA, …)
- Rb controls the transition from G1- to S-phase
- Rb is present all the time, however, its activity is modulated by de-/phosphorylation by MAPK/cdk pathways
  - phosphorylated Rb = inactive
  - dephosphorylated Rb = active

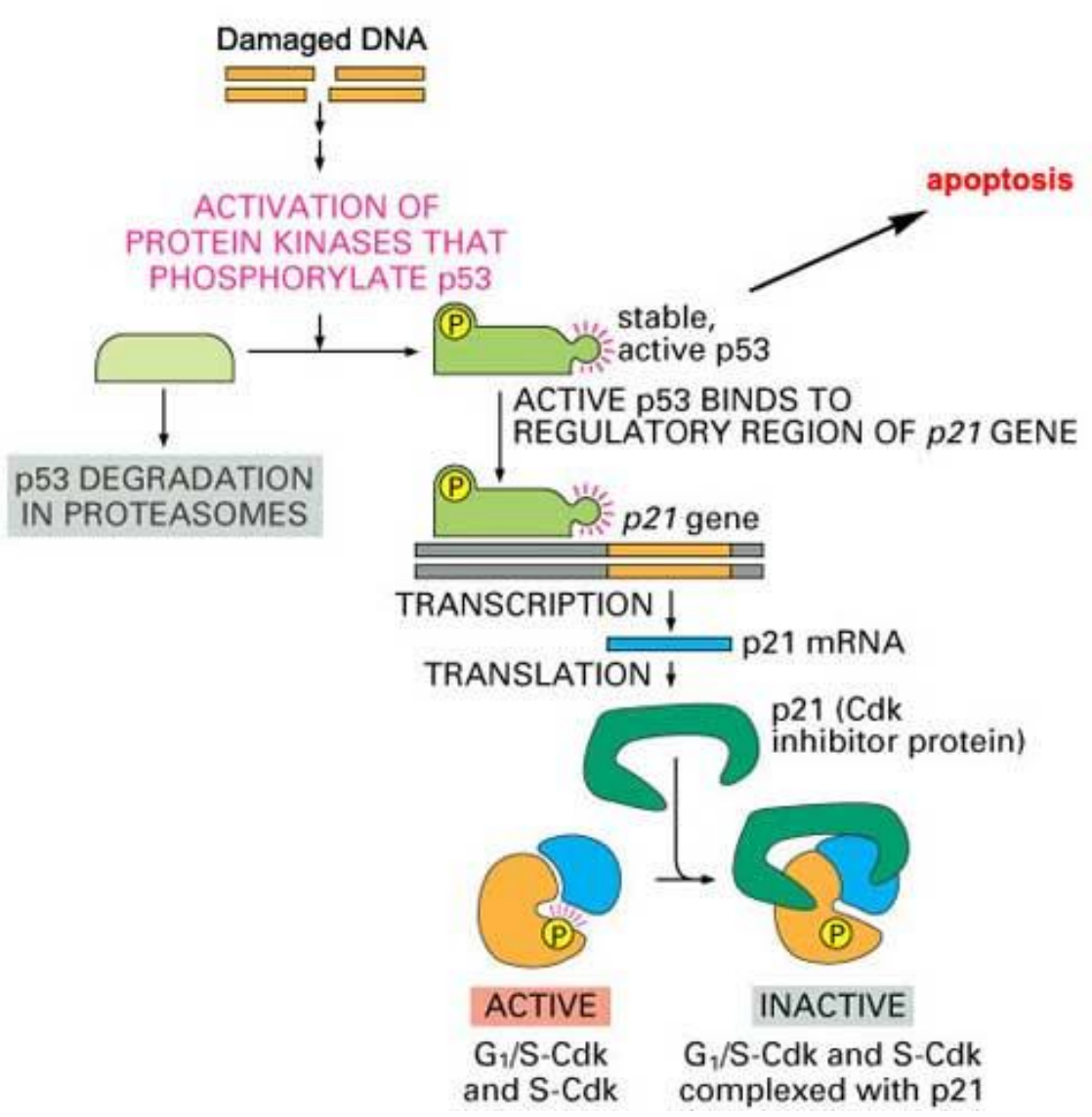
Partial summary – CC “kick-off”

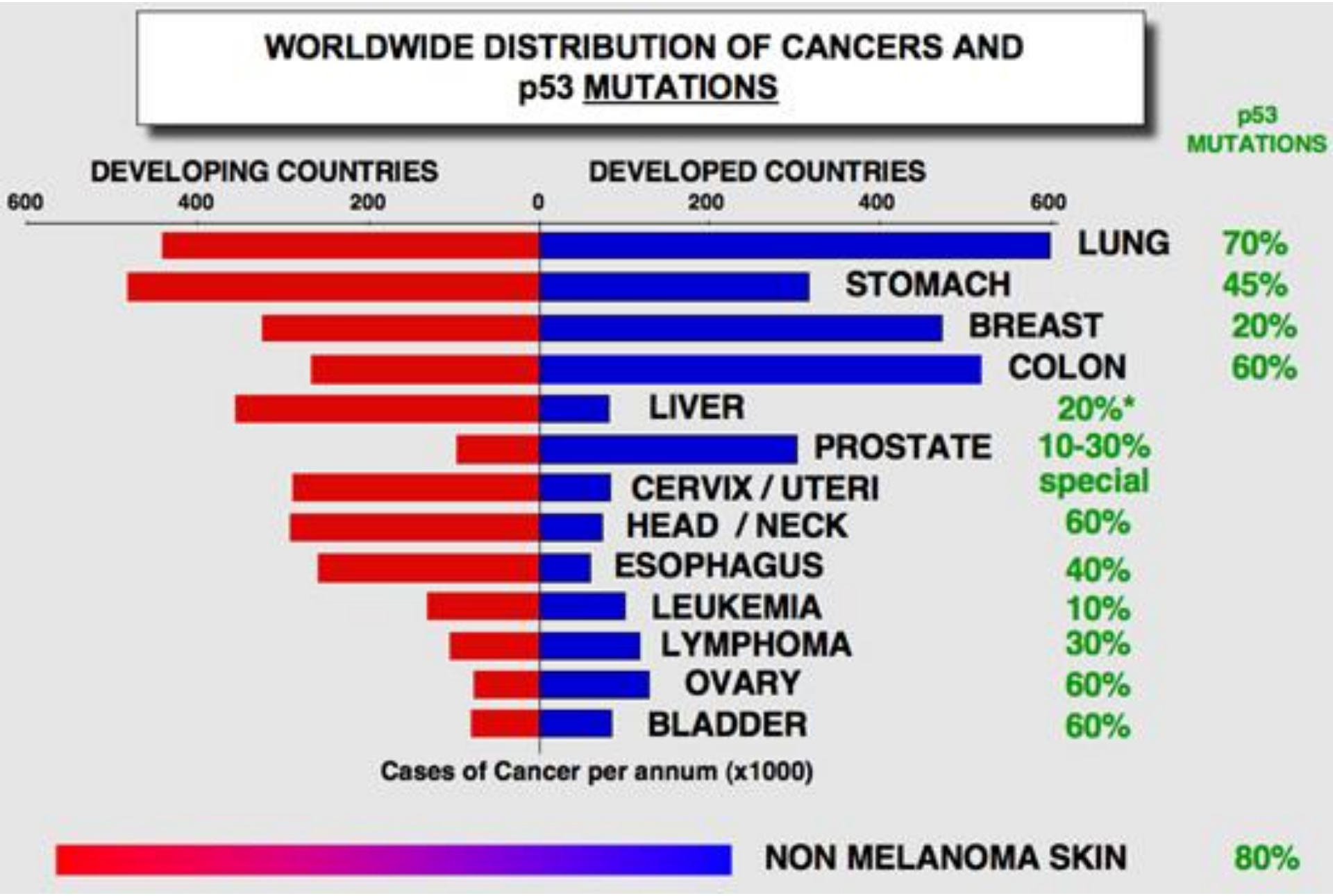


- mitogens drive CC progression by induction of cyclin D and inactivation of the retinoblastoma (Rb) protein
  - CC is driven by the co-ordinated activation of CDKs (expressed throughout the CC) and their activating subunits – the cyclins (oscillating between rapid synthesis and degradation)
  - the interface between mitogens and the cell cycle is cyclin D (and to a lesser extent cyclin E), whose expression is induced by mitogens
    - cyclin D- and cyclin E-dependent kinases phosphorylate (P) and thereby disable the Rb tumour suppressor protein, which is a principal checkpoint controlling the progression from G1 to S phase
  - inactivation of the Rb protein marks the restriction point at which cell-cycle progression becomes independent of mitogens
    - inactivated Rb releases E2F transcription factors, which stimulate the expression of downstream cyclins and other genes that are required for DNA synthesis

Protein p53 (ch. 17p13)

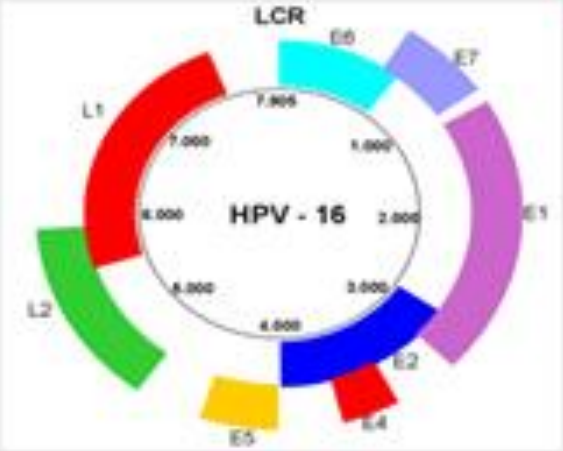
- nuclear protein, active as a phosphorylated tetramer, acts as a transcription factor
- main controller of genome stability
- if DNA is mutated or incompletely replicated p53 becomes activated and:
  - ↑ expression of CC inhibitors → temporary **CC arrest** in G1/S check point enabling DNA reparation ("major repair")
    - (CIP1/WAF1 gene → p21 protein)
  - ↑ GADD 45 (Growth Arrest and DNA Damage) → **DNA excision repair**
  - Bax expression → **apoptosis**
- p53 mutations are the most frequent genetic abnormality found in human cancer
  - ~50% of all cancers!!!
- there are also some familiar forms of cancer due to inherited p53 heterozygous mutations
    - LOH mechanism



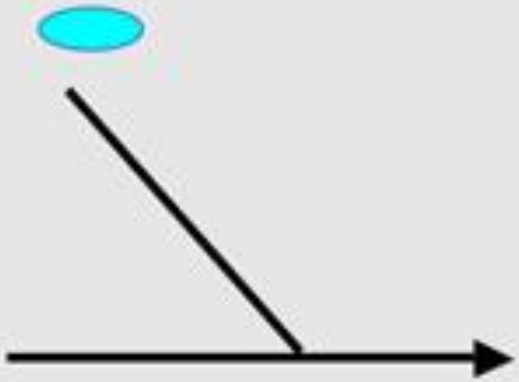

WORLDWIDE DISTRIBUTION OF CANCERS AND
p53 MUTATIONS
p53
MUTATIONS
DEVELOPING COUNTRIES
DEVELOPED COUNTRIES
600
400
200
0
200
400
600
LUNG
70%
STOMACH
45%
BREAST
20%
COLON
60%
LIVER
20%*
PROSTATE
10-30%
CERVIX / UTERI
special
HEAD / NECK
60%
ESOPHAGUS
40%
LEUKEMIA
10%
LYMPHOMA
30%
OVARY
60%
BLADDER
60%
Cases of Cancer per annum (x1000)
NON MELANOMA SKIN
80%

INDIRECT INACTIVATION OF p53

PAPILLOMAVIRUS INFECTION

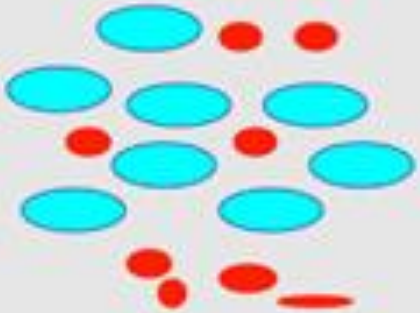


E6 PROTEIN FROM HPV



p53

ACTIVE p53

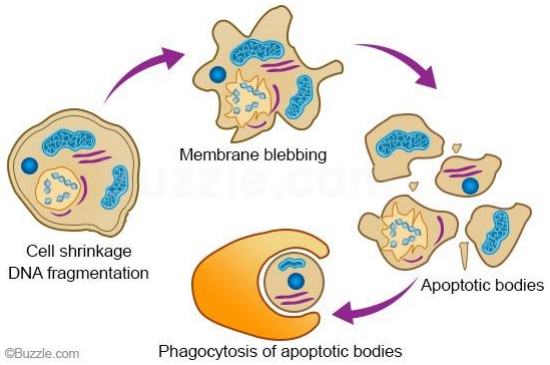


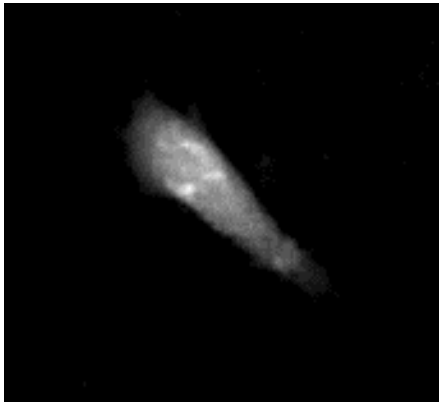
p53 DEGRADATION

The E6 viral protein expressed by HPV specifically binds to the p53 protein and induces its degradation. This observation explains the rarity of p53 mutations in cervical cancers.

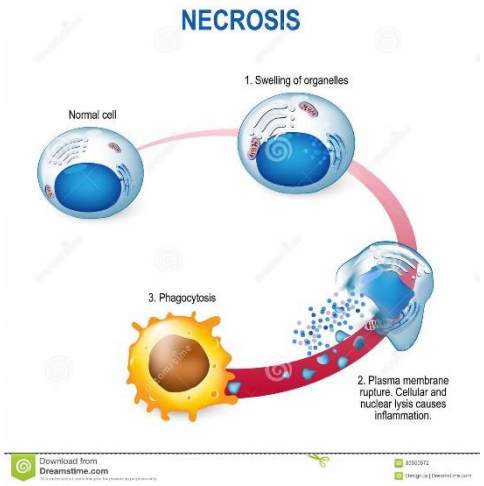
Cell death – apoptisos, necrosis, necroptosis

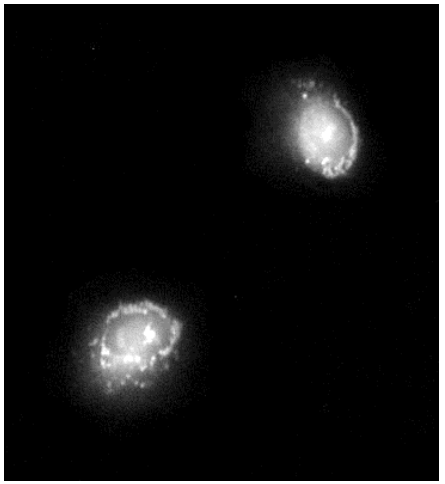
Apoptosis
Active (= energy requirement), programmed cell death. The action of caspases and other apoptotic enzymes (proteases and nucleases) leads to cell fragmentation to apoptotic bodies that are removed by macrophages.





Necrosis
Accidental cell death caused mainly by external factors (infections, toxins, etc.). Cellular content is released into the environment and damage surrounding tissues. Necrosis has proinflammatory and tumor-promoting potential.

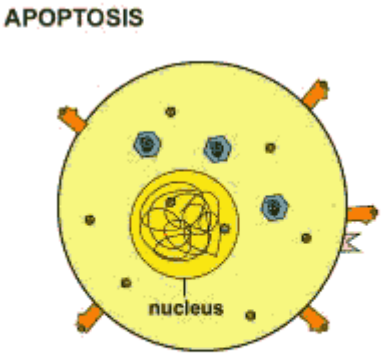




Necroptosis
Controlled form of necrosis driven by kinases RIP1 and RIP3.

Apoptosis

- type of active (=energy requirments), programmed cell death affecting isolated cells
- induction of apoptosis
  - **extrinsic (receptor) pathway**
    - DEATH receptors (FAS, TNFR) and their ligands (TNFa, LTA - lipoteichoic acid , TRAIL - TNF-related apoptosis inducing ligand) = DISC (death-inducing signalling complex)
    - Tc lymphocytes and NKbb. (granzyme)
    - absence of growth stimuli
  - **intrinsic (non-receptor) pathway – mitochondria having the main role**
    - ROS, hypertermia, DNA damage, hypoxia, starvation, …
    - permeabilisation of mitoch. membrane (Bax, …), release of cytochrome-c and Ca
    - formation of apoptosome in cytoplasma – cytochrome-c + Apaf + Ca ions and activation of "upstream" caspases (pro-caspase 9)
- both pathways converge on the level of caspase 3, both regulated by Bcl family members
  - anti-apoptotic (Bcl-2, Bcl-X, …)
  - pro-apoptotic (Bax, Bak, Bad, …)
- execution of apoptosis
  - caspases (cystein aspartases)
    - upper caspases (receptor path c-8, non-receptor path c-9)
    - lower caspases (-3, -6, -7)
    - substrates: cytosceleton, membrane proteins
  - endonucleases
    - fragementation of DNA
- morphology of apoptosis
  - rounding of cell
  - budding/blebbing
  - apoptotic bodies



Formation of apoptosome and convergention of both pathways

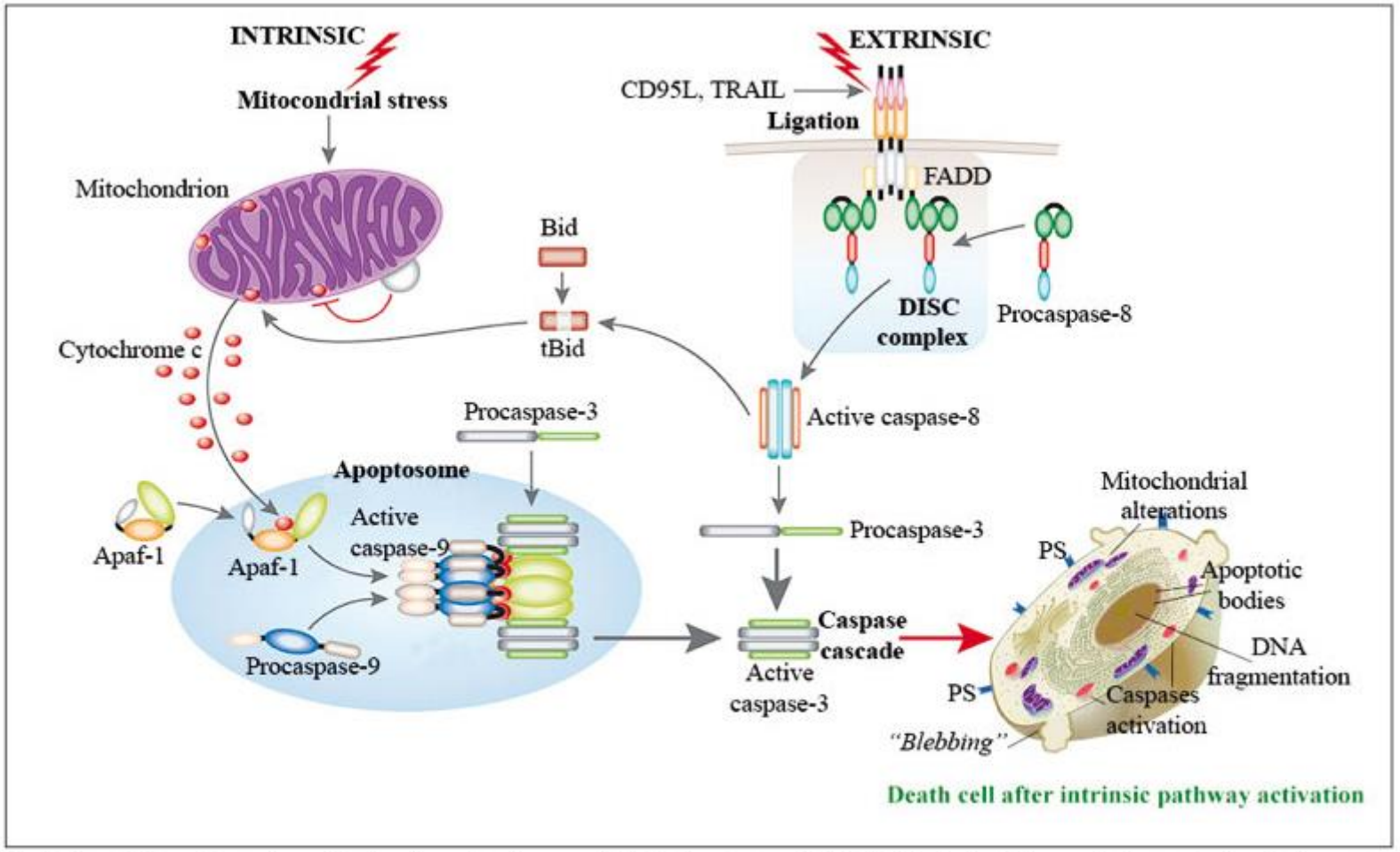
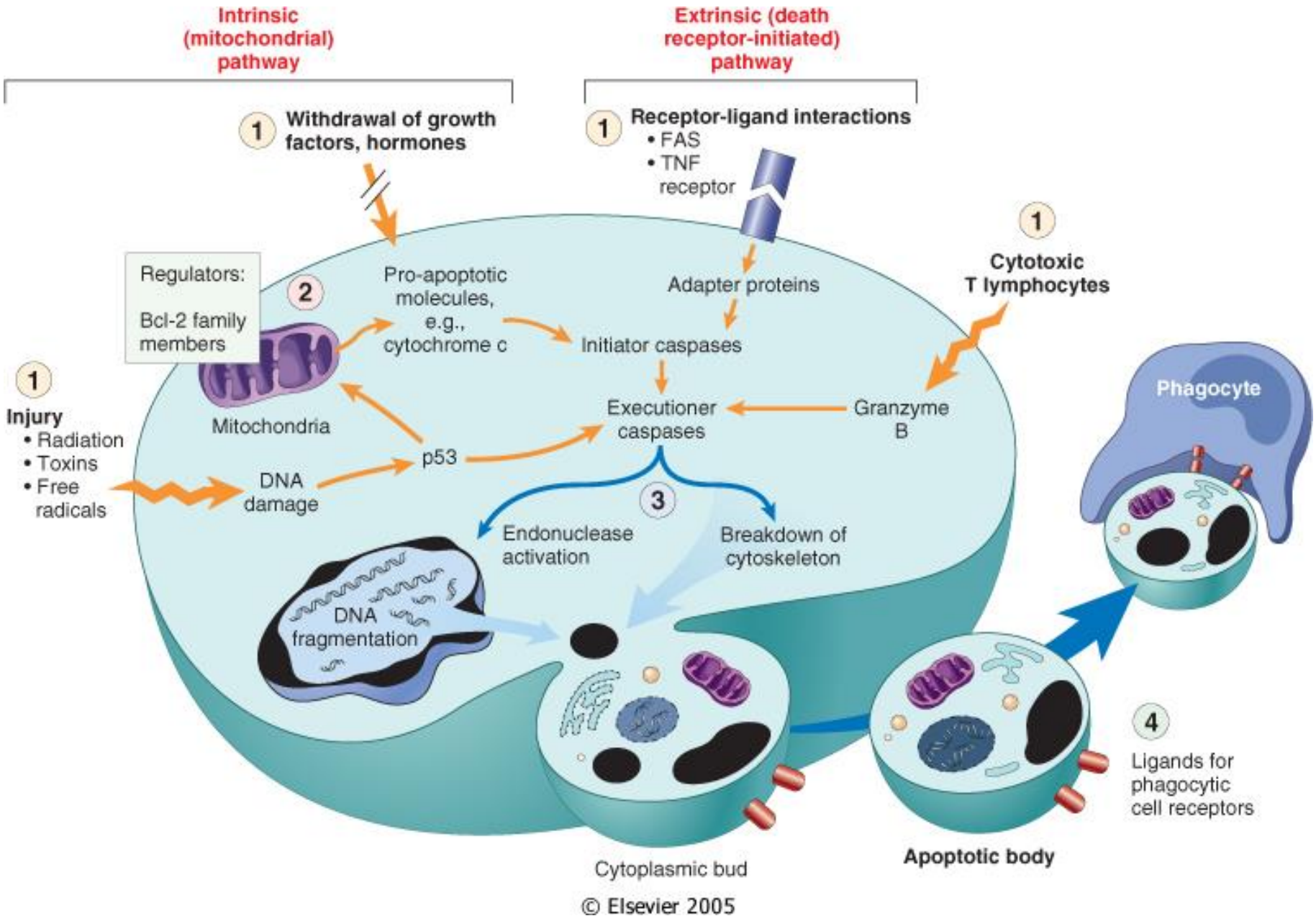


Fig. 3. *Apoptotic pathways:* the extrinsic pathway involves so-called death receptors (CD95, TRAIL); the intrinsic one involves mitochondrial granules. Both pathways converge at caspase-3 activation, where classic biochemical and morphological changes in association with the apoptotic phenotype are originated.


Intrinsic (mitochondrial) pathway
Extrinsic (death receptor-initiated) pathway
1 Withdrawal of growth factors, hormones
1 Receptor-ligand interactions
• FAS
• TNF receptor
1 Cytotoxic T lymphocytes
Regulators:
Bcl-2 family members
2
Pro-apoptotic molecules, e.g., cytochrome c
Adapter proteins
Initiator caspases
1 Injury
• Radiation
• Toxins
• Free radicals
Mitochondria
DNA damage
p53
Executioner caspases
Granzyme B
3
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
Phagocyte
4 Ligands for phagocytic cell receptors
Cytoplasmic bud
Apoptotic body
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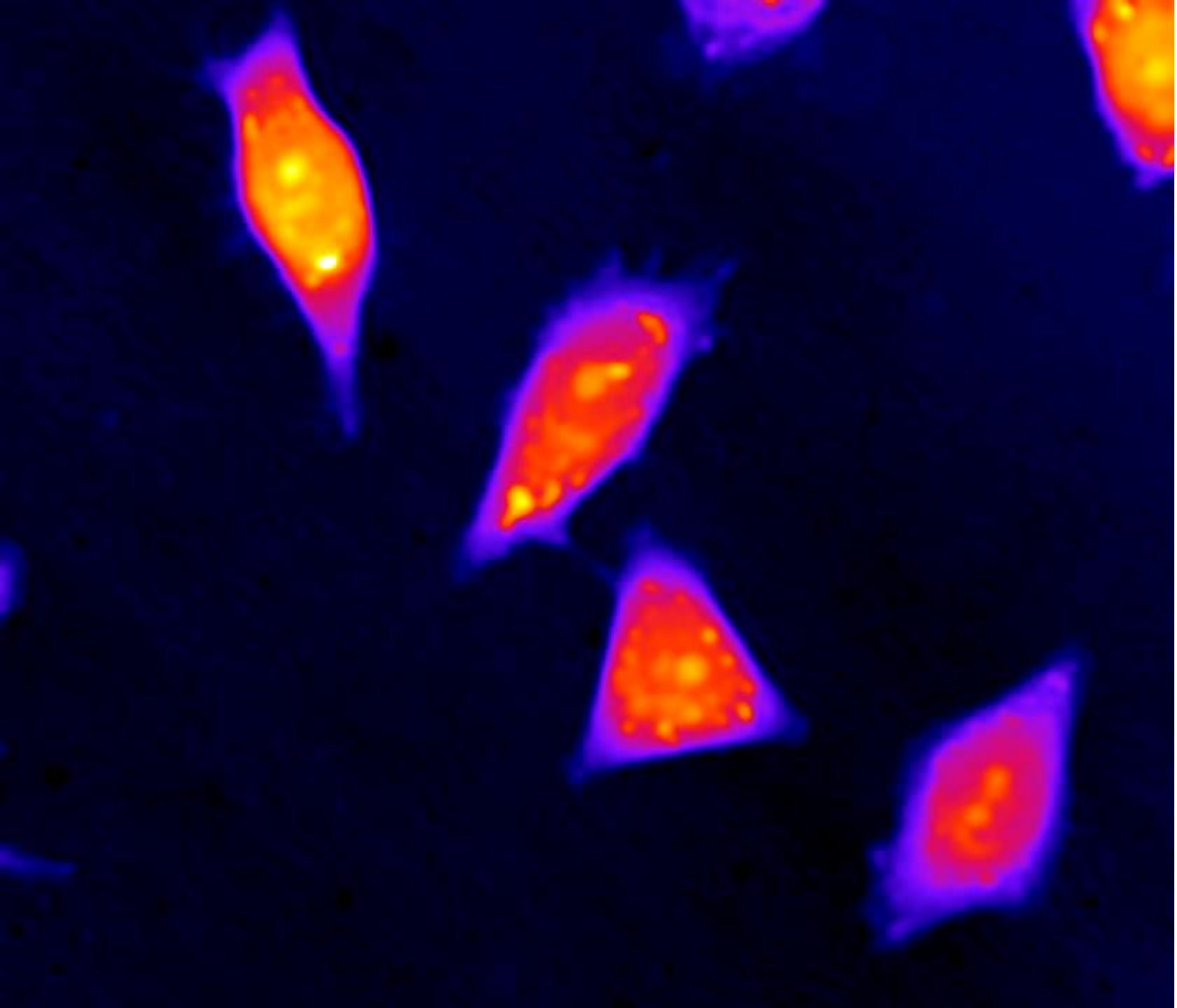
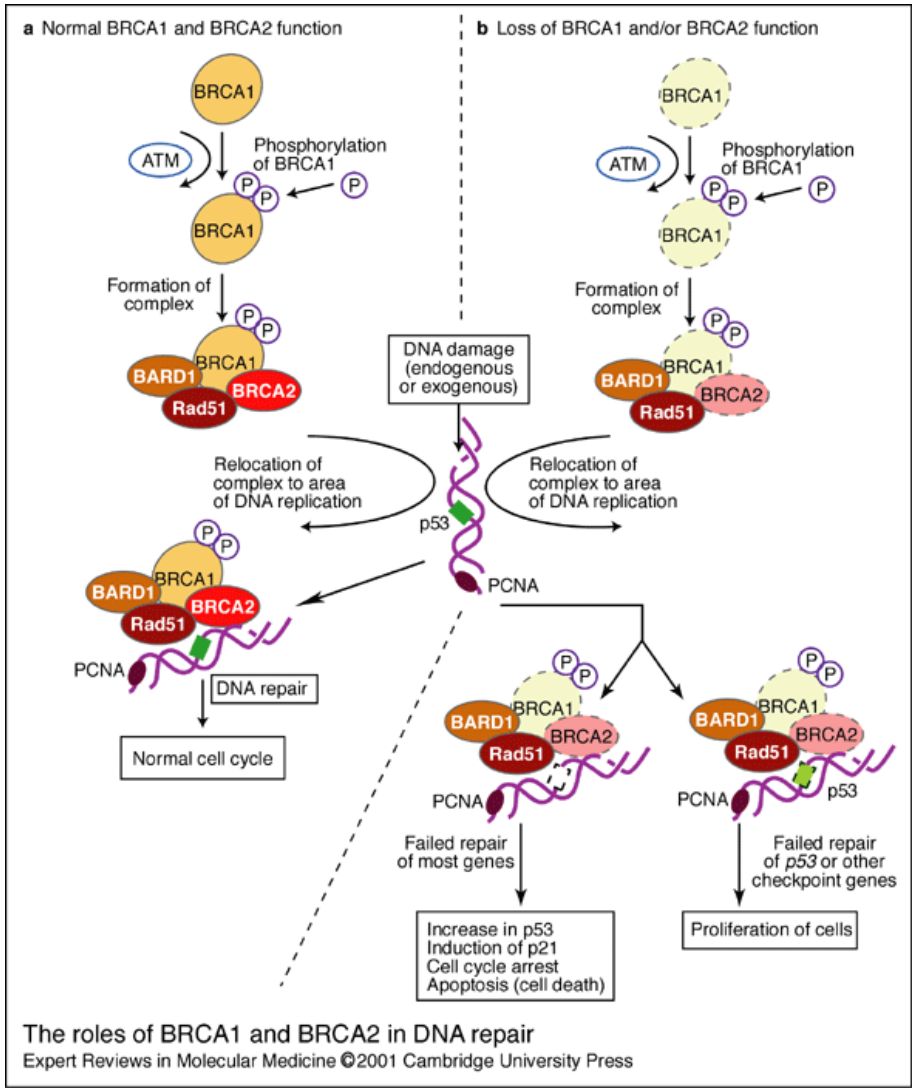
Resisting cell death

- Tumor cells evolve a variety of strategies to limit cell death. Most known are:
  - loss of **p53**
  - **increased expression of antiapoptotic** regulators (Bcl-2, Bcl-xL) and survival signals (insulin-like growth factors; Igf1/2)
  - **downregulating of proapoptotic** factors (Bax, Bim, Puma)
  - opportunistic modes of behavior (cell fusion)



(C) DNA repair (stability) genes

- (1) MMR genes/proteins ("Mismatch repair")
  - enzymes can repair erroneous base pair
  - defect in respective genes leads to the microsatellite length instability (MIN)
    - variable length of microsatellites (e.g. $(CA)_n$ repetition) leads to the DNA replication errors
  - example is **HNPCC** (Hereditary Non-Polypous Colon Cancer)
- (2) excision repair (single strand break)
- (3) genes of homologous recombination
  - main pathway activated on DNA damage (double strand break) involves:
  - **BRCA1** and **BRCA2**
  - **ATM** and **ATR** (ATM-related) kinases
  - **CHK2** and **CHK1** checkpoint kinases
    - ATM, ATR/CHK2 (CHK1) → p53/MDM2 → p21 → "growth arrest"
- inborn defects lead to several forms of familiar cancers
  - ataxia telengiectatica
  - Bloom syndrome
  - Fanconi anemia
  - xeroderma pigmentosum
  - fragile X syndrome

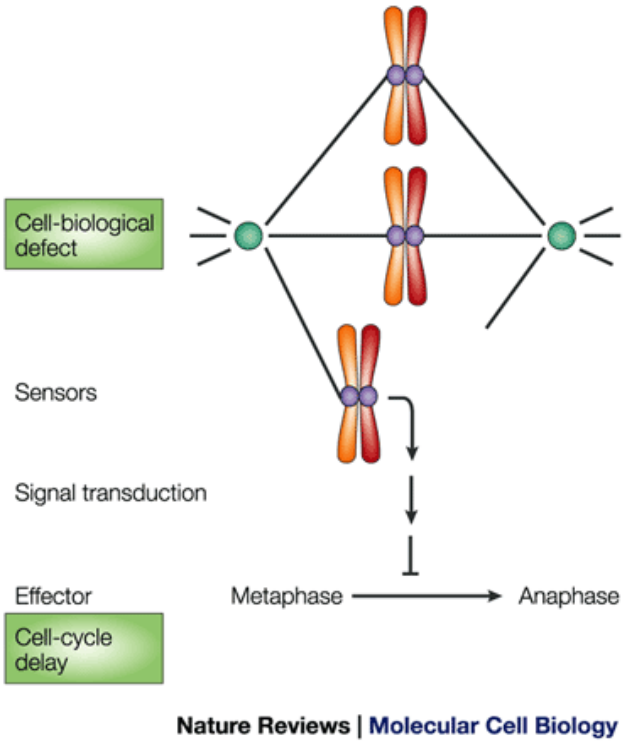


The roles of BRCA1 and BRCA2 in DNA repair

Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

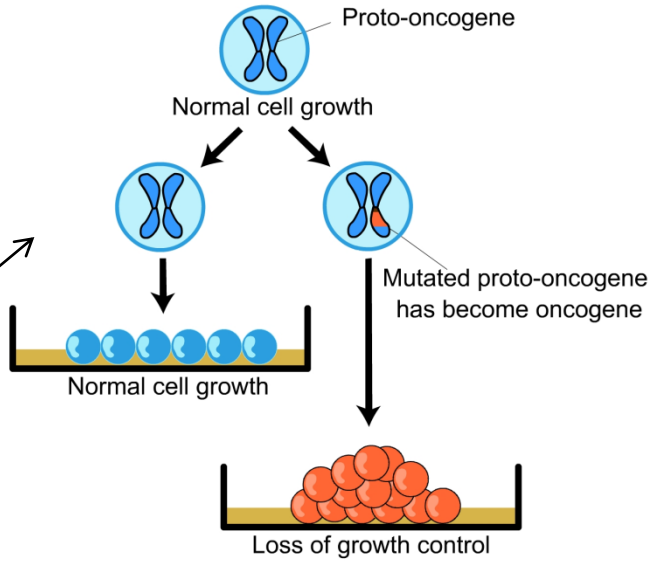
Mitotic (spindle) checkpoint

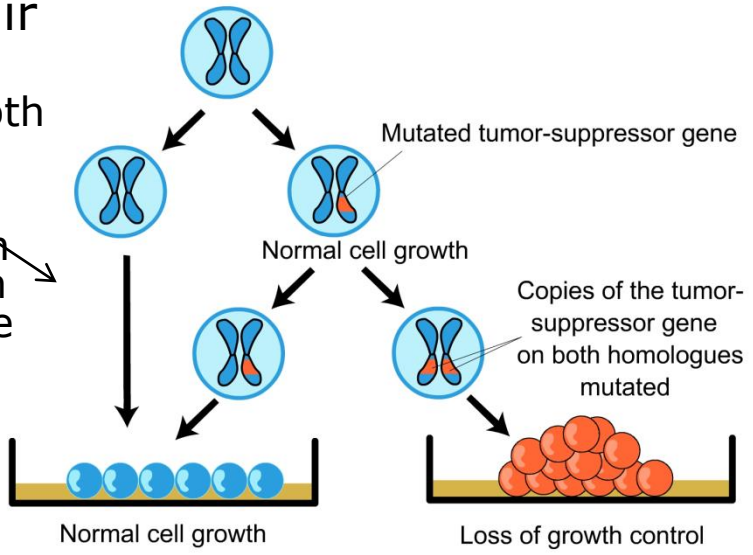
- majority of tumor cells show aneuploidy
  - consequence of failure of M-spindle checkpoint = chromosome non-disjunction
  - aneuploidy contributes to further chromosome instability and cancerogenesis
  - higher dose of oncogene or loss of tumor supressor, …
- marker of poor prognosis
- higher risk of cancer in syndromes of constitutive aneuploidy (e.g. Down syndrome, …)
- M-control is assisted by tumor supresor APC
  - mutated in familiar multiple adenomatous polyposis syndrome (FAP)



Process of cancer transformation

- mutations in critical DNA positions contributing to CC regulation as a result of:
  - exposure to mutagens / carcinogens
  - spontaneous error during replication
- mutation with carcinogenic potential leads to:
  - hyperactivity of proto-oncogene (transformation to oncogene)
    - „gain of function" = dominant effect (i.e. single mutated allele sufficient to produce pathologic phenotype)
  - inactivation of suppressor or DNA repair genes
    - „loss of function" = recesive effect (i.e. both alleles have to be mutated)
    - either sporadic mutation in somatic cell
    - or one dysfunctional allele already in germ line cell (see familiar cancer predisposition further) and second muted later during life (so called „loss of heterozygozity", LOH)
      - ☛ random mutation
      - ☛ gene conversion (attempt to repair)
      - ☛ non-disjunction during mitosis





Types of DNA mutations

- point mutation
  - silent = no effect (alternative codon, same AA)
  - non-synonymous (missense) = different AA (change of protein function, hyper- or inactivity)
  - stop-codon (nonsense) = termination of translation (truncated protein)
  - mutation of regulatory regions of genes (promotors) = quantitative effect on transcription
- short insertions and deletions
  - "frameshift" effect
- pyrimidine dimers
  - by photochemical reaction (UV light)
  - thymine and cytosine covalent bridges = no replication

Wild type allele:

M D D Q S R M L Q T L A G V N L

atggacgatcaatccaggatgctgcagactctggccggggtgaacctg

silent (third base pair) mutation:

M D D Q S R M L Q T L A G V N L

atggacgatcaatccaggatgctgcaaactctggccggggtgaacctg

point mutation (missense):

M D D Q S R M L K T L A G V N L

atggacgatcaatccaggatgctgaagactctggccggggtgaacctg

point mutation (nonsense):

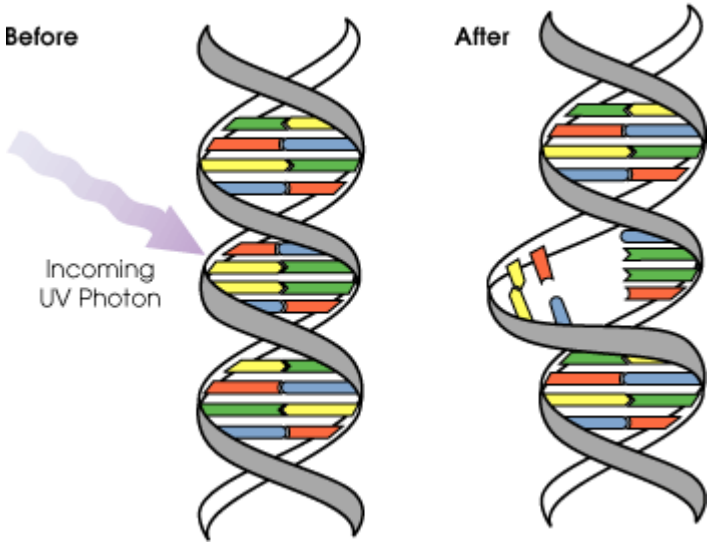
M D D Q S R M L stop

atggacgatcaatccaggatgctgtagactctggccggggtgaacctg

frameshift leading to premature termination:

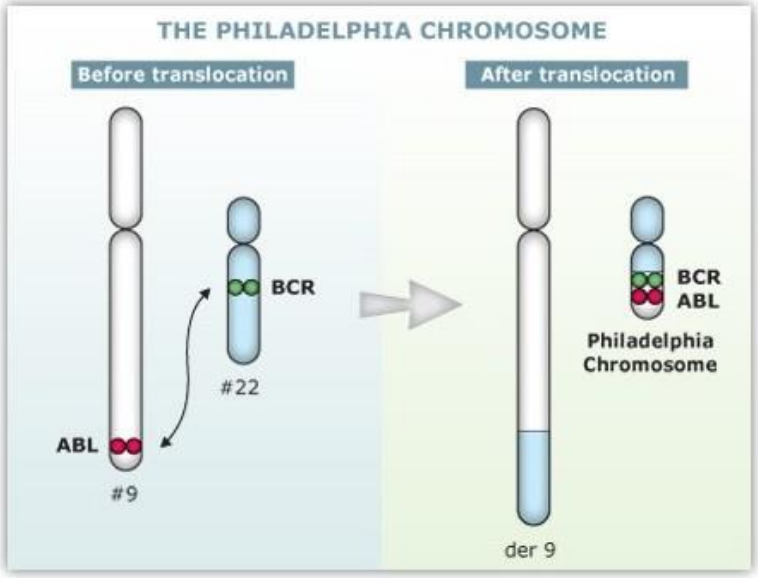
M D D Q S R M L R L W P G stop

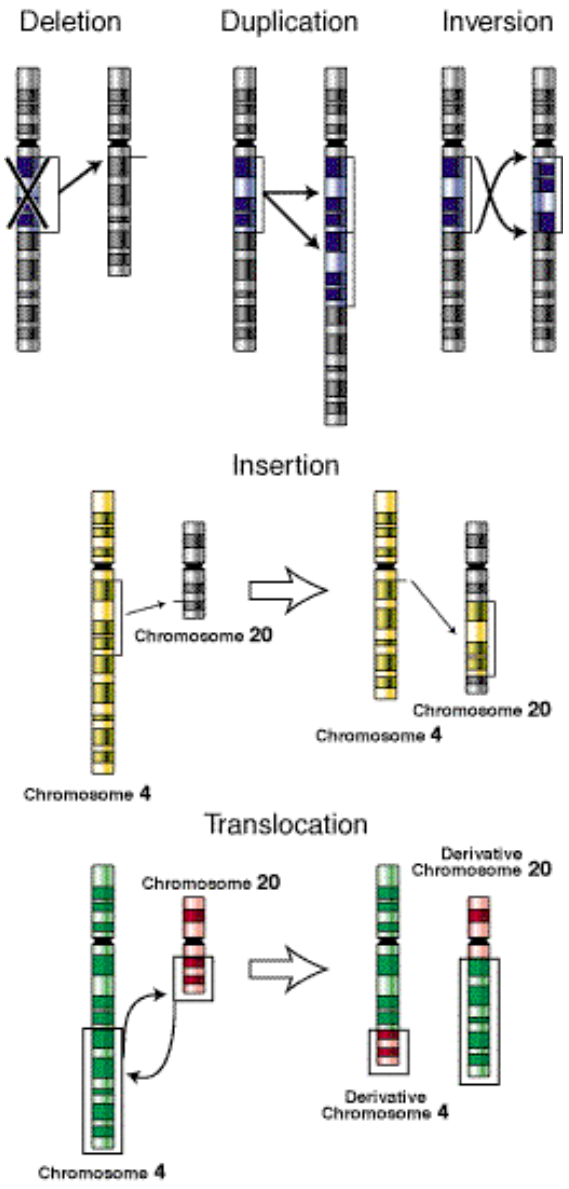
atggacgatcaatccaggatgctgagactctggccggggtgaacctg



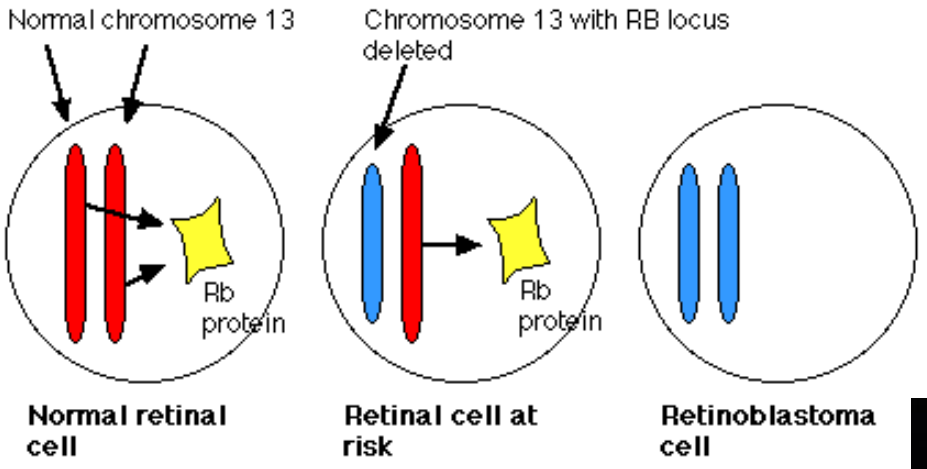
Types of DNA mutations

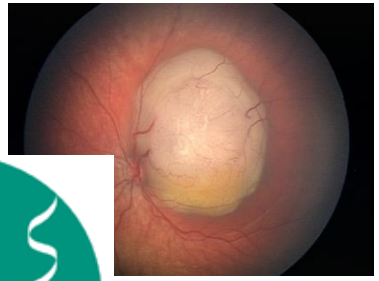
- chromosomal aberrations
  - deletions =loss of function (e.g. suppressor)
  - duplication = doubling the dose (e.g. proto-oncogene)
  - translocation = fusion gene
    - very common in haematological malignancies
    - part of the gene (e.g. proto-oncogene) attached to regulatory region of housekeeping gene

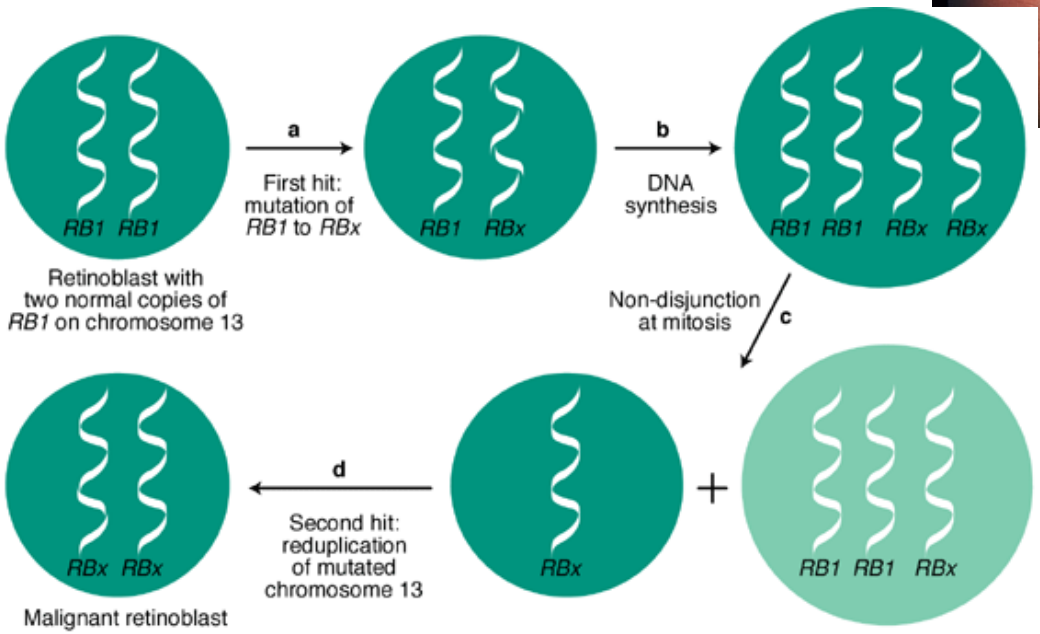




LOH example: retinoblastoma





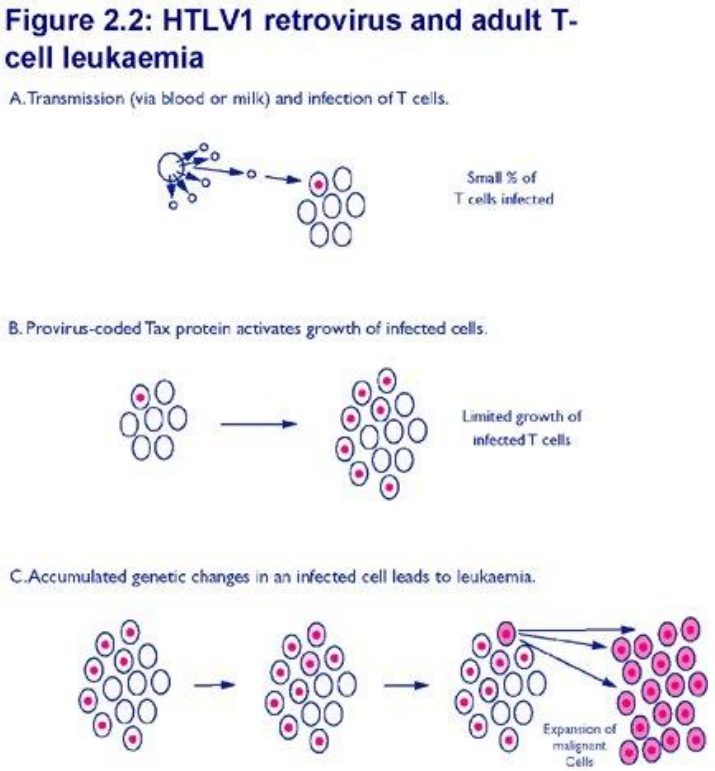


- Rb mutations (ch. 13q14) – most often microdeletions - lead to retinoblastoma (tumor of retina)
  - (1) inherited (familiar) from of retinoblastoma
    - patient inherited one mutated allele, second one is mutated early during the life (= loss of heterozygosity, LOH)
  - (2) acquired (sporadic) retinoblastoma
    - inactivation Rb by mutation of both alleles anytime during the life

Mutagens / carcinogens

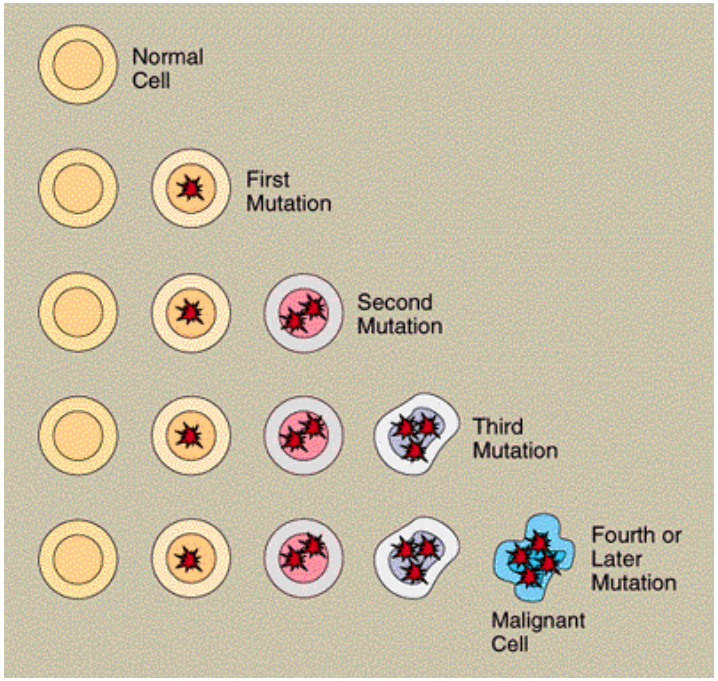
- **physical**
  - UV light (skin carcinoma and basalioma, melanoma)
  - ionising radiation and X-rays (leukaemia, thyroid gland, bones, …)
- **chemical**
  - polycyclic aromatic and chlorated hydrocarbons, aromatic amins, nitrosamins, heavy metals, mycotoxins, …
    - GIT cancer as a result of dietary toxins exposure
    - lung cancer as a result of smoking
    - alcoholic liver cirrhosis
- **biological** = viruses
  - incorporation of viral genome into the host one in critical regions
    - RNA viruses – retrovirus (activation of cellular or viral oncogenes)
      - ☛ e.g. B-lymphoma
  - inactivation supressors
    - DNA viruses (herpes, EBV, hepadnavirus, papilomavirus, adenovirus)
      - ☛ e.g. B-lymphomas, hepatocelular ca, cervical ca, larynx, oral cavity
  - indirect effect
    - increased sensitivity to mitogens - HTLV (T hairy-cell leukaemia) – ↑ expression of IL2 receptors
    - imunodeficiency - HIV (Kaposi sarcoma)
- **chronic inflammation = pre-cancerosis**
  - Baret's oesophagus in GER, ulcerative colitis and Crohn's disease, diverticulitis, ….

Figure 2.2: HTLV1 retrovirus and adult T-cell leukaemia

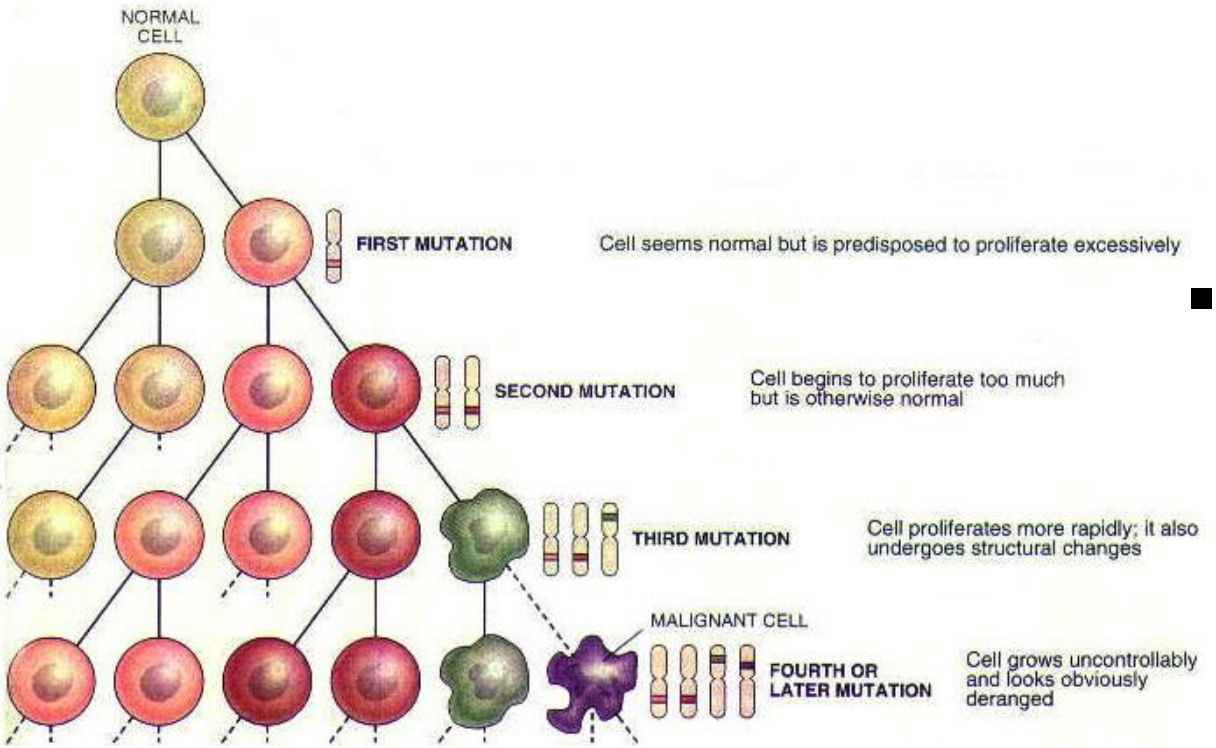


Partial summary

- cumulated mutations in 3 groups of genes contribute to the malignant transformation
  - protooncogenes (POG)
    - physiologically promote cell division by stimulating transition through cell cycle phases and transmitting the mitogenic signals
    - mutation of 1 allele is sufficient to produce uncontrolled cell division
  - suppressor genes (TS)
    - physiologically control cell division by arresting cell cycle or by inducing apoptosis
    - 1 functional copy is sufficient to exert the function
    - inactivation of both alleles contributes to tumorigenesis
  - DNA stability genes (SG)
    - not immediately involved in the tumorigenesis, but lack of their function leads to the higher mutation rate in general incl. POG and TS



Malignant transformation is a multistep process

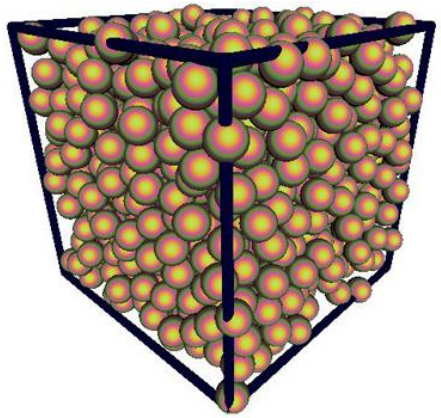


- multistage process of subsequent changes of genome - mutations in the critical DNA region
  - usually 6 – 10 necessary
- takes time
  - typical incidence of cancer in advanced age
  - the younger the age the more probable the role of inherited susceptibility

Tumor growth kinetics

- our body composed of ~10^{14} cells
  - daily billions of cells made !!!
    - 1 cell division = 6 billions of nucleotides
    - = approx. 700 kg material during lifetime
  - such a number of divisions surely brings lots of errors, however cancer affects only ~1/3 people
    - removal of damaged cells by immunity, apoptosis, desquamation, ...
    - restriction of cell division by external factors
- cell divisions in the clone of tumor cells: $N=2^n$
  - 2, 4, 8, 16, 32, …..
    - 10 divisions = ~1 000 cells
    - 20 divisions = ~1 000 000 cells (m=1mg)
    - 30 divisions = ~1 000 000 000 cells (m=1g)
    - 40 divisions = m=1kg

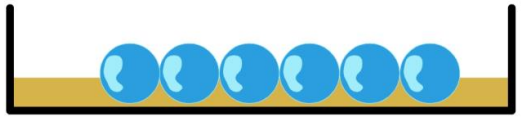
      ☛ **given ~12-hr cell cycle in approx. 20 days**
- in reality the growth is much more slower due to death of variable proportion of tumor cells and other factors:
  - prolongation of cell cycle duration
  - non-proliferating fraction of cells (differentiated)
  - tumor cell death (malnutrition, cytotoxic lymphocytes, NK cells)
  - mechanic loss of cells (desquamating e.g. in intestine)
- tumor grows only after formation of stroma and capillary network (= angiogenesis)
  - in that case growth overbalance the loss of cells



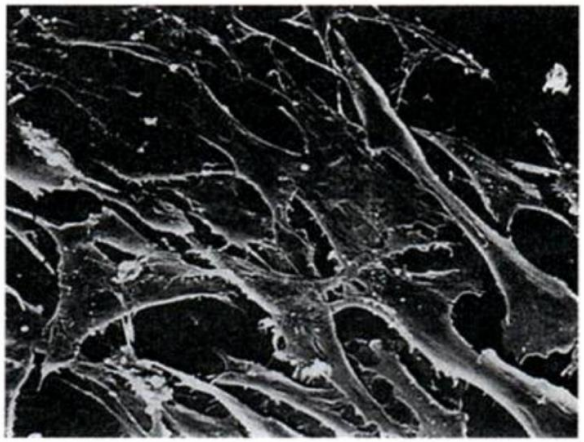
A computer-generated random packing of hard spheres.

Abnormalities of tumor cell growth – in vitro

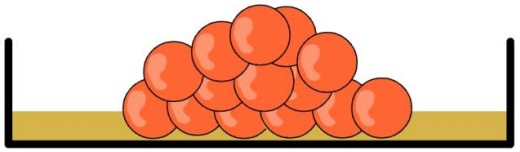
Normal cells



Normal cells grow in monolayer



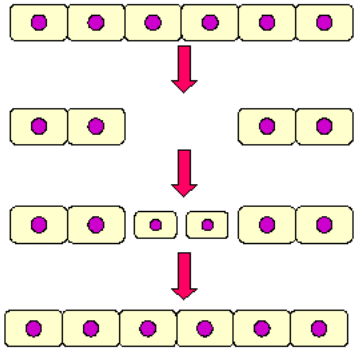
Cancer cells

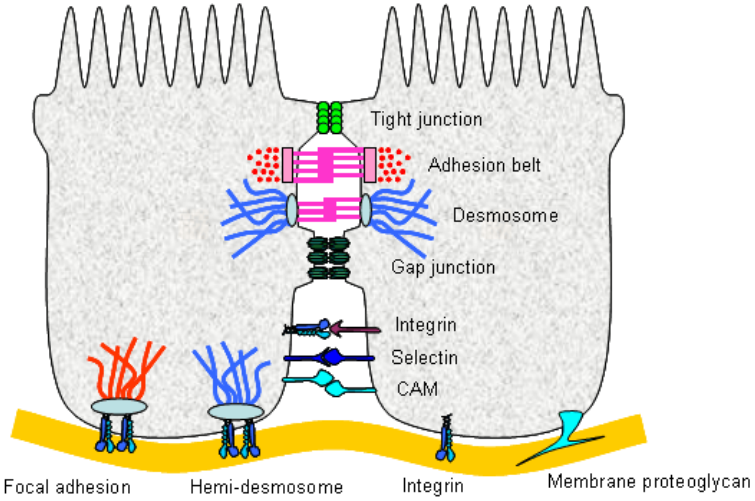


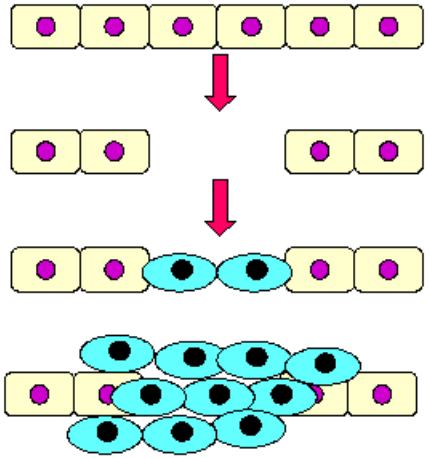
Cancer cells grow in clumps(foci)

Loss of contact inhibition, anchoring and intercellular communication

- integrins
  - with ECM
- cadherins (supressors)
  - cell to cell
  - inhibit β-catenins (oncogene)
    - act as transcription factors

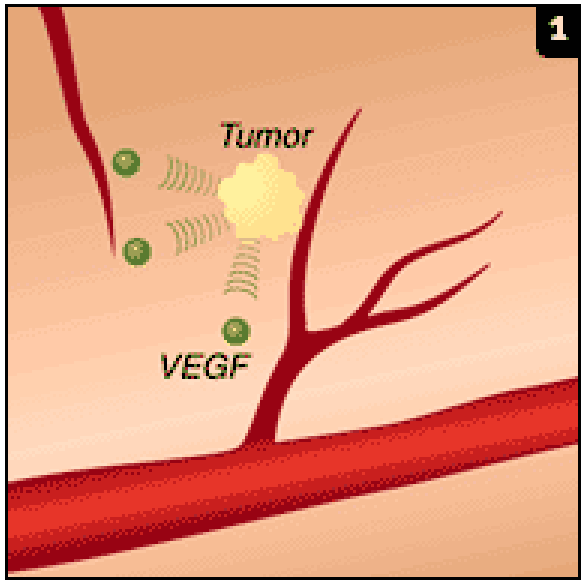




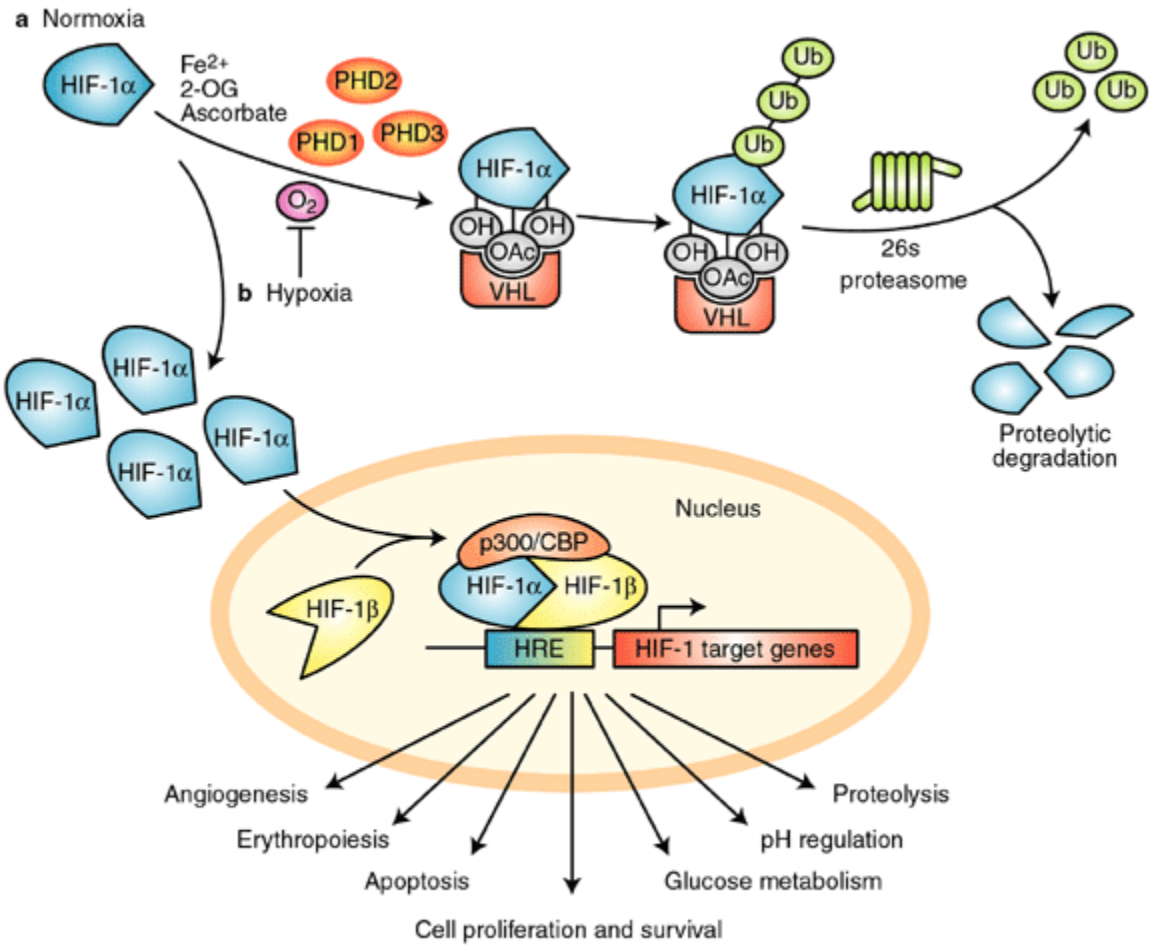


Tumor growth - angiogenesis

- ↑ cell proliferation/↓ cell death in tumor
  - **need for energy** (oxygen and substrates)
    - cell mass ~1mm^3 (~1×10^6 of cells) can't grow further without vascularisation (proliferation = apoptosis)
    - as a response to **hypoxia** hypoxia-inducible factor-1 (HIF-1) is produced
      - ☛ HIF-1 has 2 subunits - hydroxylation of HIF-1a (under the normoxia conditions leads to the rapid degradation)
      - ☛ under the hypoxia conditions HIF-1a migrates to the nucleus, binds to HIF-1b and HIF-1 complex functions as a transcription factor
      - ☛ after the translocation into nucleus HIF-1a stimulates transcription of many genes, e.g. vascular endothelial growth factor (VEGF)
    - VEGF stimulate formation of new vessels (**angiogenesis**)
    - proteolytic enzymes produced by tumor (matrix metalloproteinases) degrade extracelular matrix and enable "budding" of new vessels from the existing ones
    - proliferation and migration of endotelial cells is further potentiated by angiogenic factors secreted by tumor (e.g. VEGF, basic fibroblast growth factor (bFGF), transforming growth factor-b (TGF-b), and platelet-derived growth factor (PDGF)
    - new vessels enable invasion of tumor cells into circulation and distant metastases

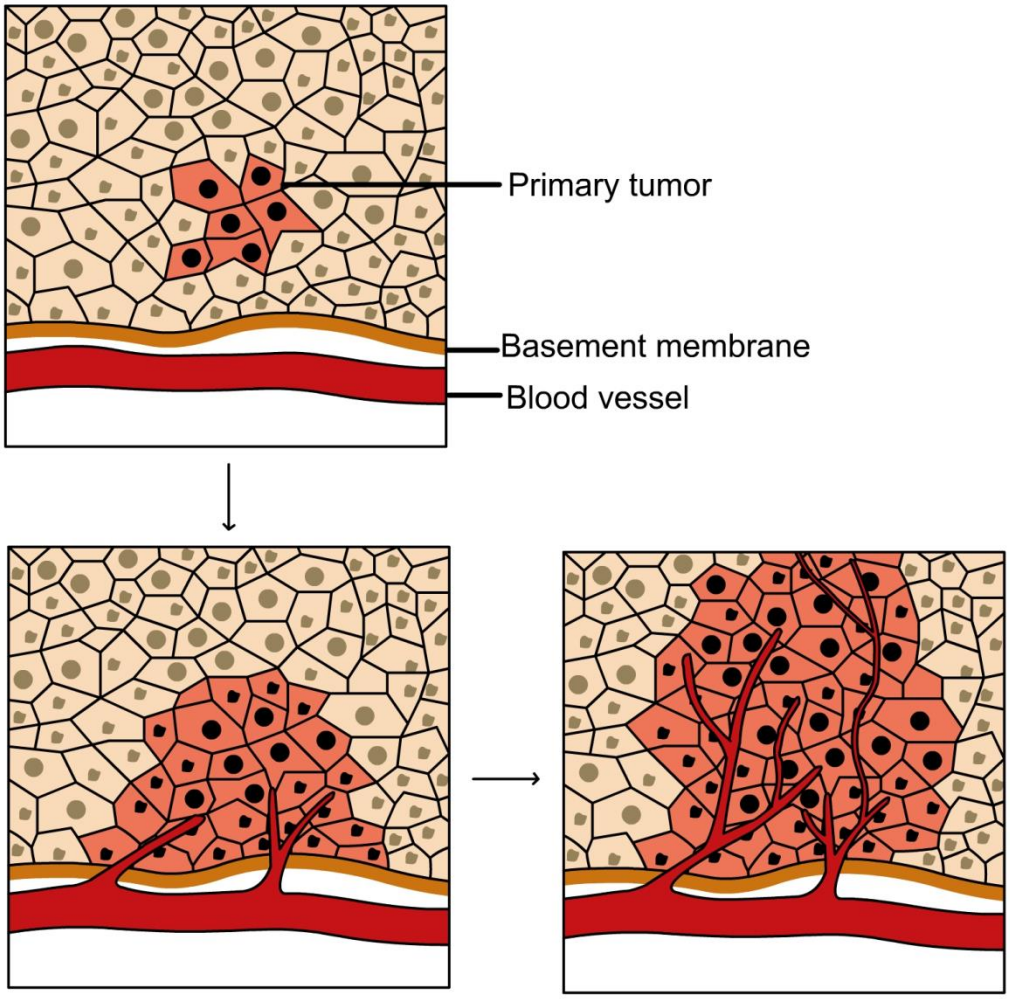


Hypoxia-induced gene transcription



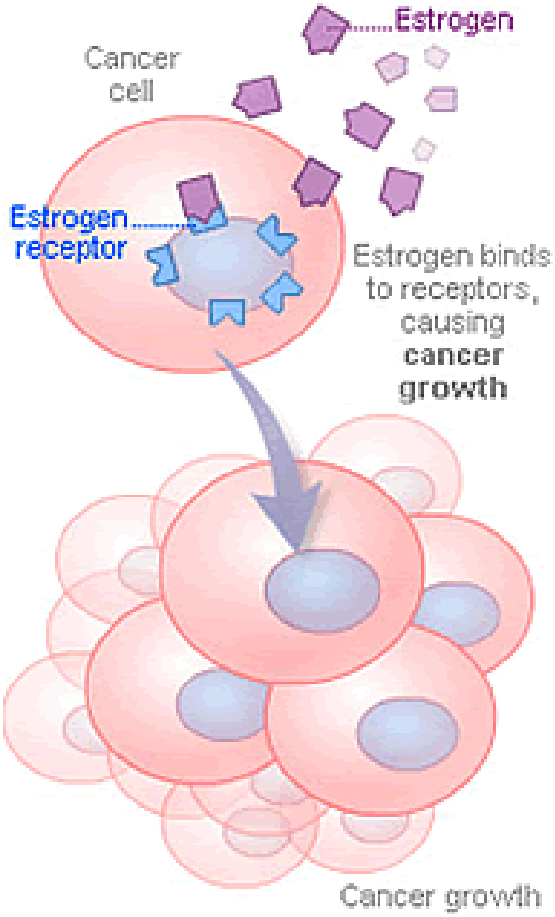
HIF-1α regulation by proline hydroxylation

Cancer angiogenesis



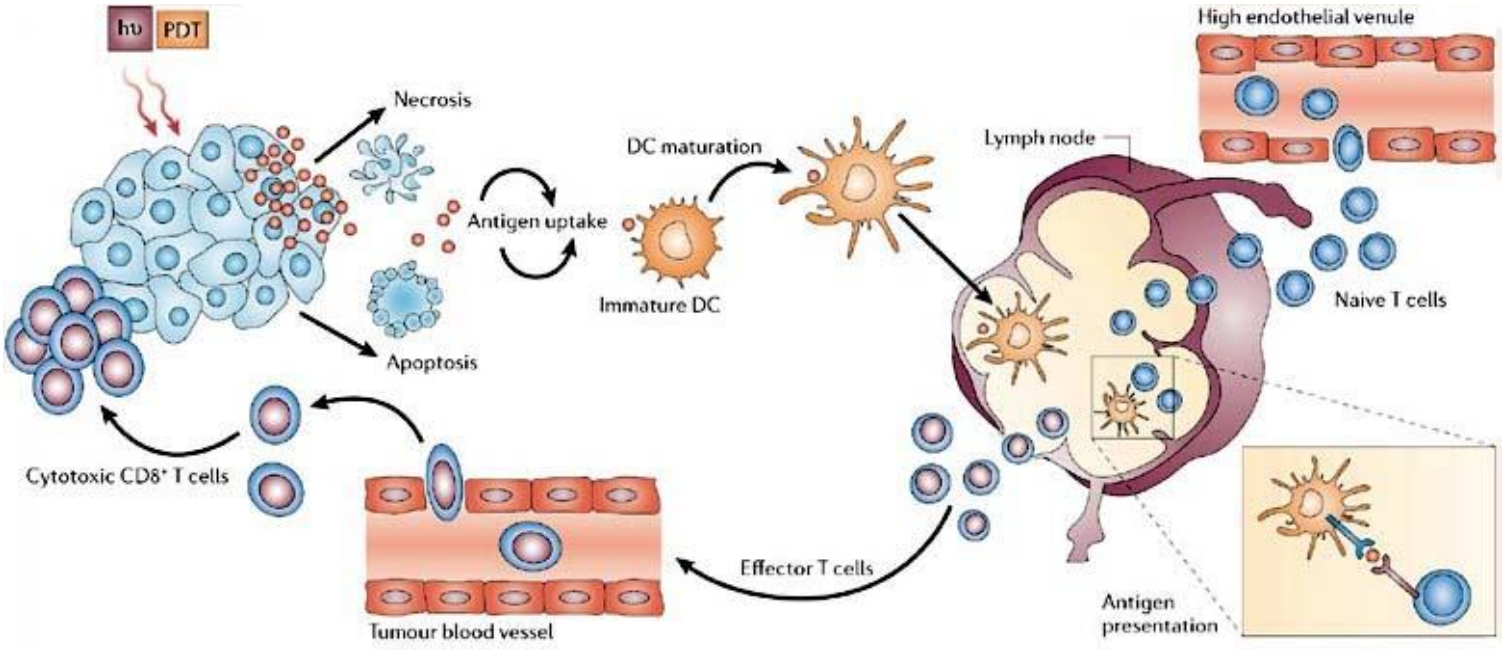
Hormonal stimulation

- growth of some tumors is significantly potentiated by hormones, typically by sex hormones
  - breast, uterus, ovary, prostate



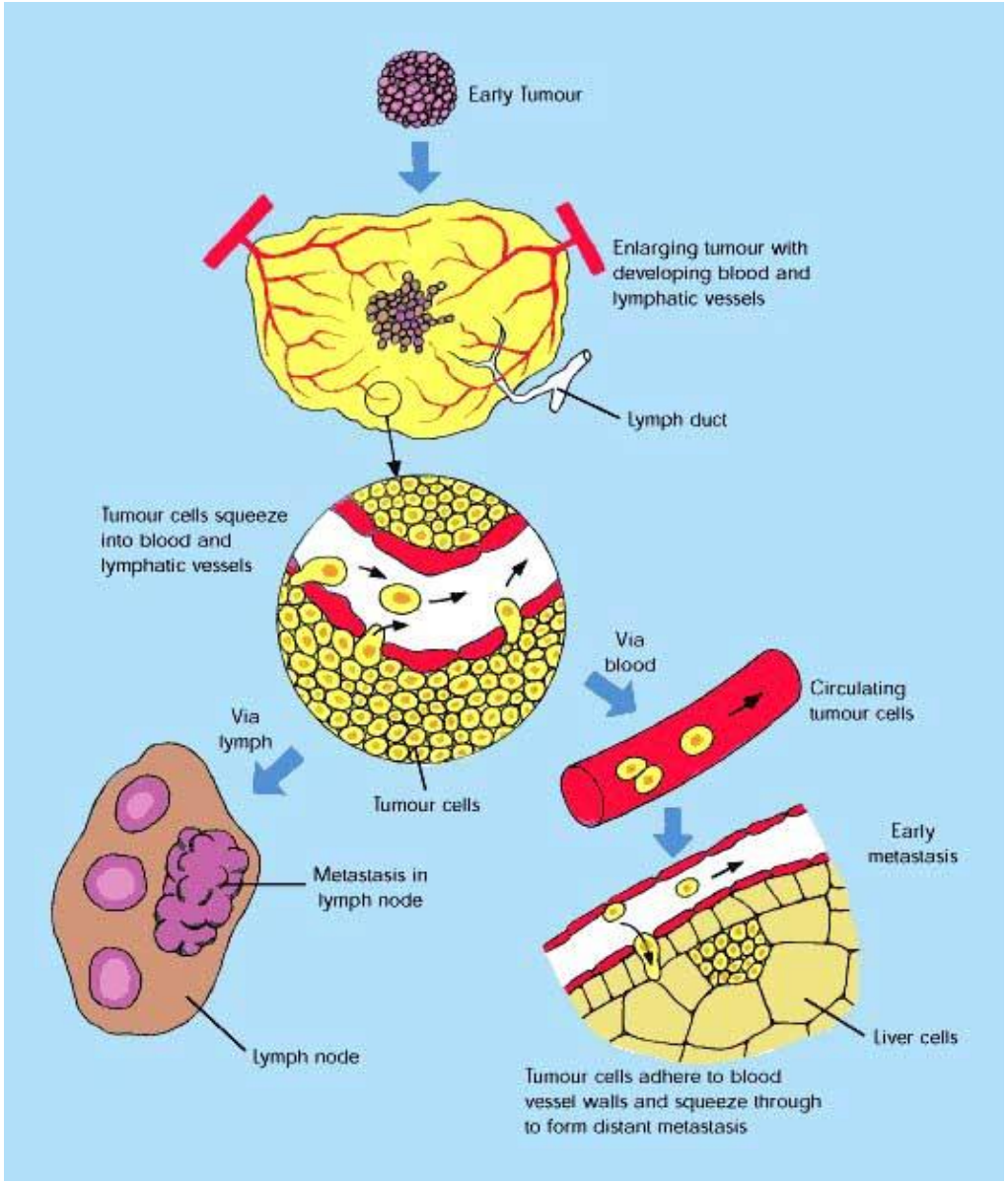
Immune system vs. tumor

- tumor cells has several immunological abnormalities
  - quantitative changes in the expression of surface antigens (↓ MHC)
    - tumor cells thus escape immune recognition and liquidation
  - qualitative – expression of neo-antigens ("oncofetal")
    - diagnostic markers (e.g. CEA, α-fetoprotein etc.)
- cytotoxic mechanisms are a major tool of anti-tumor immunity
  - CD8+ T-lymphocytes
  - NK-cells
- although immune system on its own is not powerful enough to seal with advanced tumor, the role of immunity in the anti-tumor surveillance is very important
  - people with immunosuppression has a high rate of cancer
    - e.g. Burkitt's lymphoma in Central Africa (malnutrition)

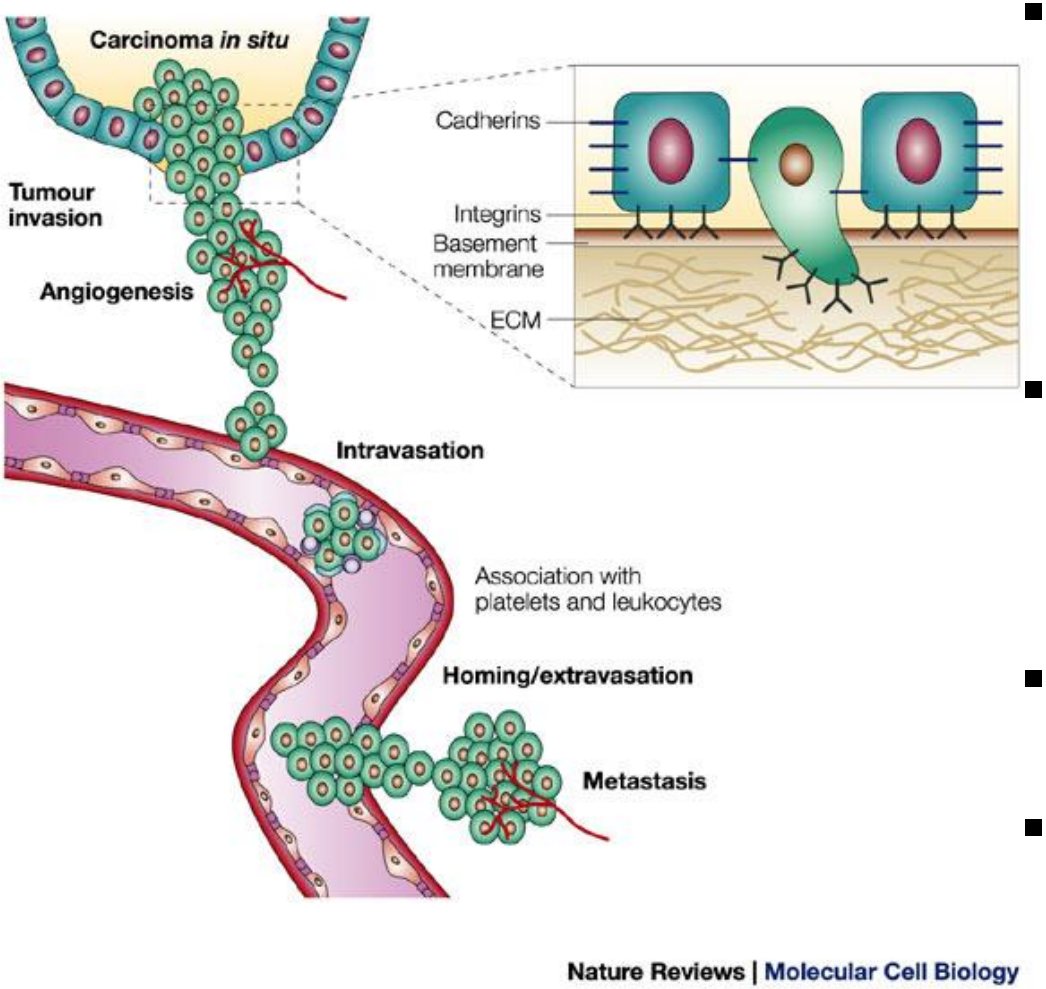


Metastasing

- formation of daughter tumors distant from original site
- several ways of spreading
  - blood
    - very often in the direction of flow
      - ☛from GIT to the liver
      - ☛by venous blood to the lungs
      - ☛from lungs by artery blood to bones and brain
  - lymphatic
    - first neighbouring lymph nodes, than distant

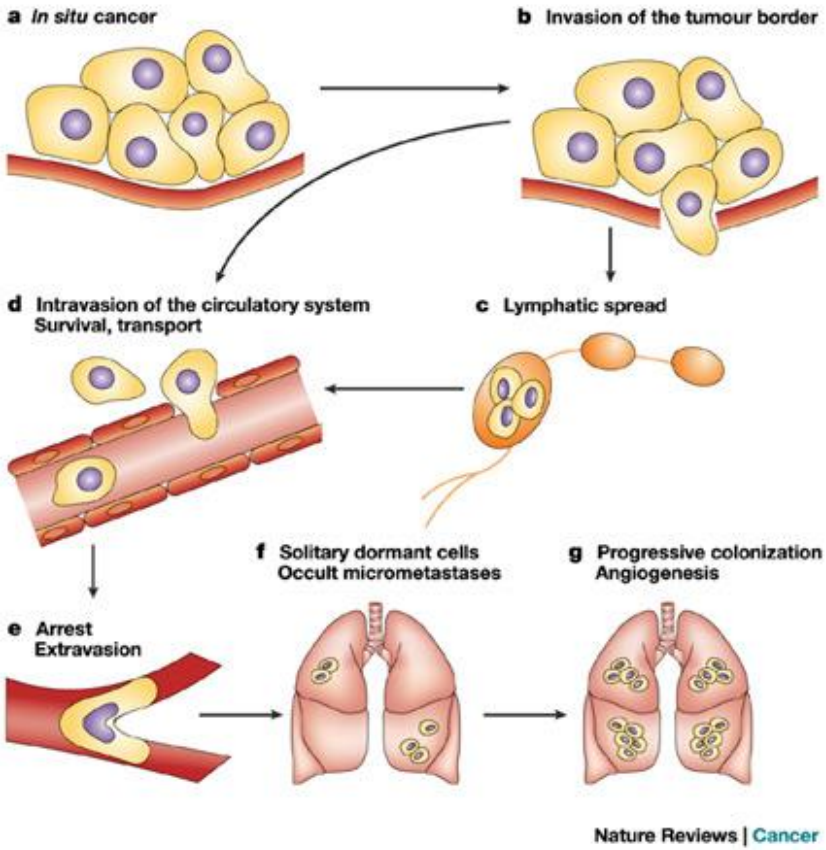


Changes in adhesion during the metastatic process

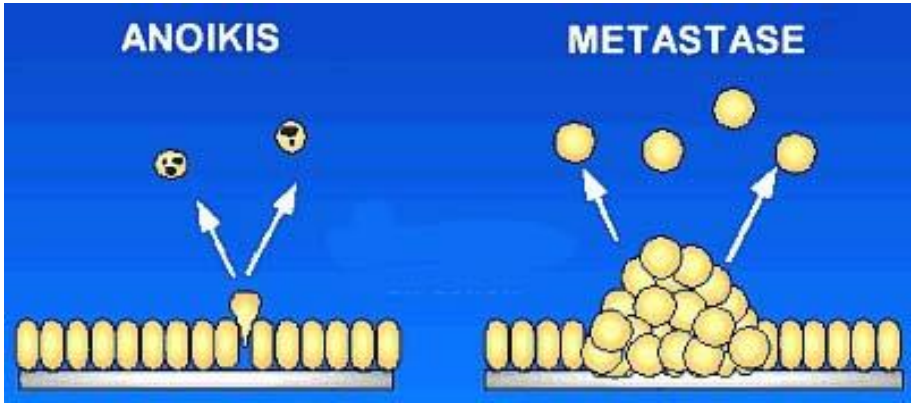


- cancer cells lose E-cadherin-dependent intercellular adhesions, acquire a **migratory phenotype,** penetrate the basement membrane, and invade the interstitial matrix
  - production of MMPs
- tumour angiogenesis then allows cancer cells to enter the bloodstream, either directly or through the lymphatic system, by a process called intravasation
- in the circulation, tumour cells form small aggregates with platelets and leukocytes
- finally, after stopping in the microcirculation of the target organ, tumour cells exit the bloodstream, by a process called extravasation, and undergo local expansion

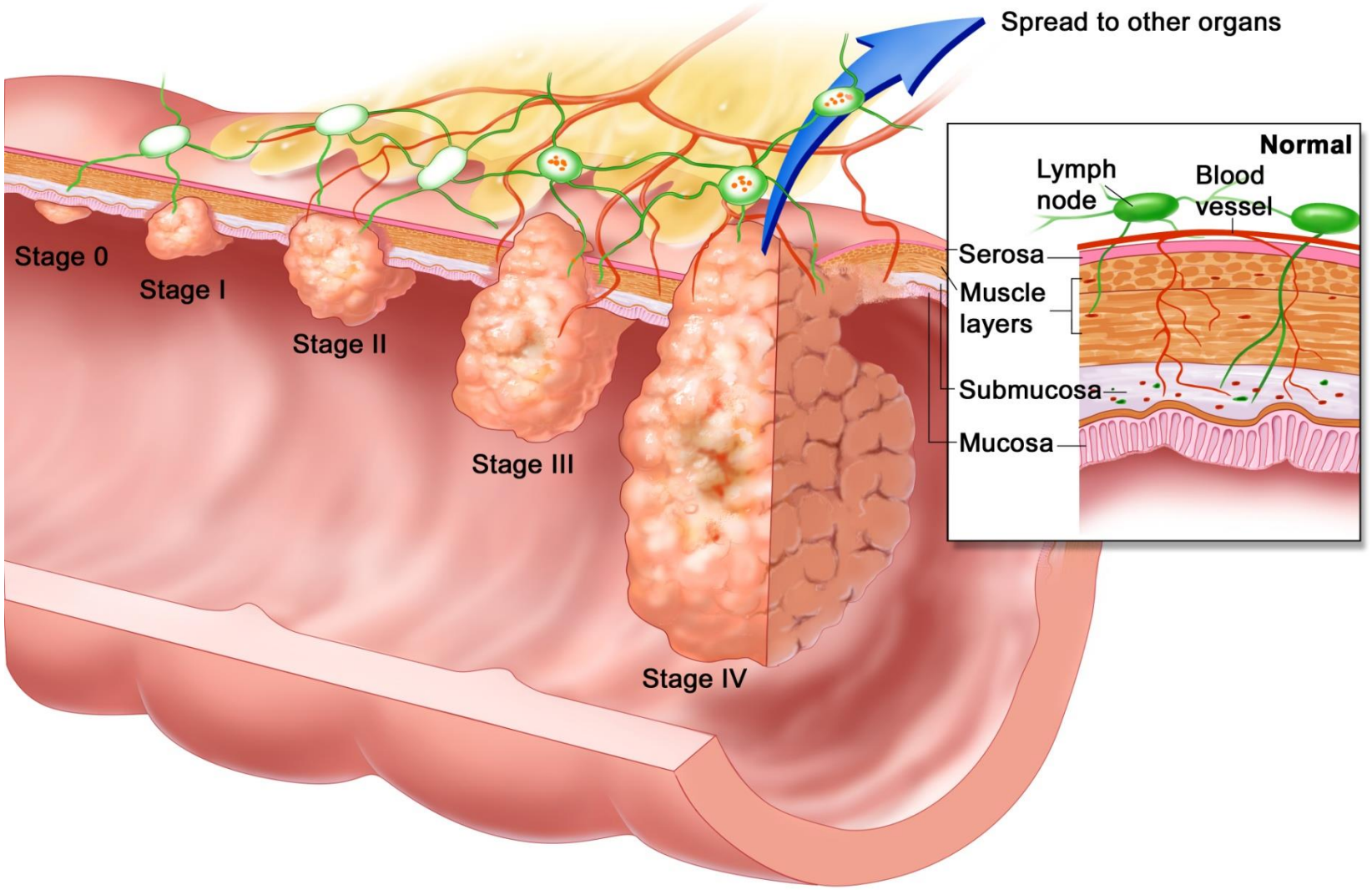
Resistance to anoikis



Anoikis is a form of programmed cell death that occurs in anchorage-dependent cells when they detach from the surrounding extracellular matrix.



Example – colorectal carcinoma

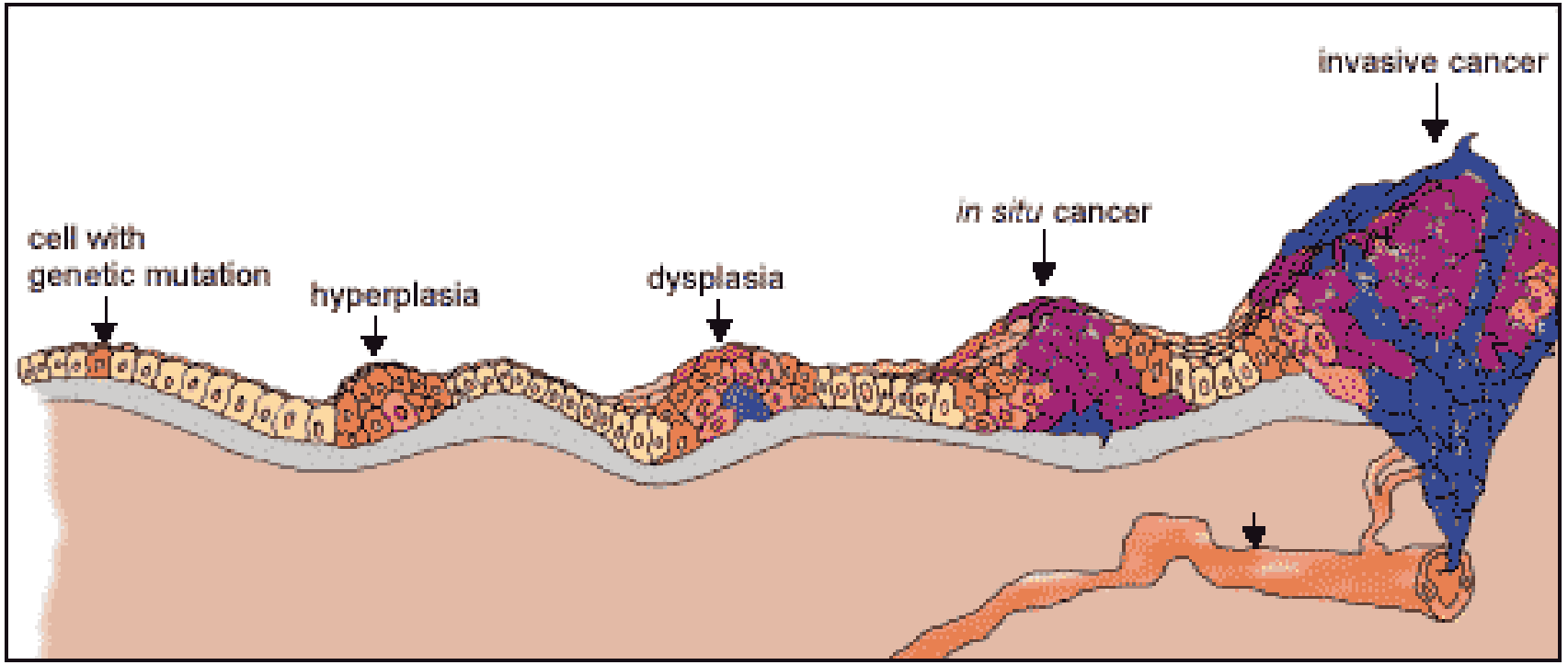


Interaction of tumor with the host

- local effect of tumor
  - mechanical compression (e.g. brain tumors)
  - obstruction (e.g. de. choledochus)
  - bleeding, anemia (leukaemia)
  - chronic blood losses into GIT (gastric and intestinal tumors)
  - oedema (e.g. lymphomas)
  - thromboses (DIC)
  - loss of vision (compression of optic nerve by hypophyseal adeoma)
  - voice change (laryngeal ca)
  - coughing (lung ca)
  - difficult swallowing (oesophageal ca)
  - pathological fractures (myeloma)
- systemic effects
  - increased temperature/unexplained fever
    - production of cytokines (pyrrogens) by tumor (IL-1, TNFα)
  - tumor cachexia
    - anorexic mediators (TNFα)
  - paraneoplastic syndromes
    - some tumors produce hormones (adenomas) – important diagnostically!
      - ☛ pigmentation
      - ☛ endokrinopathy
        - » Cushing sy, hypercalcaemia, etc.

Tumor classification

- morphologic = **typing** = histological type
- invasivity = **grading** = benign × malignant
- initial extent = **staging** = TNM classification (T = tumor, N = node, M = metastasis)



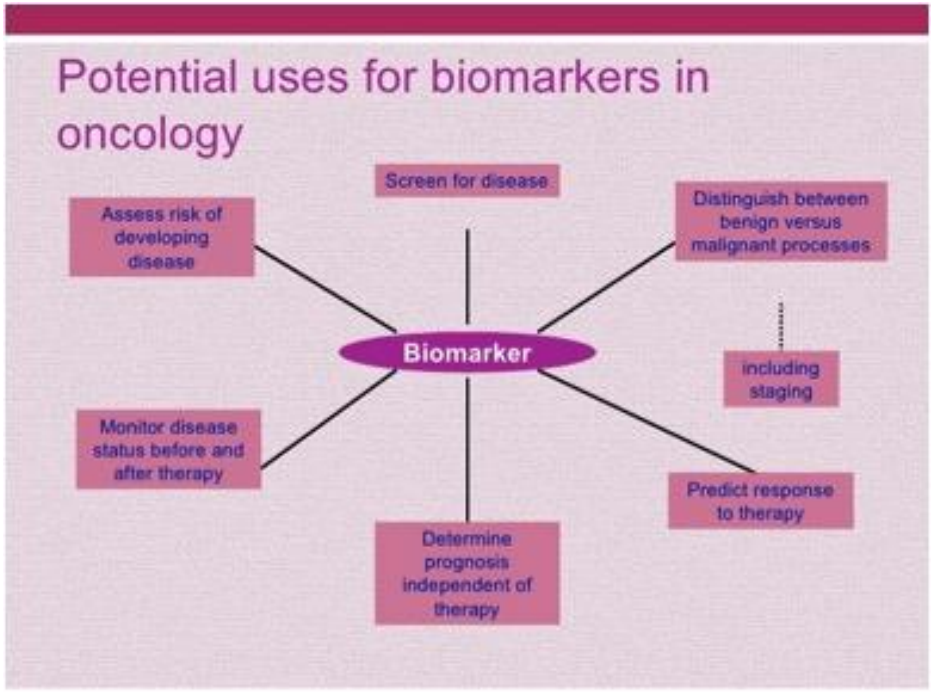
Cancer biomarkers

- Cancer biomarkers are substances that are produced in response to cancer processes.
- These substances can be found in the blood, urine, stool, tumor tissue, or bodily fluids.
- Most cancer biomarkers are proteins. However, patterns of gene expression and changes in DNA can be used.

Cancer biomarkers

Cancer biomarkers can be classified into the categories based on their usage:

- ***Predictive biomarkers*** predict response to specific therapeutic interventions (positivity/activation of *HER2* that predicts response to trastuzumab in breast cancer).
- ***Prognostic biomarkers*** aim to inform regarding the risk of clinical outcomes such as cancer recurrence or disease progression.
- ***Diagnostic biomarkers*** are used to identify whether a patient has a specific disease.



Cancer biomarkers - examples

Alpha-fetoprotein (AFP)

- Cancer types: Liver cancer and germ cell tumors
- Tissue analyzed: Blood
- How used: To help diagnose liver cancer and follow response to treatment; to assess stage, prognosis, and response to treatment of germ cell tumors

BCR-ABL fusion gene (Philadelphia chromosome)

- Cancer type: Chronic myeloid lukemia, acute lymphoblastic leukemia, and acute myelogenous leukemia
- Tissue analyzed: Blood and/or bone marrow
- How used: To confirm diagnosis, predict response to targeted therapy, and monitor disease status

Cancer antigen (CA) 15-3

- Cancer type: Breast cancer
- Tissue analyzed: Blood
- How used: To assess whether treatment is working or disease has recurred

Cancer biomarkers - examples

HER2/neu gene amplification or protein overexpression

- Cancer types: Breast cancer, gastric cancer
- Tissue analyzed: Tumor
- How used: To determine whether treatment with certain targeted therapies is appropriate

Prostate-specific antigen (PSA)

- Cancer type: Prostate cancer
- Tissue analyzed: Blood
- How used: To help in diagnosis, assess response to treatment, and look for recurrence

Carcinoembryonic antigen (CEA)

- Cancer types: Colorectal cancer and some other cancers
- Tissue analyzed: Blood
- How used: To keep track of how well cancer treatments are working or check if cancer has come back


DON'T!!!
Caspases on
the way
Z-VAD
IAP