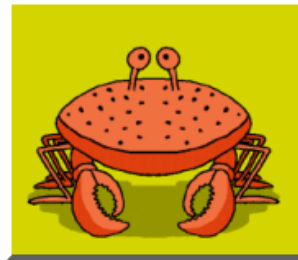


Malignant transformation of the cell - cancer

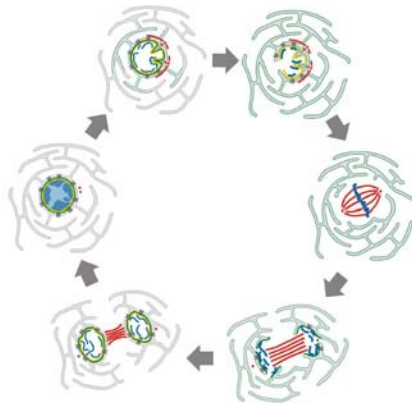
Cell cycle control
 Oncogene × suppressor genes/proteins
 Principle of malignant transformation
 Interaction of tumor and organism
 Metastases



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
 - 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 germinative 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
- All types of cancers are due to **genetic alteration** of key genes controlling cell cycle
 - however, only a few are inherited at the same time (i.e. **familial**) = due to the mutation in germinative 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)oncogenes** – genes that normally potentiate cell division and growth under the physiologic stimuli, if mutated process becomes uncontrolled
 - suppressor genes** – genes that normally inhibit cell division, if mutated growth becomes uncontrolled
 - DNA reparation 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

Cell cycle (CC)

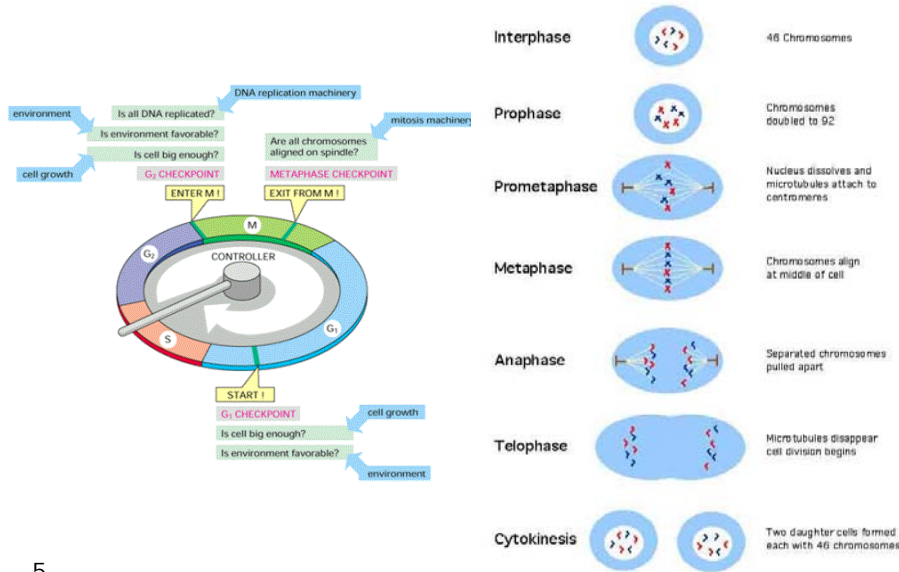


- most of the somatic cells grows, double the amount of cell organelles and divide = **cell cycle**
- CC (4 phases)
 - interphasis
 - cell growth (G1-phase)
 - DNA replication (S-phase)
 - additional growth (G2-phase)
 - mitosis (M-phase)
- duration of CC is very variable in different cell types
 - hours 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) act

CC phases

G0	Resting phase. Cell perform its function, maintain basal metabolism and does not divide.
G1	Interval between previous <u>M</u> itosis and subsequent <u>S</u> ynthesis (=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 check point (=)
S	DNA replication in the nucleus and histone synthesis in the cytoplasm. At the end the cell contains doubled the amount of DNA.
G2	Interval between previous <u>S</u> ynthesis and subsequent <u>M</u> itosis. cell further grows and proteosynthesis (mainly tubulin and other proteins necessary for mitotic apparatus). G2 check point (= completeness and correctness of DNA replication).
M	Mitosis (6 phases) – first 5 (prophases, prometaphases, metaphases, anaphases, telophases) represent division of nucleus, the last one (cytokinesis) division of the whole cell. M check point (= correct formation of mitotic spindle in metaphase/anaphase transition)

CC check points and mitosis



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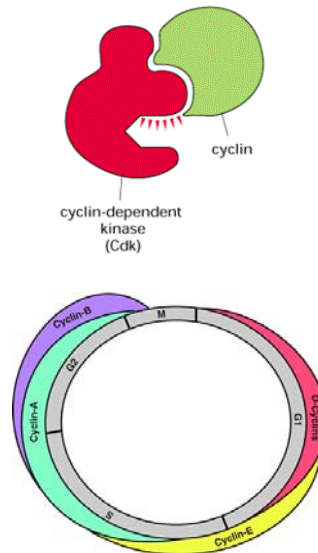
CC regulatory proteins

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

6

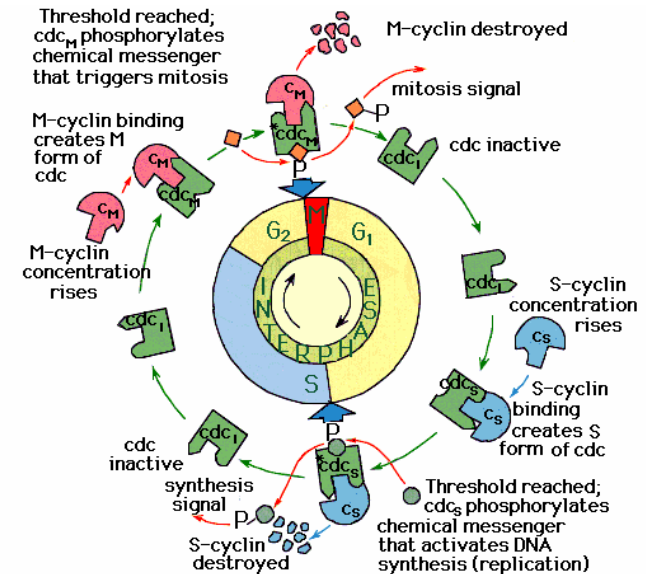
(A) - Protooncogenes

- (1) cyclins
 - 8 types – A, B, C, D, E, F, G, H
 - specific for particular CC phases
- (2) 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



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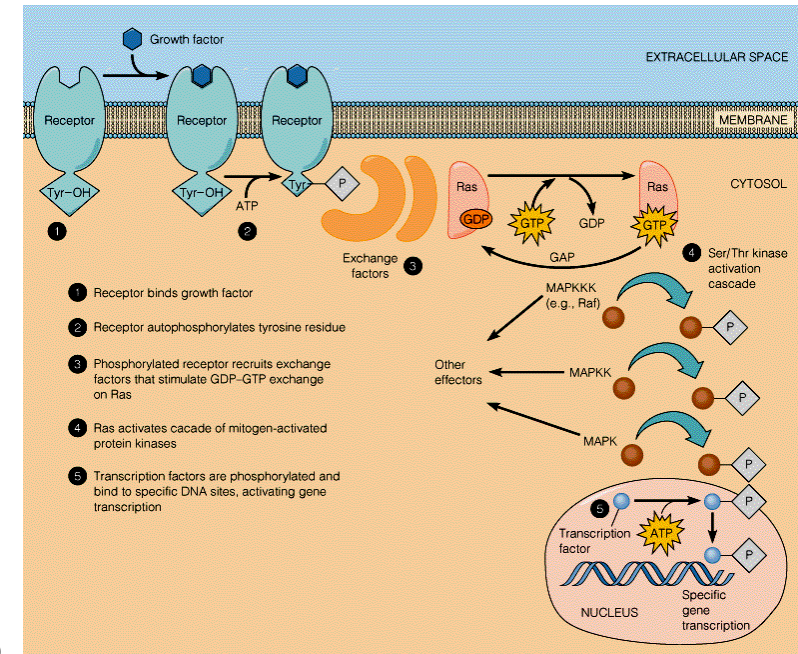
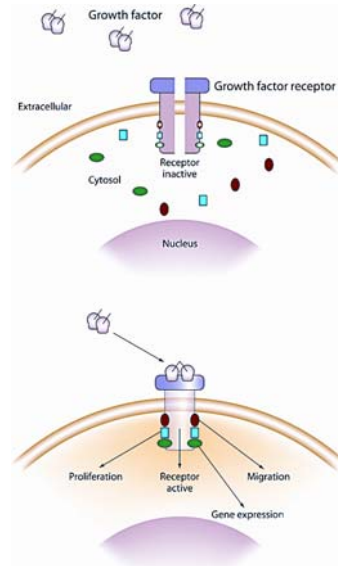
Cyclin/cdk interplay



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(A) Protooncogenes

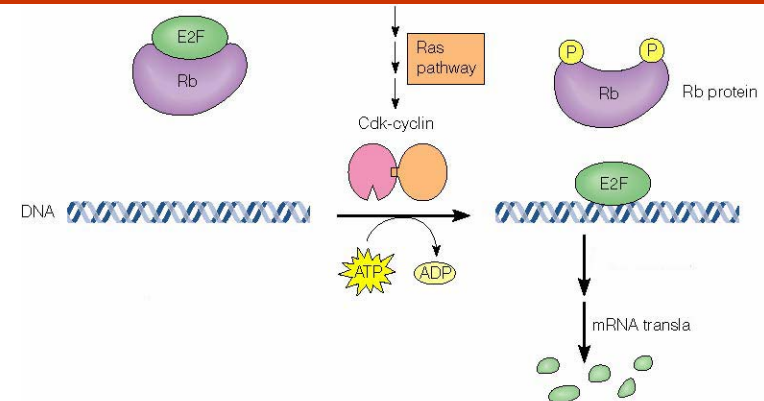
- (3) growth factors (GF)
 - e.g. TGF- β , PDGF, EGF, FGF, VEGF, erythropoietin, ...
 - GF acts in extremely low concentrations in paracrine fashion
 - different target cells according to the expression of specific receptors
- (4) GF receptors
 - receptors with tyrosinkinase activity
 - transduction cascade
 - downstream kinases (MAPK – Mitogen Activated Protein Kinase)
 - transcription of early-response genes (~15 min)
 - e.g. regulatory proteins fos, jun a myc (=products of protooncogenes **fos**, **jun** and **myc**)
 - transcription of late-response genes (~1 hrs)
 - expression of cyclins and cdk under the stimulation with fos, jun, myc regulatory proteins



(B) - Suppressor genes

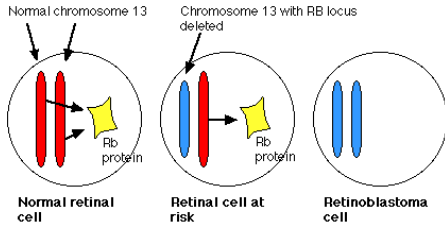
- encode inhibitory proteins of CC
 - (1) Rb protein (ch. 13q14)
 - principal negative regulator of CC, controls the G1-S-phase transition, activity modulated by de-/phosphorylation (by cdk4/6 + cyclin D complex)
 - mutations in Rb (microdeletions) predispose to the retinoblastoma
 - (2) p53 protein (ch. 17p13)
 - "gardian of 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) p21
 - main target of p53 = inhibitor of Cdk – CC arrest in G1 phase by inhibition of Cdk2/cyclin E complex
 - (4) BRCA1 and BRCA2
 - part of DNA damage detection system
- inherited mutations in suppressor genes van confer susceptibility to the inherited (familial) 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 G₁ checkpoint)



- Rb is a main inhibitor/regulator of CC
 - binding to transcription factor E2F to \uparrow 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

Rb protein - retinoblastoma



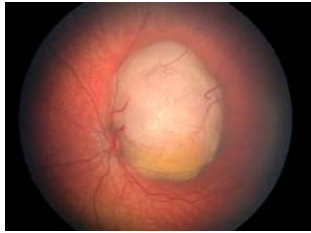
▪ Rb mutations (ch. 13q14) – microdeletions most often - lead to retinoblastoma (tumor of retina)

– inherited (familial) retinoblastoma

▪ patient inherited one mutated allele, second one is mutated early during the life (= loss of heterozygosity, LOH)

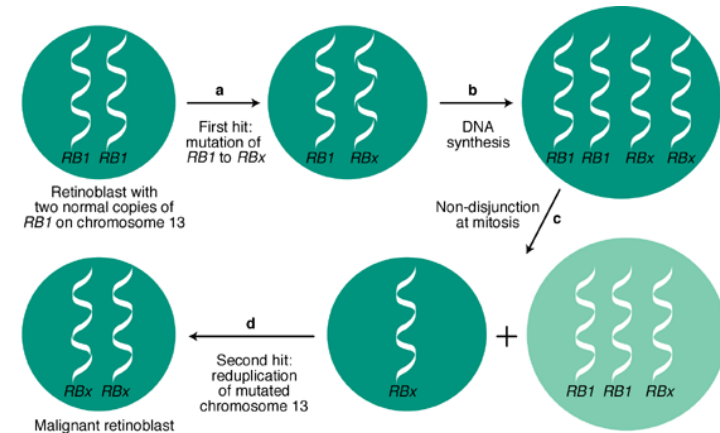
– acquired (sporadic) retinoblastoma

▪ inactivation Rb by mutation of both alleles anytime during the life



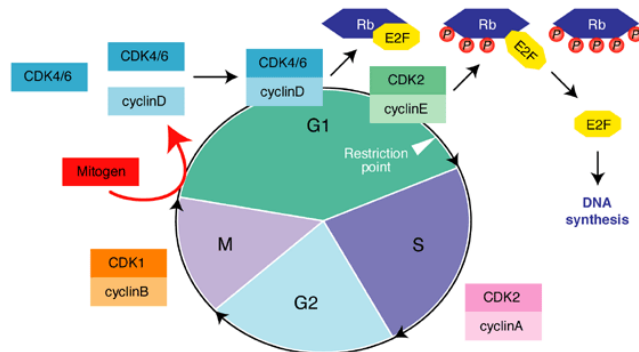
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Development of sporadic retinoblastoma by LOH mechanism



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Partial summary – CC “kick-off”



▪ mitogens drive cell cycle progression by induction of cyclinD 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 cyclinD (and to a lesser extent cyclinE), whose expression is induced by mitogens
 - cyclinD- and cyclinE-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

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Protein p53 (ch. 17p13)

▪ main controller of genome stability

▪ if DNA is mutated or incompletely replicated p53 becomes activated and:

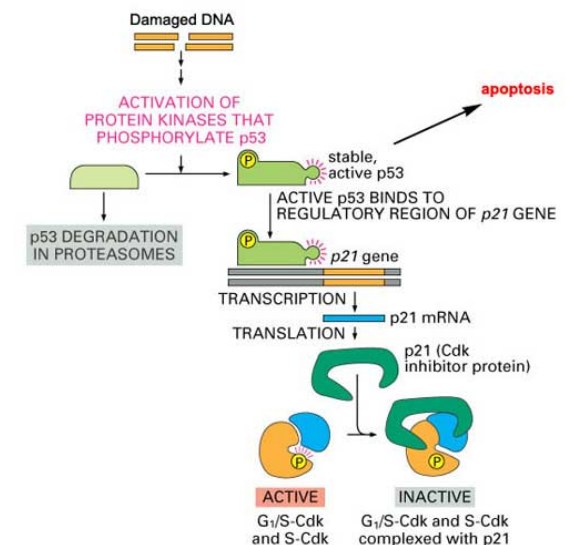
- ↑ expression of CC inhibitors → temporary **CC arrest** in G1/S check point enabling DNA repair (“major repair”)
 - (CIP1/WAF1 gene → p21 protein)
- ↑ GADD 45 (Growth Arrest and DNA Damage) → **DNA excision repair**
- Bax expression → **apoptosis**

▪ 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

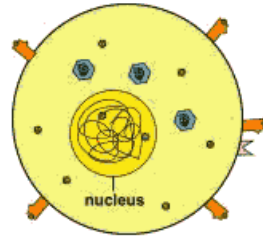
▪ LOH mechanism



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Apoptosis

APOPTOSIS

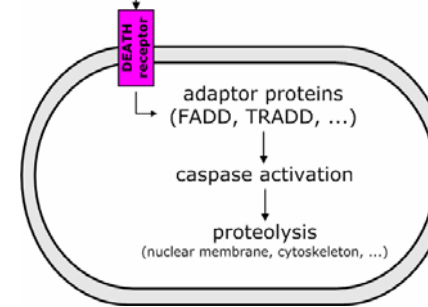


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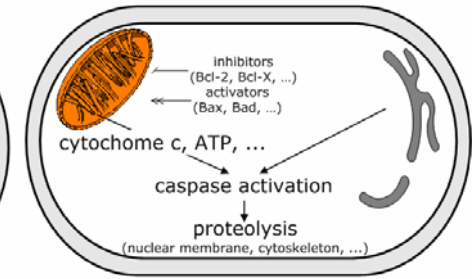
Apoptosis-initiating pathways

EXTRINSIC PATHWAY

ligands: TNF- α , LTA, TRAIL, Fas-L



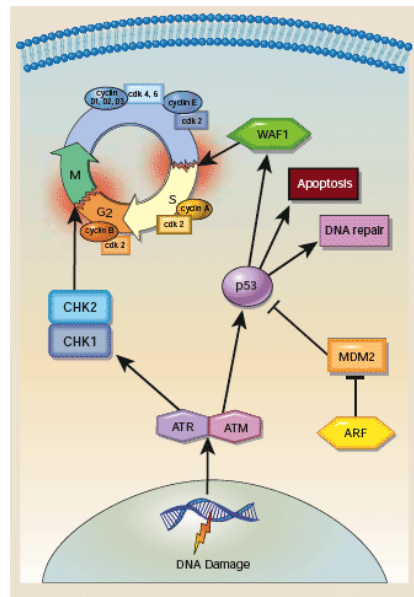
INTRINSIC PATHWAY -
mitochondria-related
ER-related



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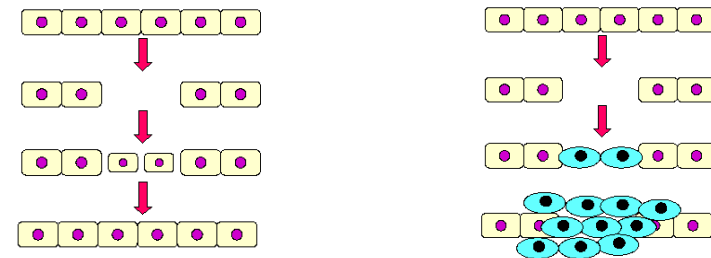
(C) - DNA repair (stability) genes

- (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
- (3) geny homologi recombine
 - main pathway activated on DNA damage (double strand break) involves: ATM (ATR)/CHK2 (CHK1) \rightarrow p53/MDM2 \rightarrow p21 \rightarrow "growth arrest"
- inborn defects leads to several forms of familiar cancers
 - ataxia telengiectatica
 - Bloom syndrome
 - Fanconi anemia
 - xeroderma pigmentosum
 - fragile X syndrome



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Other factors influencing CC

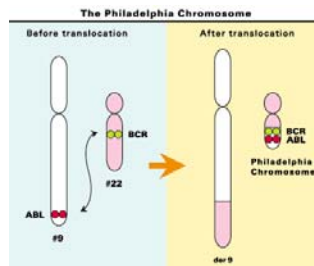
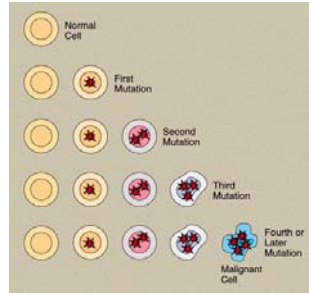


- contact inhibition
- anchoring
- intercellular communication
 - integrins, cadherins

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Malignant transformation

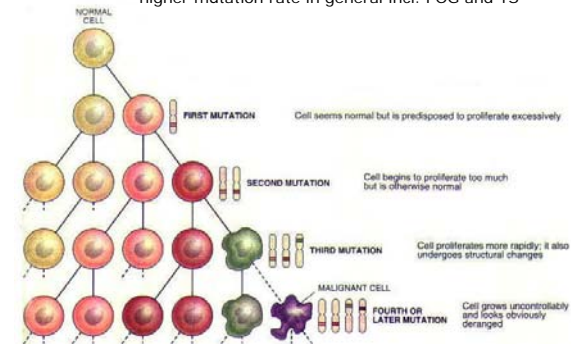
- multistage process of subsequent changes of genome - mutations in the critical DNA region (usually 4 - 6)
 - i.e. (proto)oncogen, suppressor, repair enzyme gene
- chromosomal aberrations
 - translocation, insertion, deletion, amplification
- gene mutations
 - point mutations
 - length mutations (ins/del)
- effect of mutation
 - change of transcription – quantitative change
 - synthesis of altered product – qualitative change
- 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 dietary toxins exposure
 - lung cancer as a result of smoking
 - alcoholic liver cirrhosis
 - biological = incorporation of viral genome into the host one in critical regions
 - DNA viruses
 - herpes/EBV - lymphomas
 - HBV – hepatocellular ca
 - papillomavirus – cervical ca, larynx, oral cavity
 - adnoviruses
 - RNA viruses – retroviruses
 - HIV - Kaposi sarcoma, B-lymphoma
 - HTLV – T-cell leukaemia
 - pre-cancerosis = chronic inflammation
 - Baret's oesophagus in GER, ulcer=se colitis and Crohn disease, diverticulitis, ...



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Partial summary

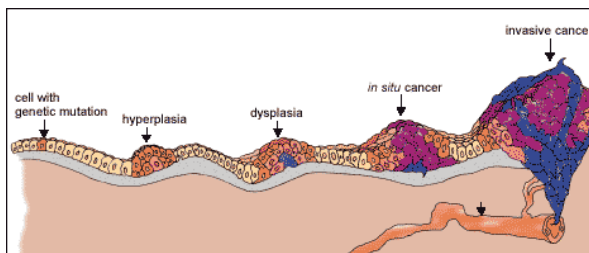
- 3 groups of genes contribute to the malignant transformation
 - protooncogenes (POG)
 - physiologically promote cell division by stimulating transition through cell cycle phases and transmitting the mitogenic signals
 - mutation of 1 allele is sufficient to produce uncontrolled cell division
 - suppressor 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



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Cancer

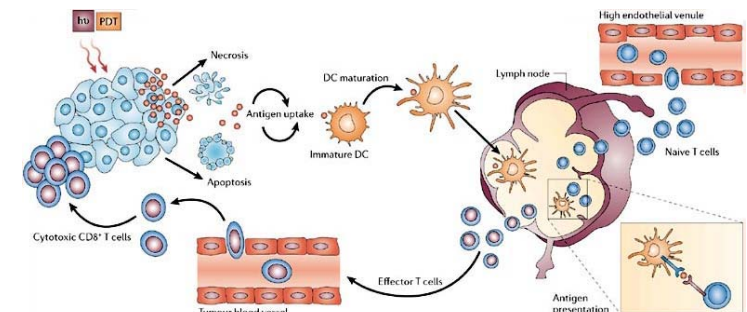
- autonomously proliferating tissue genetically altered by exposure to carcinogens
- originate from 1 cell (= **monoclonal**)
 - the whole process of malignant transformation is however multistage (i.e. subsequent accumulation of several mutations), so the tumor gradually becomes genetically **heterogeneous**
 - transition fro precancerosis – benign to malignant tumor
- histologically 3 groups
 - epithelial - skin, mucous membranes, ductal
 - papilloma, adenoma (b.), carcinoma (m.)
 - mesenchymal - connective tissue, endothelium, muscle, haematopietic and lymphatic tissue, bones,
 - fibroma, hemangioma, myoma (b.), sarcoma, lymphoma, leukaemia (m.), ...
 - neuroectodermal - CNS and peripheral nerves, pigment nevi
 - astrocytoma, glioma, blastoma, neurinoma, melanoma
- tumor classification
 - morphologic = **typing** = histological type
 - invasivity = **grading** = benign x malignant
 - initial extent = **staging** = TNM classification (T = tumor, N = node, M = metastasis)



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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)



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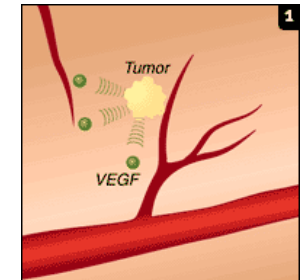
Tumor growth kinetics

- 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

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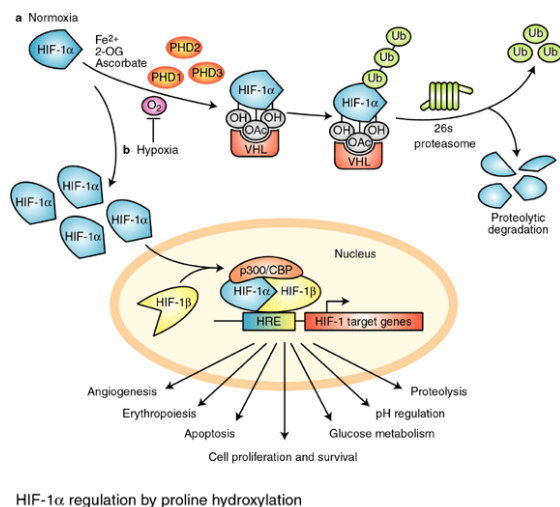
Tumor growth - angiogenesis

- ↑ cell proliferation/↓ cell death in tumor
 - need for energy (oxygen and substrates)
 - cell mass ~1mm³ (~1×10⁶ 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-1α (under the normoxia conditions leads to the rapid degradation)
 - under the hypoxia conditions HIF-1α migrates to the nucleus, binds to HIF-1β and HIF-1 complex functions as a transcription factor
 - after the translocation into nucleus HIF-1α 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 extracellular matrix and enable "budding" of new vessels from the existing ones
 - proliferation and migration of endothelial cells is further potentiated by angiogenic factors secreted by tumor (e.g. VEGF, basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF))
 - new vessels enable invasion of tumor cells into circulation and distant metastases



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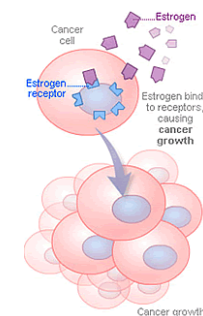
Hypoxia-induced gene transcription



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Hormonal stimulation

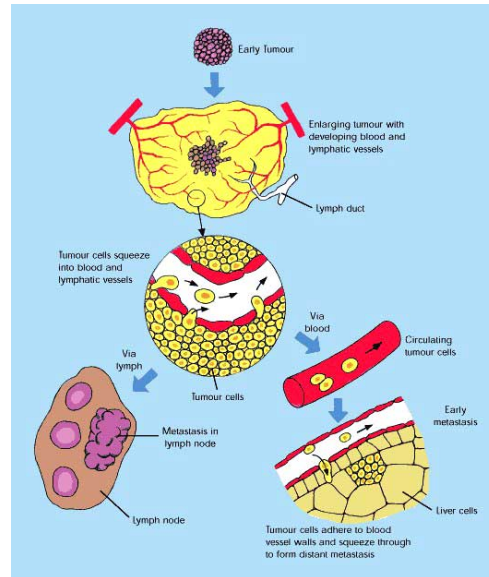
- growth of some tumors is significantly potentiated by hormones, typically by sex hormones
 - breast, uterus, ovary, prostate



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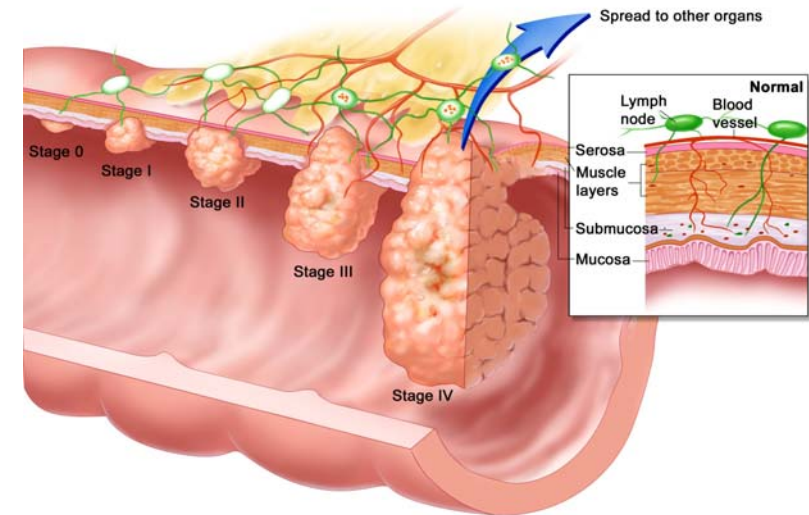
Metastasing

- 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, then distant



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Example – colorectal carcinoma



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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 (pyrogens) by tumor (IL-1, TNF α)
 - tumor cachexia
 - anorexic mediators (TNF α)
 - paraneoplastic syndromes
 - some tumors produce hormones (adenomas) – important diagnostically!
 - pigmentation
 - endokrinopathy
 - Cushing sy,
 - hypercalcaemia, etc.

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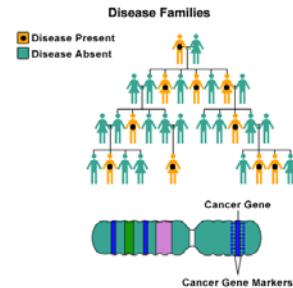
Tumor anorexia / cachexia

- initial anorexia might be a part of non-specific defence mechanisms (energetic deprivation of growing tumor)
 - gradually if becomes advert complication leading to progressive cachexia and further compromise of self-defence
 - i.e. tumor cachexia is an end-result of tumor anorexia and secondary effects (energy deprival, obstruction, treatment side-effects)
- initial tumor anorexia (also experimentally inducible - TNF α) is different from nausea and sickness as a side-effect of treatment
- also consequences of tumor anorexia are more serious than effect of simple starvation in otherwise healthy man
 - energetic requirements of growing tumor are increased and compromise other organs
- pathophysiology of tumor anorexia/cachexia
 - altered activation of regulatory centres in hypothalamus regulating food intake (n. arcuatus) due to cytokines produced by tumor or by the host's immune system (IL-1, IL-6, TNF- α)
 - cytokines stimulate release of serotonin and thus persistent activation of POMC/CART neurons
 - hypothalamic centres respond by a long-term decrease of concentration of NPY (orexigenic) and, conversely, over-activation of system POMC/CART (anorexigenic)

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Hereditary cancer predisposition

- (1) sporadic cancers
- (2) some rare types of cancer due to inborn mutation (manifested usually in childhood):
 - retinoblastoma (retina)
 - Wilm's tumor (kidney)
 - Li-Fraumeni syndrome (various types of cancer incl. sarcomas, brain tumors and leukaemia)
 - familial adenomatous polyposis (~1% of all colon cancers)
- (3) other mutations increase susceptibility/probability of common types of cancer (+ exposure to the environmental factors):
 - 5 – 10% of all cancers
 - colon
 - ovary or breast cancer (BRCA1 gene)
 - nevertheless, because breast and colon cancer are so widespread, even a small fraction of the total equals a very large number
 - it is estimated that as many as 1 in 300 women may carry inherited mutations of breast cancer susceptibility genes, and approximately the same proportion carry mutations that make them susceptible to colon cancer
- genetic prediction possible – genetic screening and counselling



Syndrome	Major tumor types	Mode of inheritance	Type of gene	Genes
Adenomatous polyposis of the colon	Colon, thyroid, stomach, intestine, hepatoblastoma	Dominant	TS	APC
Juvenile polyposis	Gastrointestinal	Dominant	TS	SMAD4/DPC4
Peutz-Jeghers syndrome	Intestinal, ovarian, pancreatic	Dominant	TS	STK11
Neurofibromatosis type 1	Neurofibroma, optic pathway glioma, peripheral nerve sheath tumor	Dominant	TS	NF1
Neurofibromatosis type 2	Vestibular schwannoma	Dominant	TS	NF2
Tuberous sclerosis	Hamartoma, renal angiomyolipoma, renal cell carcinoma	Dominant	TS	TSC1/TSC2
Xeroderma pigmentosum	Skin, melanoma, leukemia	Recessive	SG	XPA, B, C, D, E, F, G, POLH
Bloom syndrome	Leukemia, lymphoma, skin	Recessive	SG	BLM
Fanconi anemia	Leukemia, squamous cell carcinoma, gynaecological system	Recessive	SG	FANCA, B, C, D ₂ , E, F, G
Nijmegen breakage syndrome	Lymphoma, medulloblastoma, glioma	Recessive	SG	NBS1
Ataxia telangiectasia	Leukemia, lymphoma	Recessive	SG	ATM
Von Hippel-Lindau syndrome	Retinal and central nervous hemangioblastoma, pheochromocytoma, renal cell carcinoma	Dominant	TS	VHL
Wilms tumor syndrome	Wilms tumor	Dominant	TS	WT1
Retinoblastoma	Retinoblastoma, osteosarcoma	Dominant	TS	RB1
Li-Fraumeni syndrome	Soft tissue sarcoma, osteosarcoma, breast, adrenocortical carcinoma, leukemia, brain tumor	Dominant	TS	TP53
Multiple exostosis	Chondrosarcoma	Dominant	TS	EXT1/EXT2
Werner syndrome	Osteosarcoma, meningioma	Recessive	SG	WRN
MEN 1	Pancreatic islet cell tumor, pituitary adenoma, parathyroid adenoma	Dominant	TS	MEN1
MEN 2	Medullary thyroid carcinoma, pheochromocytoma, parathyroid hyperplasia	Dominant	OG	RET

TS - tumor suppressor gene; OG - oncogene; SG - stability gene; OMIM - online Mendelian inheritance in man

