Molecular biology of the tumor

Cancer - definition

- name **cancer** ("cancer") derived from the Latin word for crab (greek: karkinos = crab, onkos = expense, burden)
- disease caused by a **malignant tumor**
- tumor - neoplasia, neoplasm - pathological unit created in the tissue of a multicellular organism whose growth is out of control
- affects plants, animals, humans (not a disease of modern times)

Historical overview

- 400 B.C. **Hippocrates** described the cancer as long extensions (like crayfish feet) jutting into the healthy tissue:
  Gr: karkinos = crayfish; onkos = crab
  Lat: cancer = crayfish
- descriptive (**epidemiological**) findings:
- 1848 - increased incidence of breast cancer among nuns (associated with childlessness and no breastfeeding)
  - **1775** - Scrotal cancer among chimney sweepers (in connection with the occurrence of harmful substances in soot; connection with hygiene habits)
  - 1902 - connection of x-rays and development of cancer
  - begin. 20. cent. - family history of cancer

Historical overview

- 1909 - Rous - Infectious tumor transmissions in chickens
- study of tumor viruses (**oncogene** - a fragment of viral genes which cause tumor) (1961 - Nobel Prize)

- 1976 – Bishop, Varmus – discovered c-src (**protooncogenes**)
- associated with mitogenic signaling pathways
- slowly transforming viruses
- Henry Harris (cells fusion) – **tumor suppressors** – recessive genes (brakes)
- Knudson – retinoblastoma – „two hits hypothesis"
- DNA transfer (transformation, transfection)

tumor, neoplasm

- It is new and abnormal tissue in a multicellular organism, which has no physiological function in this organism and grows in unregulated manner.

- is a genetically conditioned abnormal growth of cell tissue mass of clonal nature. Its growth is not coordinated with the growth of surrounding tissue, and the equilibrium state of the organism.

Basic characteristics

at the cellular level, genetic disease (a consequence of mutations that are transmitted to the daughter cells)

- ✓ phenotype of tumor cells is heritable (transmitted to other cell generations)

- ✓ manifests by the change of growth and differentiation properties of cells and by changing their viability

- ✓ begins at a single cell level

Danger of cancer

reproduce **regardless of the needs of the organism** (unresponsive to conventional cellular signals)

colonize the body areas that are reserved for other cell types

disrupt the function of the affected organs

rapidly dividing tumor cells exhaust the organism

it is difficult for immune system to distinguish from healthy cells

tumor is formed by heterogeneous and continuously further developing population of cells that exhibit different (and variable) sensitivity to drugs

Are tumors hereditary?

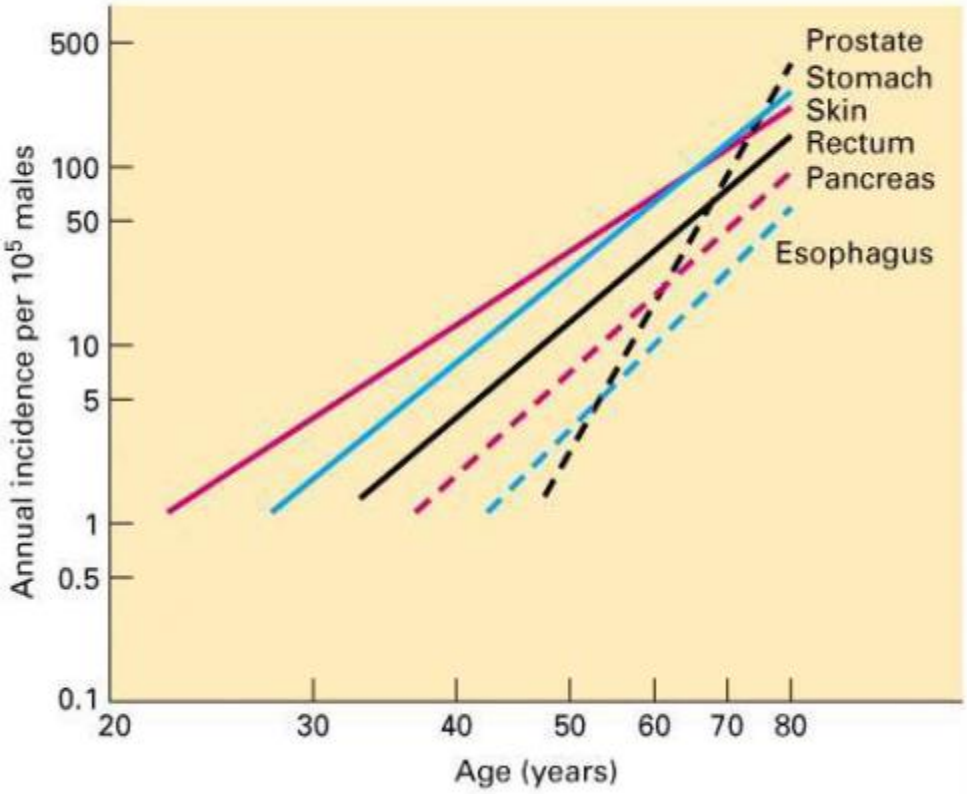
- predisposition to tumorigenesis can be inherited: inherited germline mutations are recorded at rare **familial cancer syndromes** (e.g. mutations in the RET proto-oncogene MEN syndrome causes - "multiple endocrine neoplasia" or thyroid tumors)

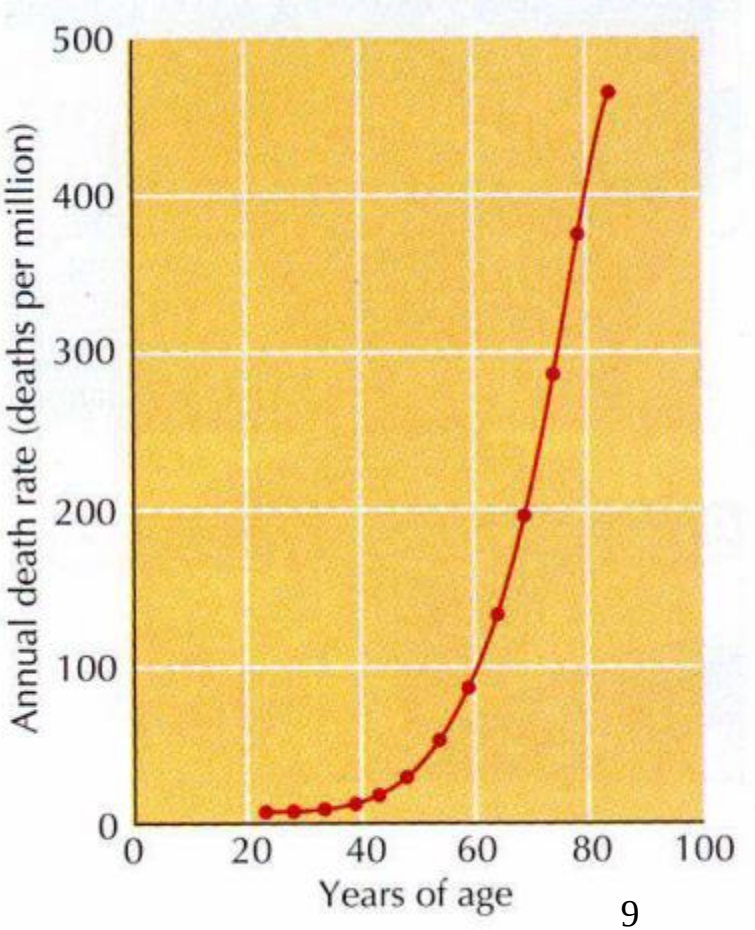
- common are tumors derived from somatic cells, which have experienced undesirable combination of tumor mutations

- increased frequency of mutations / genomic instability increase the risk of cancer

Development of tumors - process of gradual accumulation of genetic changes

- Incidence of tumors - advanced age





Three characteristics that describe a malignant tumor (Richard Klausner 2002)

- **genome instability** disease
- (Exceptions: leukemia, certain lymphomas, Ewing's sarcoma)
- **altered cell behaviour** disease
- **modified tissue behaviour** disease

Special properties of tumor cells in *in vitro* cultivation

1. **They do not need anchorage**

- Most of healthy cells need a substrate and form a monolayer in culture
- Cancer cells can grow in suspension

2. **Reduced sensitivity to contact signals (contact inhibition)**

- A healthy cell stops dividing when there is no place (in culture are only monolayers)
- Cancer cell is further divided, and oppresses the surrounding tissue (in culture grows into multiple layers, 3D shapes)

Classification of tumors I:

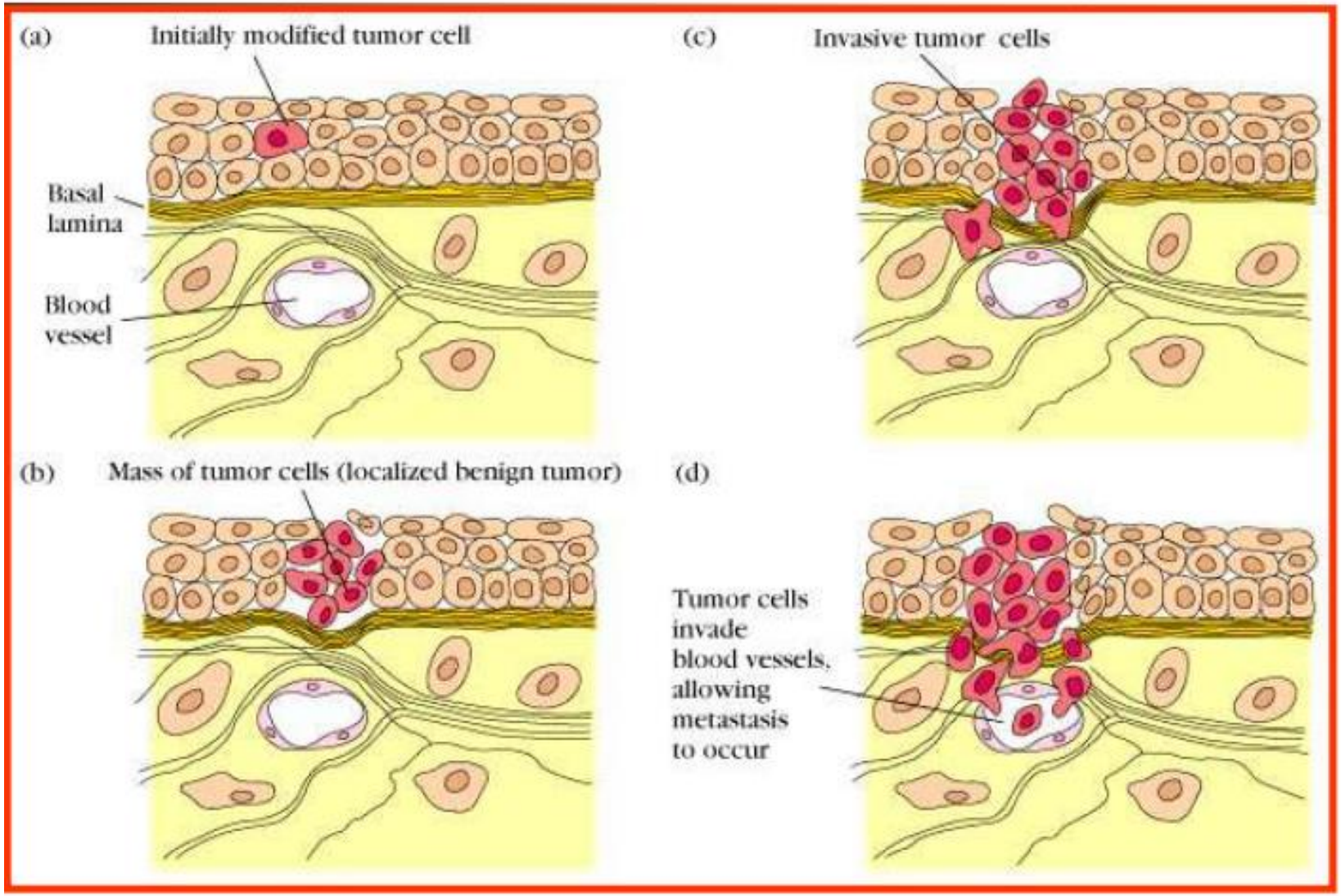
by their ability to infiltrate other tissues

- **Benign (noncancerous):** remain in their place of origin, they do not migrate, do not invade other tissues. The similarity with the original tissue. Usually not life-threatening

- **Malignant (cancerous):** penetrate into surrounding tissues through the blood and lymphatic system to the whole body in new tissues induce the formation of **secondary tumors (metastases).** A lower degree of differentiation. High proliferation (large nuclei, nucleoli, creation polyribosomes). A change in the morphology, size and shape of cells.

- From this perspective tumors can be classified into **primary** and **secondary**.

(Attention: secondary - therapy-related: the development from other less serious conditions.)


(a)
Initially modified tumor cell
Basal lamina
Blood vessel
(b)
Mass of tumor cells (localized benign tumor)
(c)
Invasive tumor cells
(d)
Tumor cells invade blood vessels, allowing metastasis to occur

Even benign tumors can be fatal

- **overproduction of important biologically active molecules (e.g. hormones)**

Example: glandular tumor cells - Islets of Langerhans - excessive secretion of insulin - hypoglycemia – Death

location of the tumor interferes with a vital function

Example: brain lining - disturbance in the functioning of vital centers of the brain - death

Classification of tumors II:

according to cell type (tissues) which they arise from

name reflects the original tissue where the tumor arose
suffix determines whether the tumor is benign or malignant
-**om** (benign)
-**karcinoma** (malignant epithelial tissue)
-**sarkoma** (malignant connective tissue or muscle)

- **Carcinomas** - tumors of the epithelial cells (about 90% of human cancers)
- **Sarcomas** - solid tumors connective tissues - muscles, bones, cartilage
- **Leukemia and lymphomas** - derived from hematopoietic cells and cells of the immune system
- **Gliomas** - tumors derived from neural tissue

Origin	Benign	Malign
Epithelial/Endothelial	liver adenoma, pancreas, colon, kidneys etc.	liver adenoma, pancreas, colon, kidneys etc.
Mesenchymal connective tissue	Lipoma	Liposarkoma
	Fibroma	Fibrosarkoma
	Chondroma	Chondrosarkoma
		Neuroblastoma
		Retinoblastoma
Germ	Teratoma	Teratokarcinoma
		Embryonal karcinoma
Other		Melanoma
		Leukemia

- tumors of **epithelial cells** (carcinomas) represent the largest group of human tumors (more than 80% of deaths from cancers in the Western world)

Classification of tumors III:

according to the affected organ or tissue

- lung cancer
- colorectal cancer
- breast cancer
- acute myeloid leukemia

- and many others

Carcinogenesis

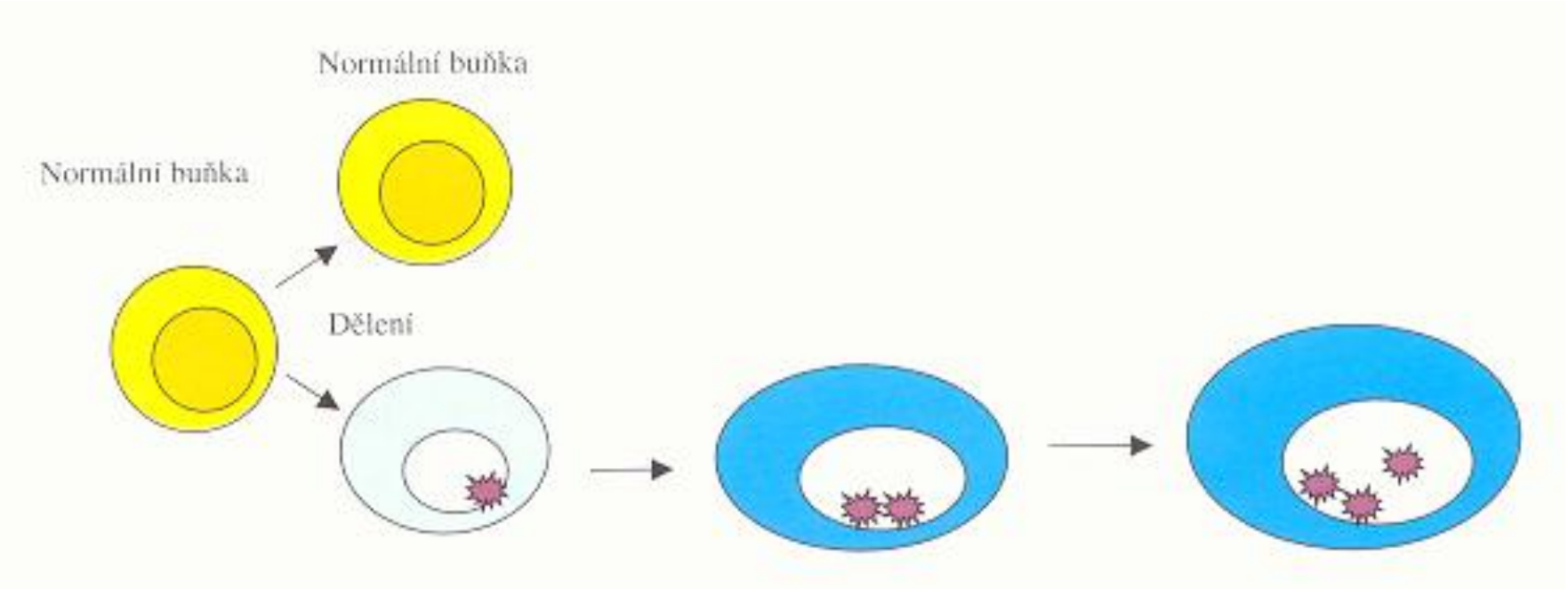
- the process of formation and tumor development

- it is a multistep process

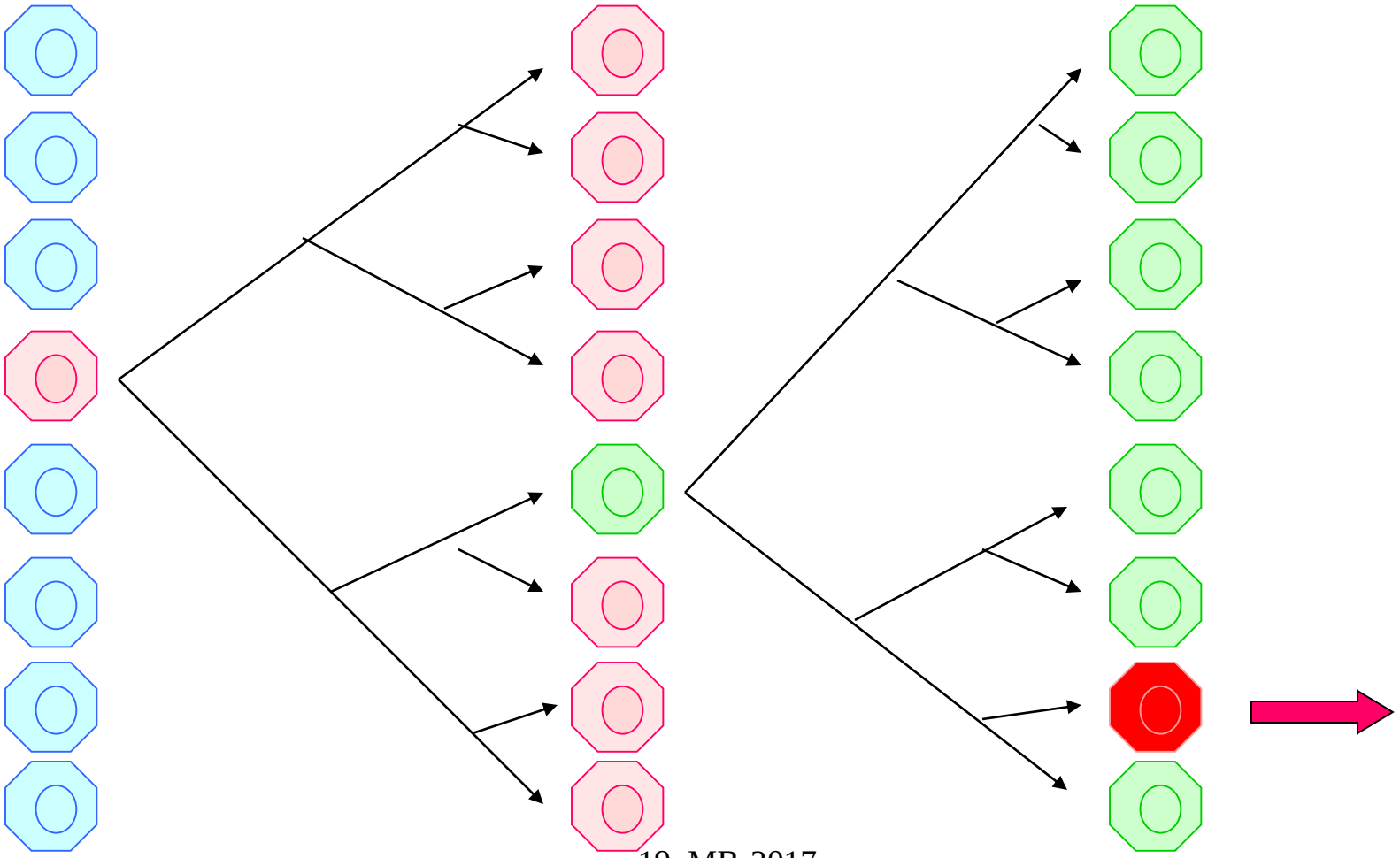
- the essence of carcinogenesis is the gradual accumulation of genetic (and epigenetic) changes

Neoplastic transformation - is transformation of somatic cell in the tumor cell

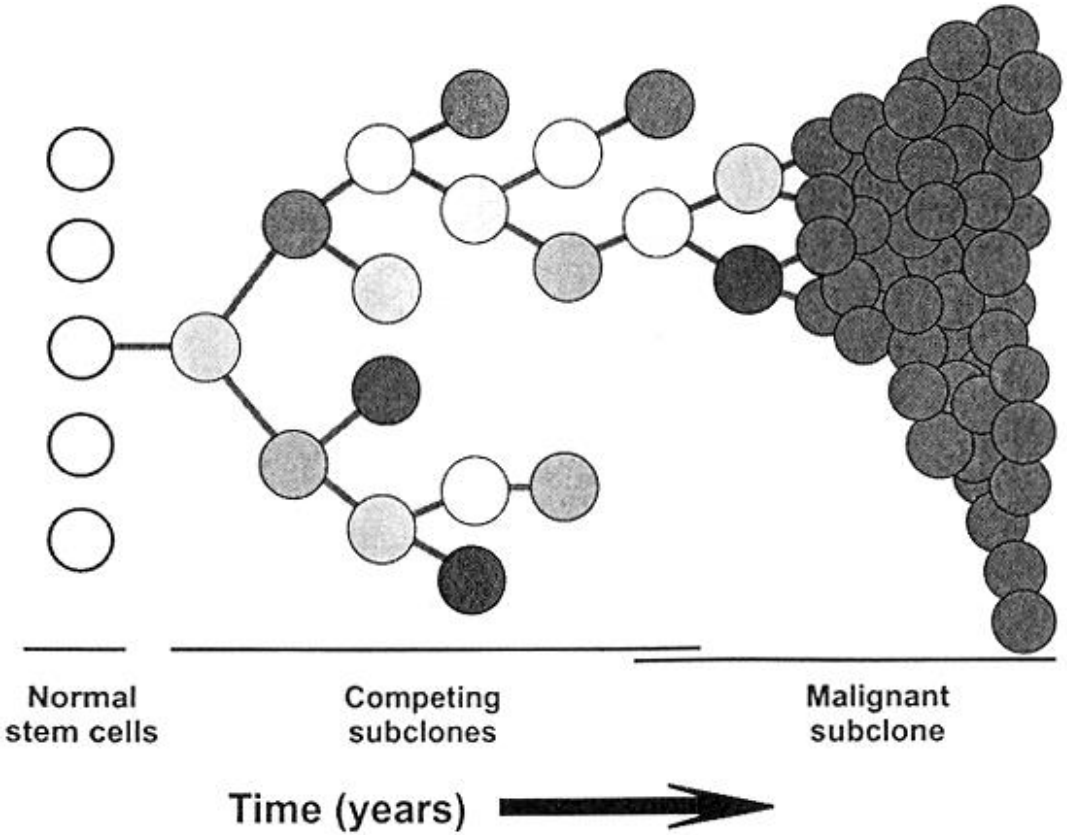
The essence of carcinogenesis is the gradual accumulation of genetic changes



Multistage carcinogenesis associated with clonal expansion steps



Clonal model of tumor development: selection, clonal expansion



How many and which genes are altered in carcinogenesis?

- Cancer is not a homogenous disease.
- It is estimated that 4-7 targets need to be hit in carcinogenesis.
- Dozens of particular genes can be targeted during carcinogenesis.
- Overall there are six (seven?) basic characteristics to a malignant tumor:

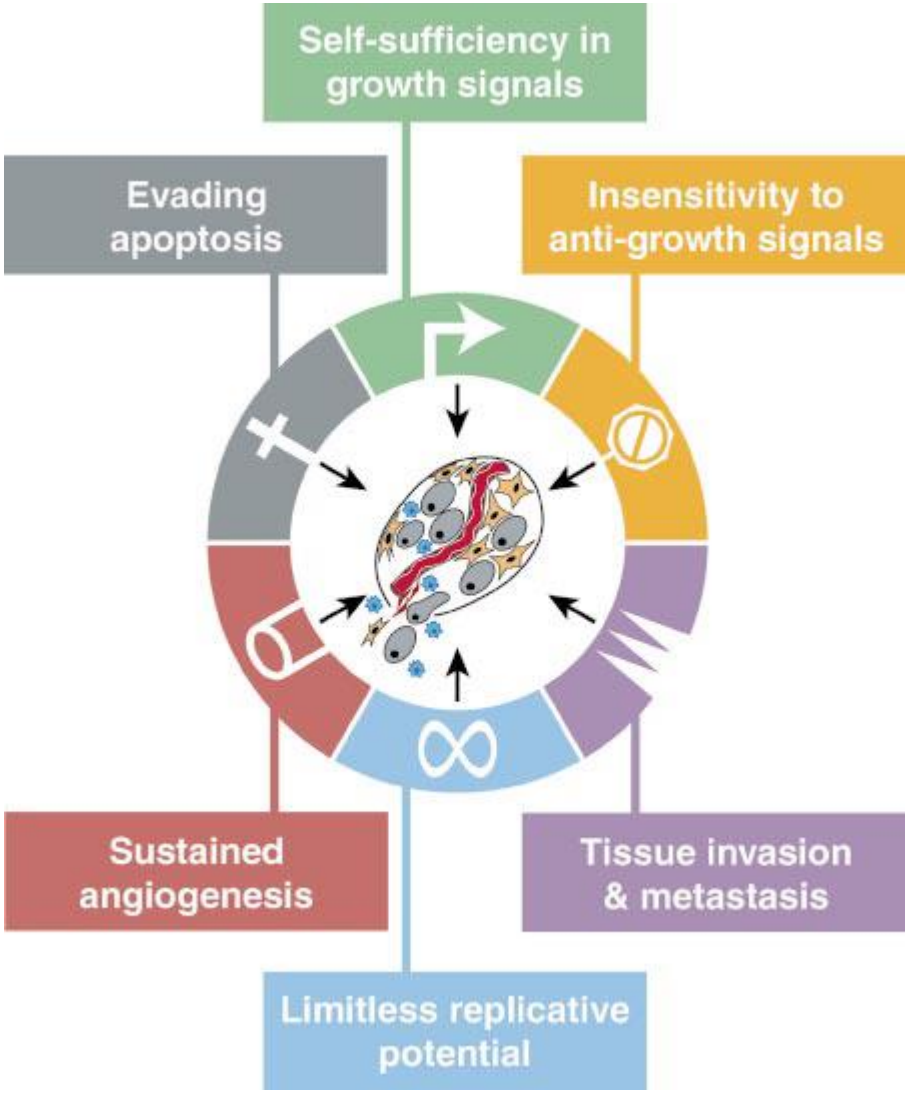
- https://www.youtube.com/watch?v=MWr20ZZipNA

Six acquired characteristics of a malignant tumor (Robert A. Weinberg 2000)

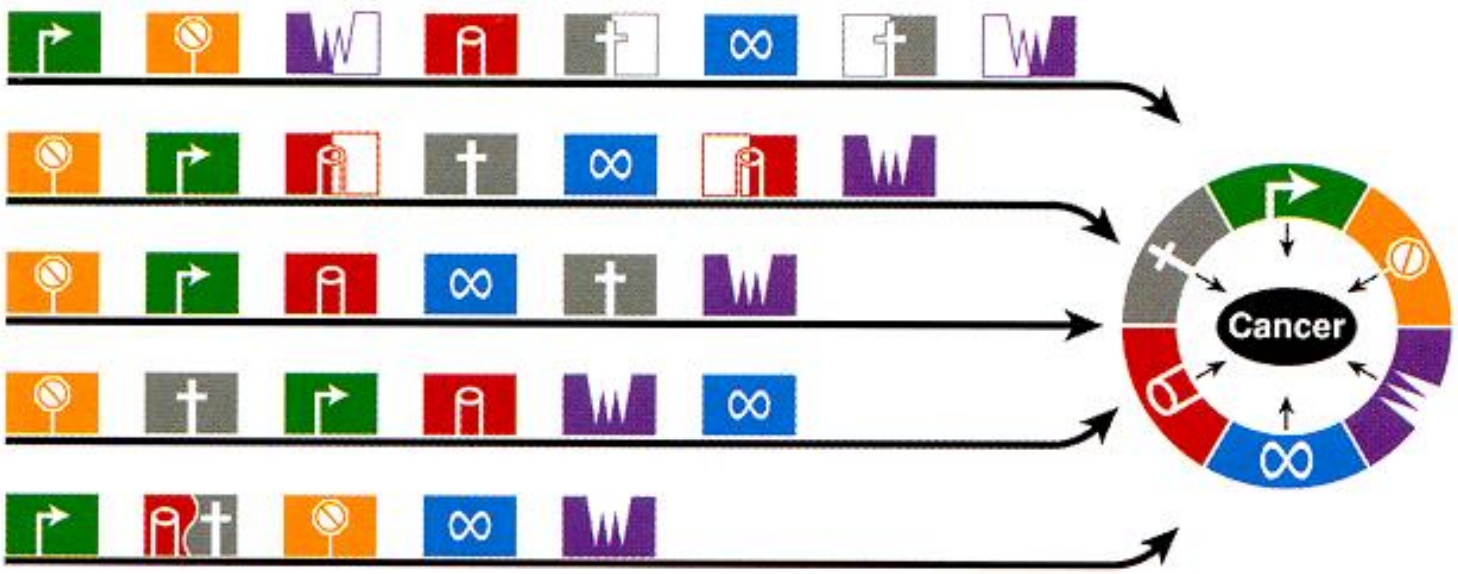
characteristic	example
Self-sufficiency in growth signals	H-*ras* loss
Insensitivity to anti-growth signals	RB loss
Evading apoptosis	IGF production
Limitless replicative potential	telomerase activation
Sustained angiogenesis	VEGF production
Activating invasion and metastasis	E-cadherine inactivation

Genome instability is a required feature to achieve these characteristics.

Hallmarks of cancer



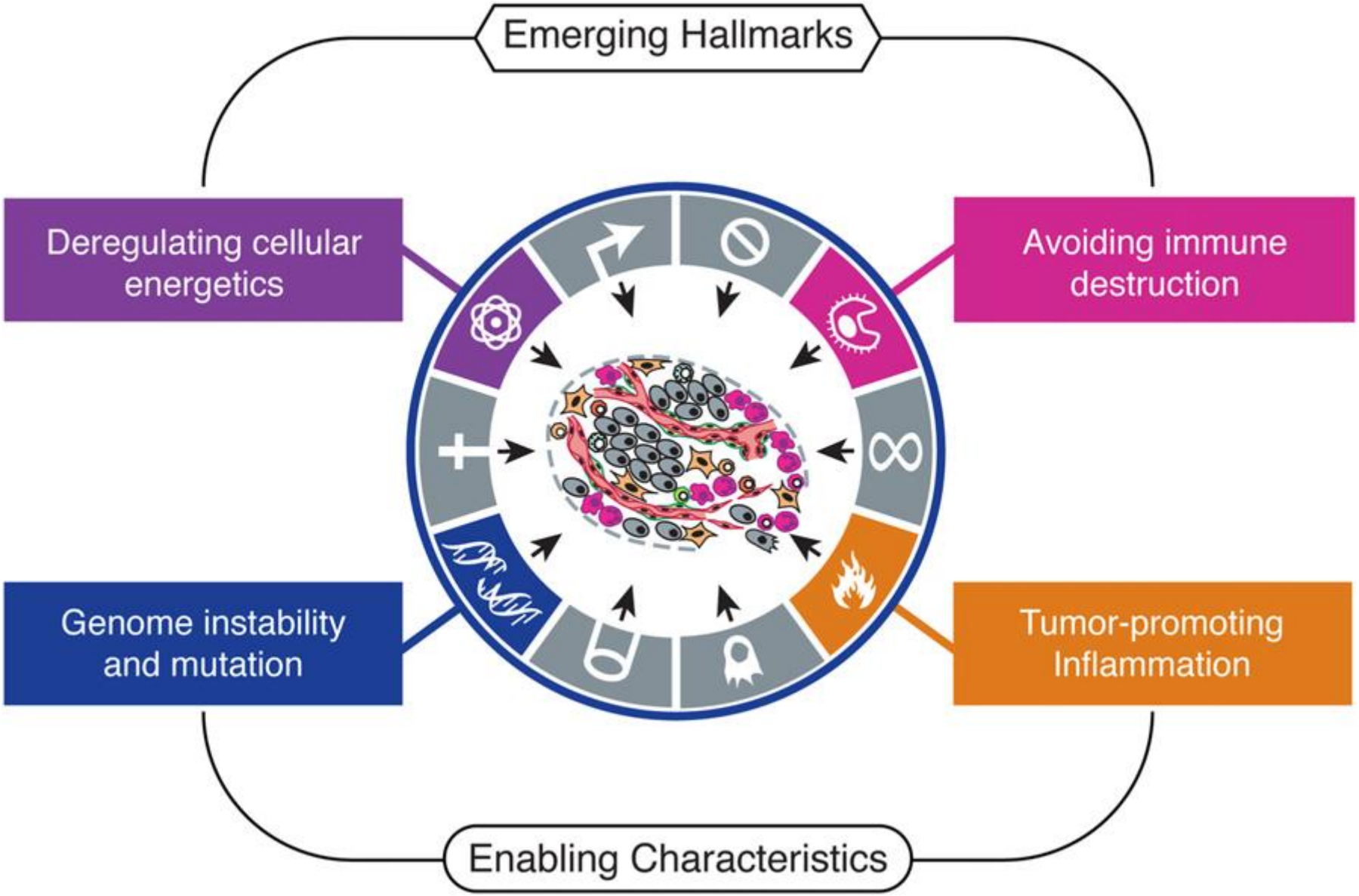
Carcinogenesis has individual progression



Individual – **order** in which hits occur

- number of hits

- genes that are hit


Emerging Hallmarks
Deregulating cellular energetics
Avoiding immune destruction
Genome instability and mutation
Tumor-promoting Inflammation
Enabling Characteristics

(A) Self-sufficiency in growth signals

- healthy cells cannot proliferate without **growth signals**
- many oncogenes stimulate signal pathways that are usually active only in the presence of growth factors
- reduced dependency on growth factors is observable also for tumor cell lines propagated *in vitro*

Three strategies to sustain proliferative signaling

alter growth factors or the way they are produced

Healthy cells usually produce growth factors utilized by other cells (heterotypic signalization), while tumor cells gain the ability to synthesize growth factors to which they are themselves sensitive (autocrine signaling) e.g.. PDGF – produced by glioblastoma

alter transmembrane receptors

a) Increased expression of receptor gene increases cellular sensitivity to low concentrations of growth factors (e.g. EGF receptor expression is increased in stomach, brain and breast cancer),

b) Change to receptor structure: *constitutive activity, even without signal*

alter intracellular component of signal pathway

Main role : Ras-Raf-MAPK cascade

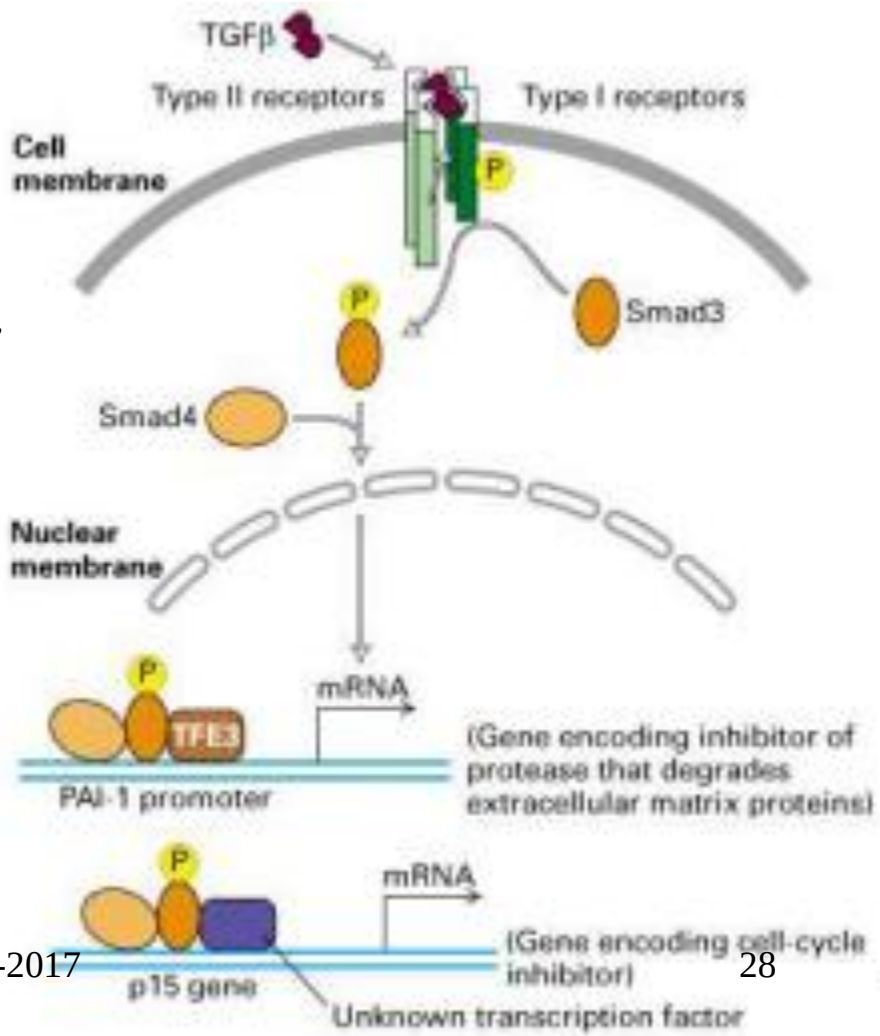
Ras proteins are altered in 25% of human tumors

It is likely that growth factor pathways are somehow deregulated in all tumor types.

(B) Insensitivity to anti-growth signals

Healthy tissue
reacts to both pro- and anti-proliferative (e.g. TGFβ) signals that are dissolved in body fluids and exert their function through membrane receptors and intracellular signal cascades

Tumor cells can loose the sensitivity to TGFβ by different means:
Lower the expression of TGFβ receptors, mutate TGFβ receptor, mutate proteins of intracellular signal cascade (SMAD proteins)



(C) Metastasis and invasion

- primary tumors can be chirurgically removed

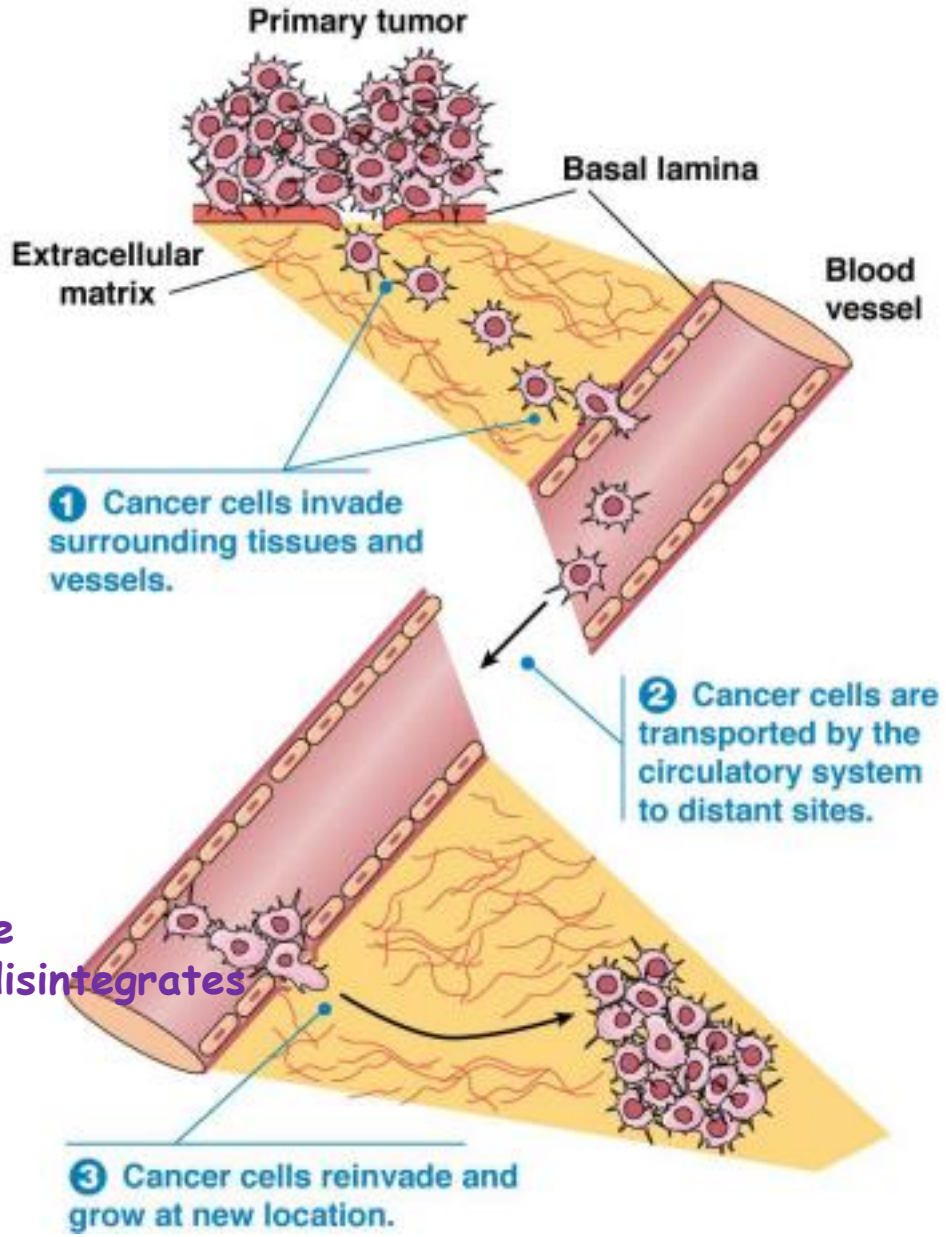
Invasion

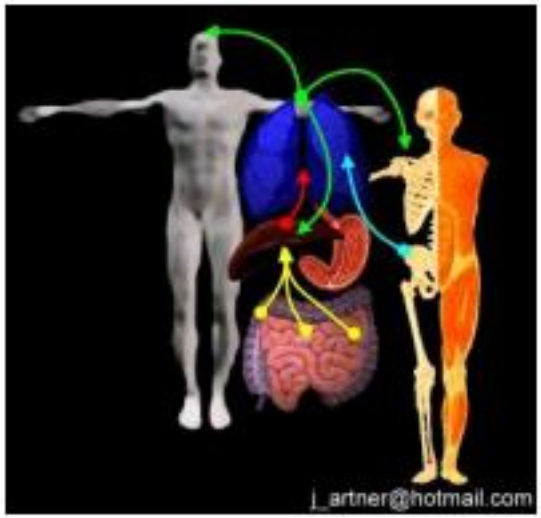
Tumor cells penetrate to neighboring tissue

metastasis

Tumor cells migrate by bloodstream and create secondary tumors

- requires changes to adhesion





Metastatic cascade

basal membrane disintegrates

cells separate

cells move

invasion

vascular system penetration

Tumor cells circulate

leave bloodstream (TEST)

(C) Metastasis

tumor cells travel from primary tumor to new locations, that at least at the early phases have enough room and resources to support tumor growth enabled by changes in two protein types:

- **Proteins that are responsible for cell adhesion** to neighboring cells (CAM) and to **matrix (integrins)**
- **Extracellular proteases** (protease overexpression, protease inhibitor inhibition)

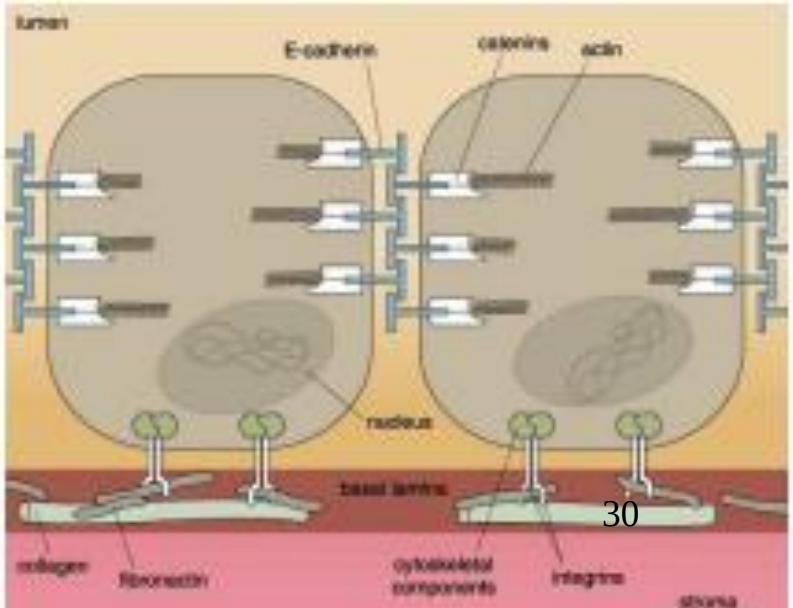
cell detachment

Tumor cells have reduced cohesion as a result of reduced expression of adhesion genes (**cadherins, catenines**)

regular cells of the same type do not detach from each other inside a tissue

Transfecting invasive cells with cDNA for E-cadherin decreases their metastability

E-cadherin



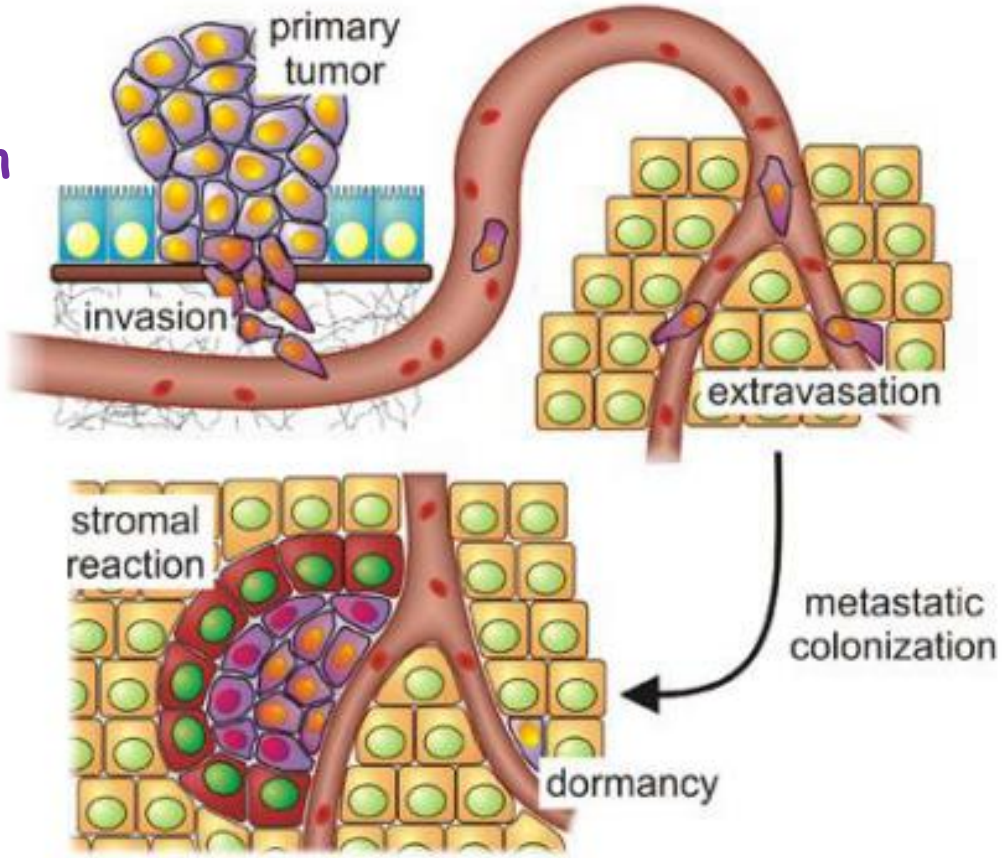
Metastasis and invasion

- Cancer cells can sustain in secondary tumors in **dormant stage**, hard to be discovered by diagnostic tools – causing **relapse** years after initial treatment

Metastasis cascade
- basal membrane disintegration
- cells separate
- cells migrate

invasion

vascular system penetration
- circulation in bloodstream
- leaving bloodstream



Extravasation *is the movement of cells from the capillaries to the tissues surrounding them*

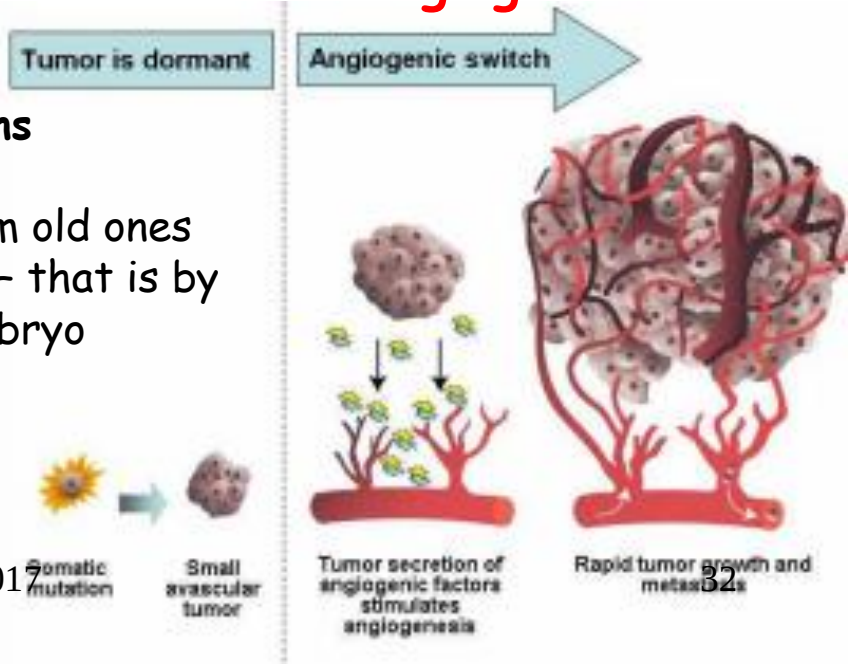
(D) Angiogenesis= growth of new capillaries

- capillaries supply (tumor) cells
- tumor needs capillaries to supply nutrients, oxygen and for waste removal, otherwise it can grow to a max. 1-2 mm
- Controlled by releasing angiogenesis factors (e.g. VEGF and FGF)
- Capillary formation is dependent on balance between **angiogenesis inductors** (e.g. FGF, VEGF) **and angiogenesis inhibitors** (e.g. trombospondine-1)

tumor growth is restricted by capillar availability. Under the lack of oxygen and other nutrients the cells start to die by necrosis, starting from tumor centre (furthest from cappilaries).

Tumor cells overproduce angiogenesis inductors and limit angiogenesis inhibitors

Tumor cells induce angiogenesis



Capillary formation under physiological conditions

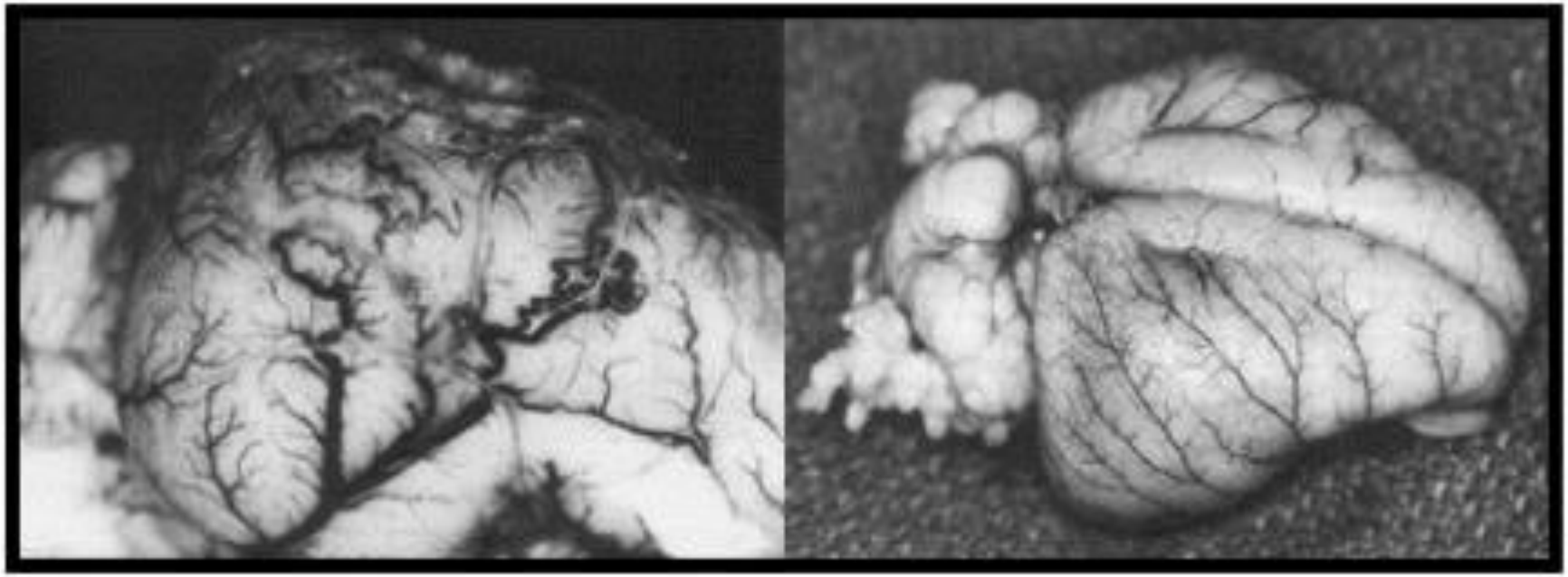
2 mechanisms:

- **angiogenesis** – new capillaries start to grow from old ones
- **vasculogenesis**– capillaries form from „nothing" – that is by differentiation of epithelial precursors inside embryo

Oxygen diffuses across 100 micron (0,1 mm)

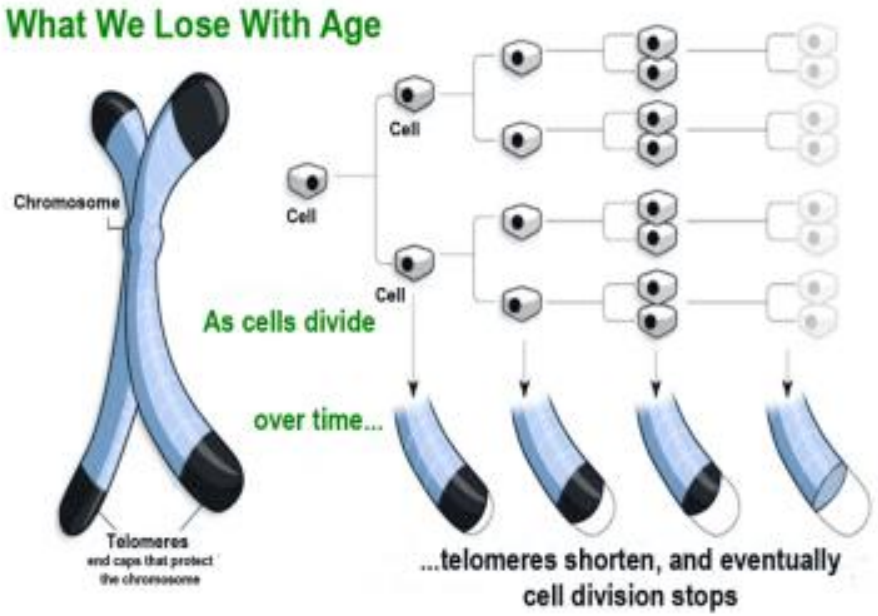
capillary formation – is regulated by the needs of metabolism

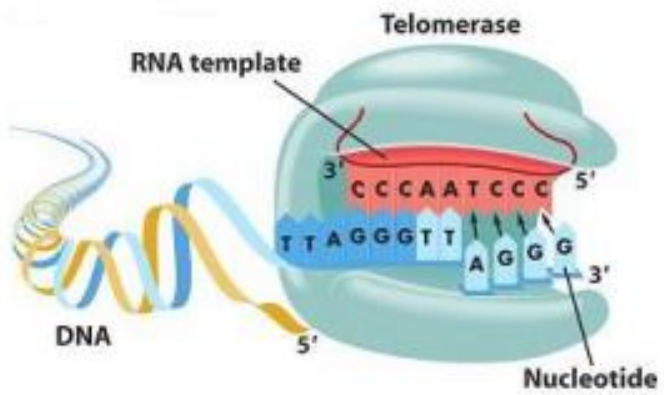
Healthy vasculature (right) is more systematically arranged compared to the tumor vasculature (left)



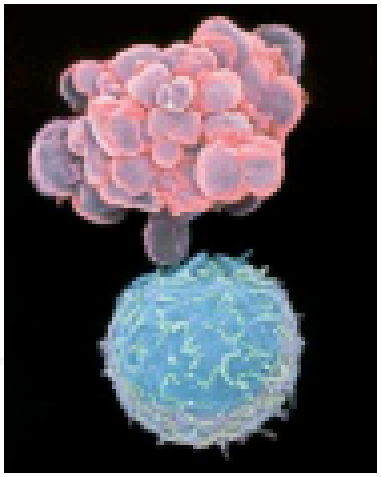
(E) Limitless replicative potential

Telomeres - repetitive sequences at the end of each chromatid
Mammals: sequence TTAGGG (repeated in humans around 2500x)
During each replication chromatids shorten. Their elongation can be performed by **enzyme - telomerase**
Most somatic cells however do not have active telomerase
Active telomerase **is a hallmark to tumor and embryonal cells – 90% immortalization**





(F) Avoiding apoptosis



Apoptosis = programmed cell death

Happens in organogenesis and during growth factor starvation
physiological cell removal without endangering neighboring cells
different than necrosis (result of physical cell injury, when cells burst, releasing their contents to intercellular space and cause inflammation)

Apoptosis trademarks:

cytoskelet breaksdown, cell squishes
Nuclear membrane decomposes
nuclear DNA cleaved into fragments
cell disintegrates into apoptotic vesicles
cell surface altered as to induce imminent phagocytosis

Tumor cells are not sensitive to signals inducing cell death
healthy cells can live only in the presence of growth factors, otherwise they die by apoptosis x tumor cells live on without growth factors
Healthy cells with damaged DNA die by apoptosis x tumor cells do not
Resistance to apoptosis is one of the reasons for increased survivability of tumor cells

Molecular basis of cancer

Changes in **genes** that control **cell cycle and DNA repair**

1. Proto-oncogenes

- Genes stimulating proliferation
- Mutations causing their hyperactivity are called oncogenic
- "Gain-of-function" mutations
- Often genes of growth signalization cascade
- **e.g. Ras, Myc...**
- Activation is **dominant** – corrupting one allele is enough to start carcinogenesis

2. Tumor-suppressors

- Genes inhibiting cell cycle
- Often dysfunctional in cancer
- "Loss-of-function" mutations
- **e.g. p53, Rb1, BRCA1 a BRCA2...**
- Activation is **recessive** – both alleles must be defective to induce cancer

Proto-oncogenes and tumor suppressors encode genes that regulate cell proliferation and growth

1. Growth factors
- e.g. PDGF, EGF...

2. Growth factor receptors
- e.g. PDGFR, EGFR...

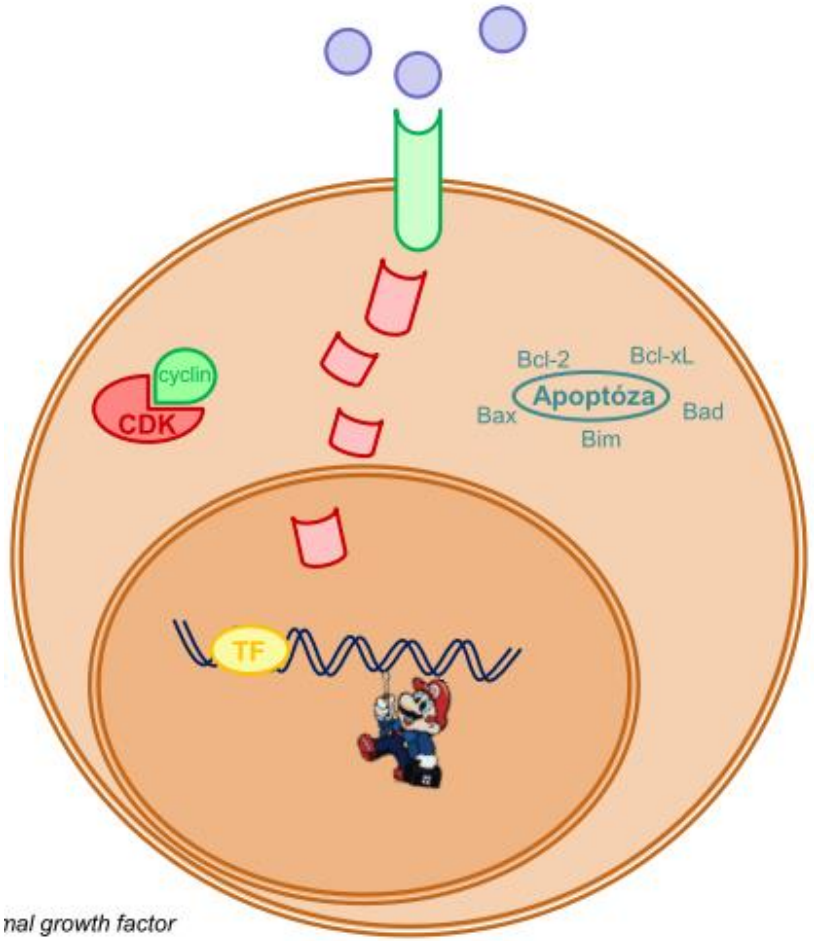
3. Intracellular carriers
- e.g. Ras, Src...

4. Transcription factors
- e.g. Myc, Fos...

5. Apoptosis regulators
- e.g. Bcl2 protein family

6. Proteins regulating cell cycle
- e.g. Cyclins and cyclin dependent kinases

7. Proteins involved in DNA repair
- e.g. BRCA, ATM, ATR, γH2AX...



Oncogenes

Proto-oncogene is a structural gene in eukaryotic cell, that is somehow connected to **cellular proliferation and differentiation**

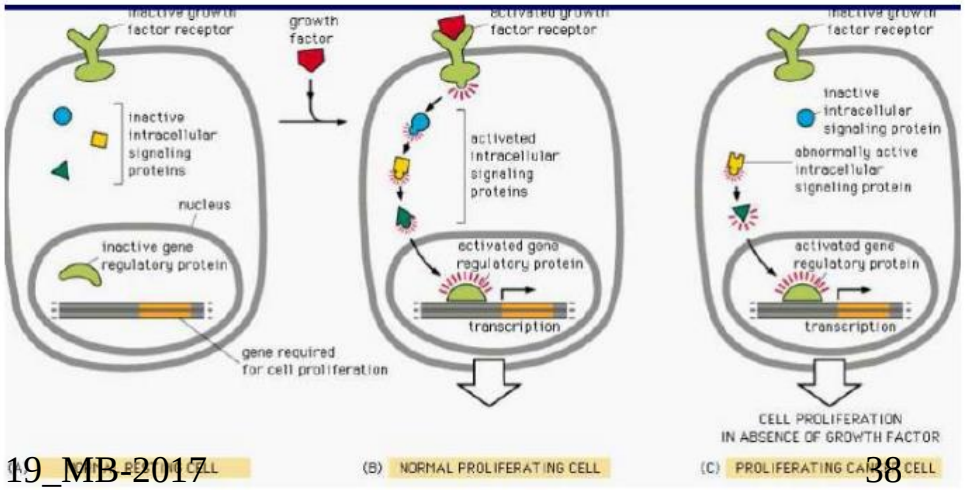
Oncogene is a proto-oncogene altered or activated in a manner that favors **neoplastic cell transformation**

Proto-oncogene activation turns proto-oncogene into oncogene.

Proto-oncogene mutations are:

- activating
- dominant
- occur in somatic and rarely also in progenitor cells

pathological oncogene activation



Tumor suppressors

Tumor suppressors (**anti-oncogenes**) regulate (inhibit) proliferation in healthy cells and keep them in non-dividing phase (G_0). Their loss is manifested by uncontrolled proliferation.

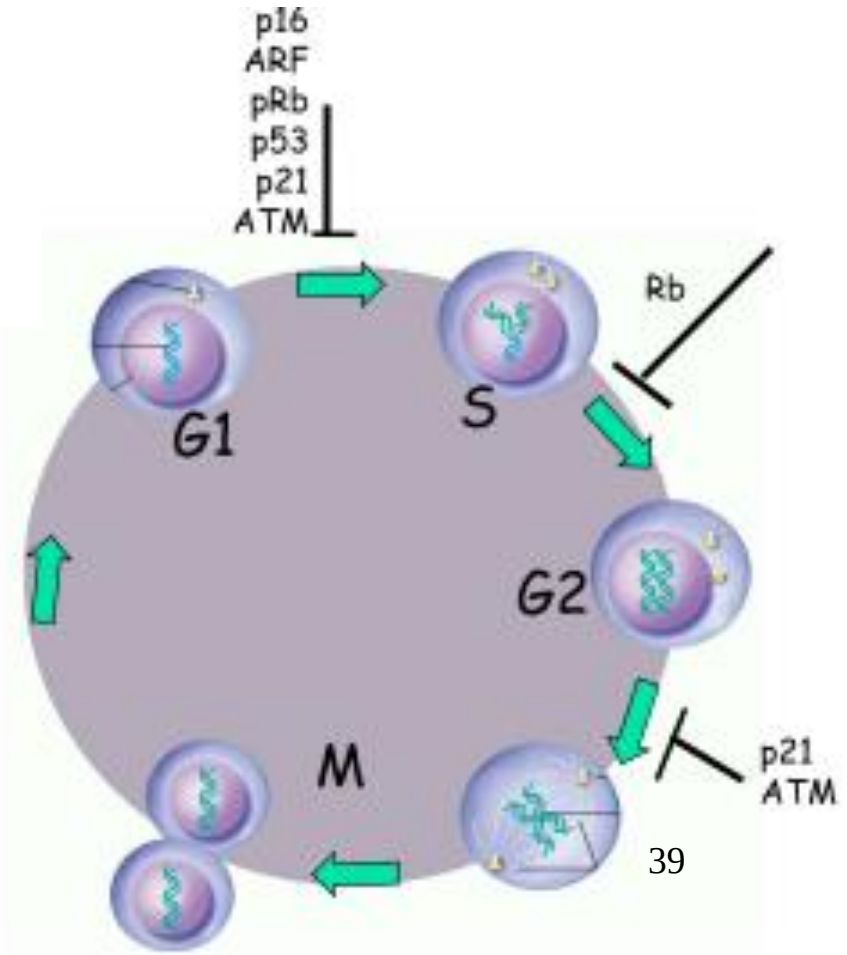
Tumor suppressor mutations are:

- inactivating
- recessive (coupled with LOH)

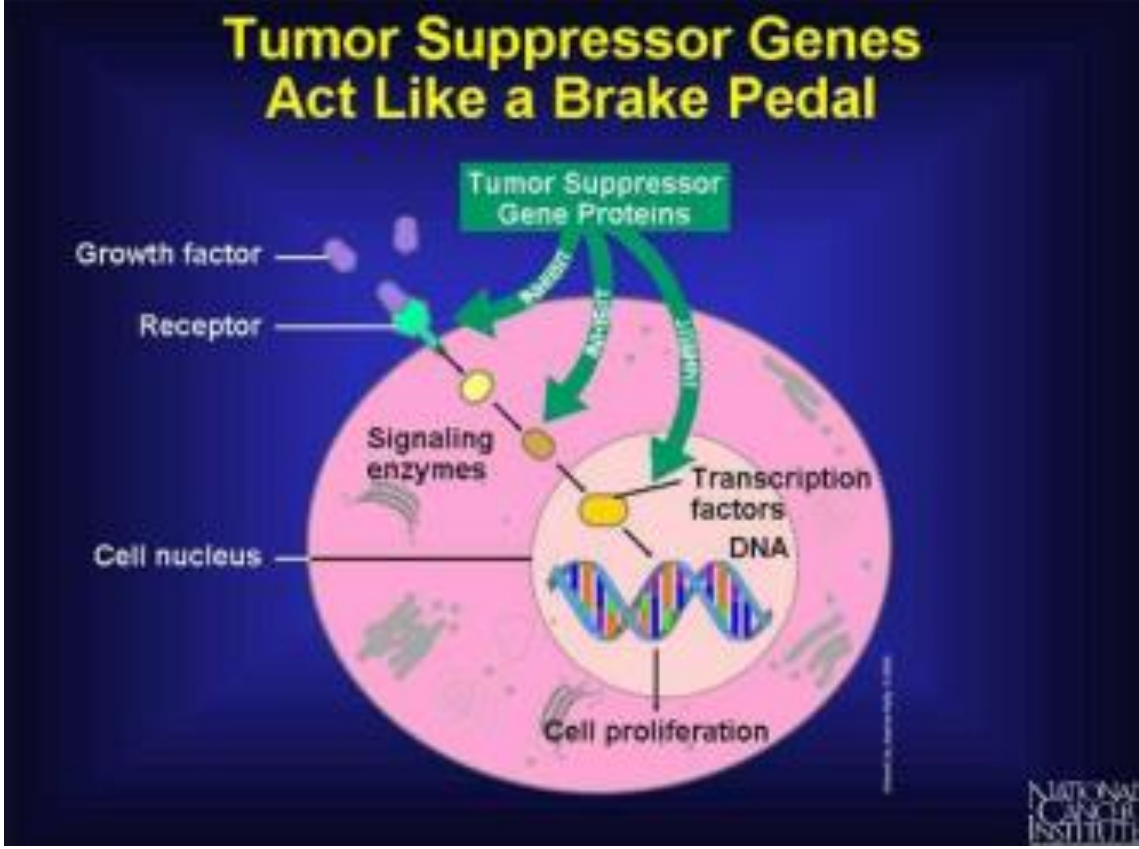
(„recessive oncogenes")

- occur both in somatic and progenitor cells

Most tumor suppressors act as cell cycle negative regulators



Tumor suppressors



- cell cycle negative regulators (Rb, p16)
- proliferation signal pathways negative regulators
- (WT-1 inhibits EGR-1; NF-1 inhibits RAS)
- intercellular adhesion negative regulators (APC, DCC)
- DNA damage repair and recognition pathways (p53, MSH2, MLH1)

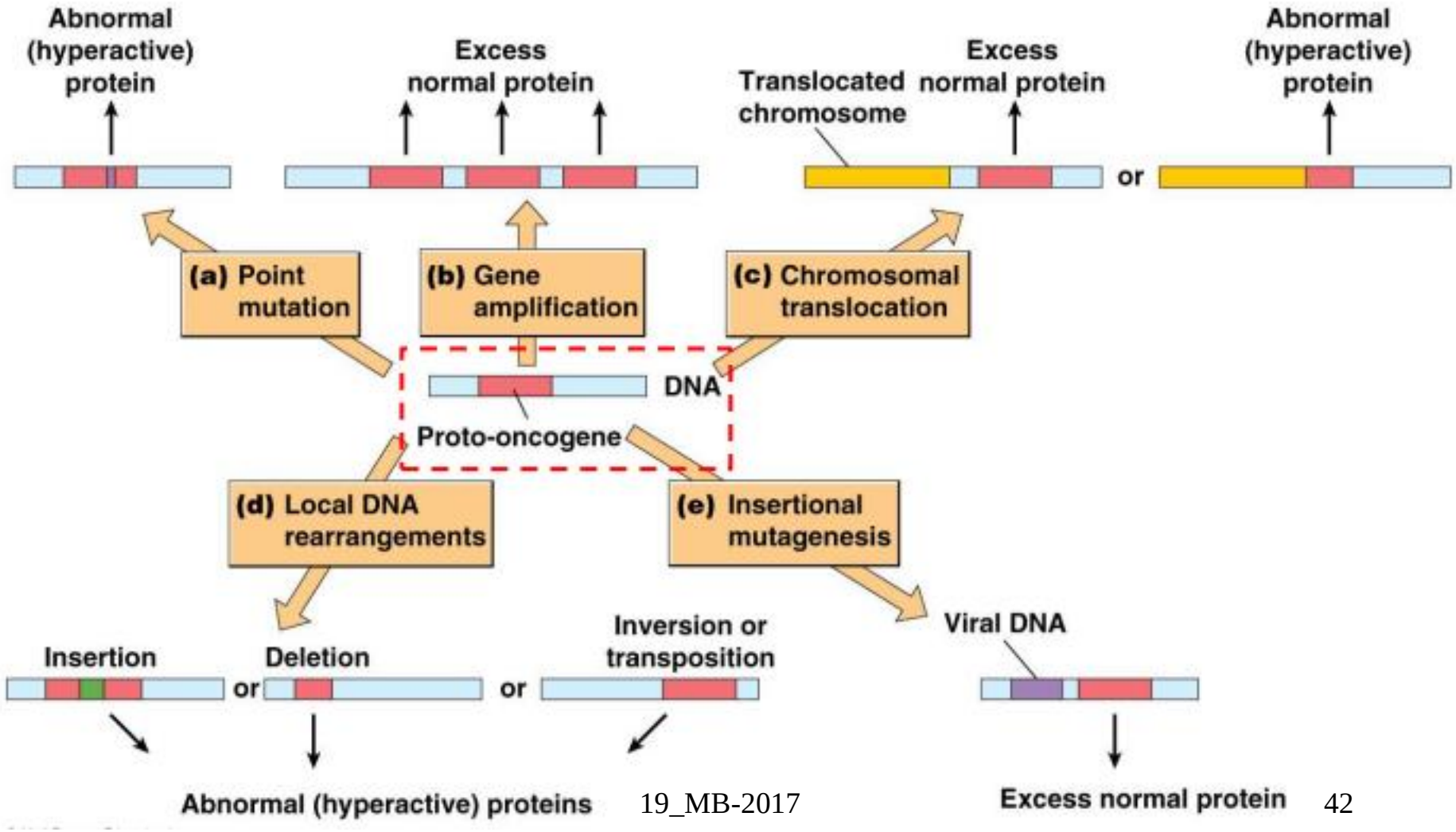
Genes targeted in carcinogenesis

1. Oncogenes
   Tumor suppressors

2. Oncogenes
   Tumor suppressors
   genes for genome stability („stability genes")

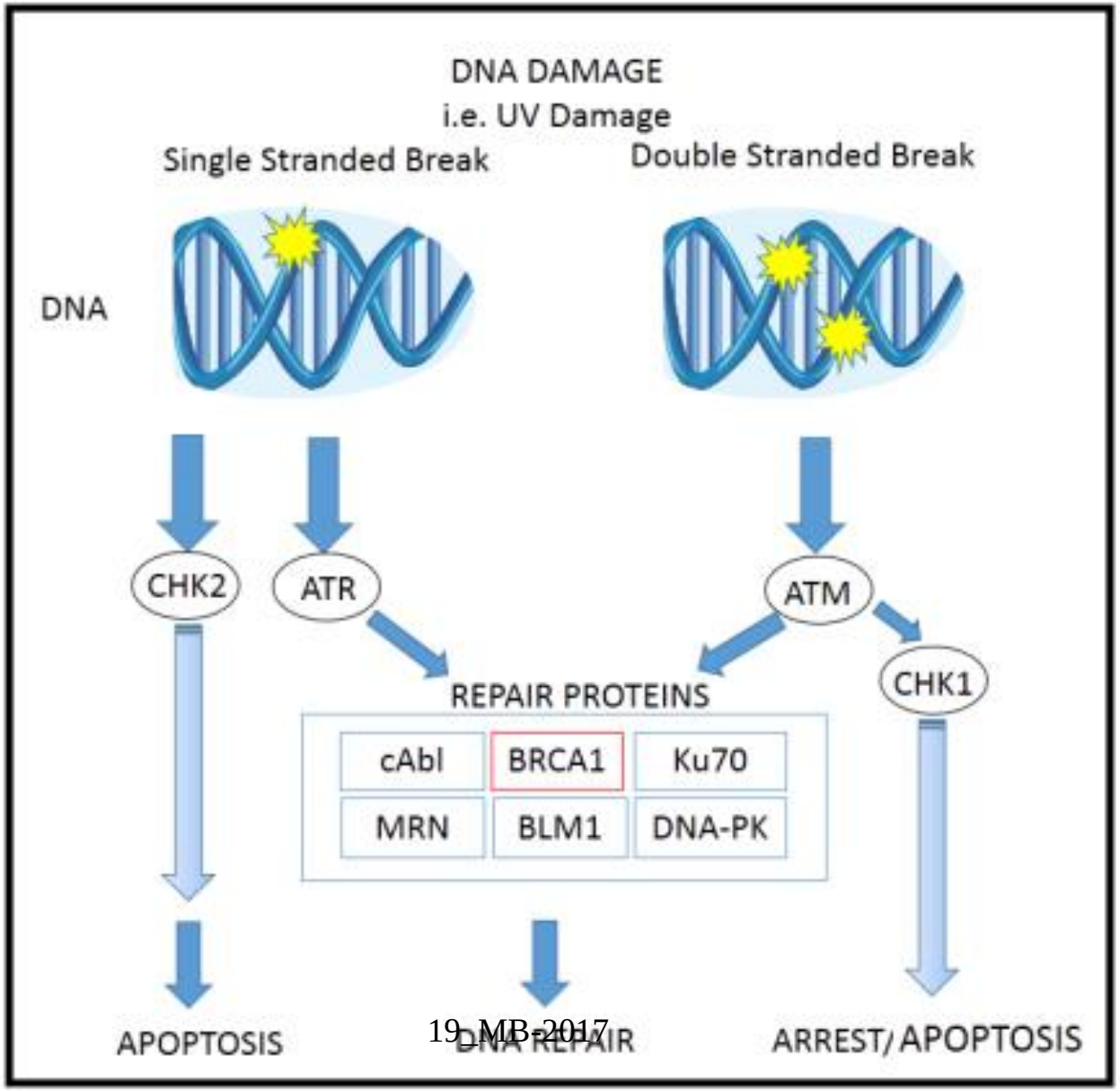
Types of mutations

a) point mutation, b) gene amplification, c) chromosomal translocation, d) the local reconstruction of DNA, e) sertional mutagenesis



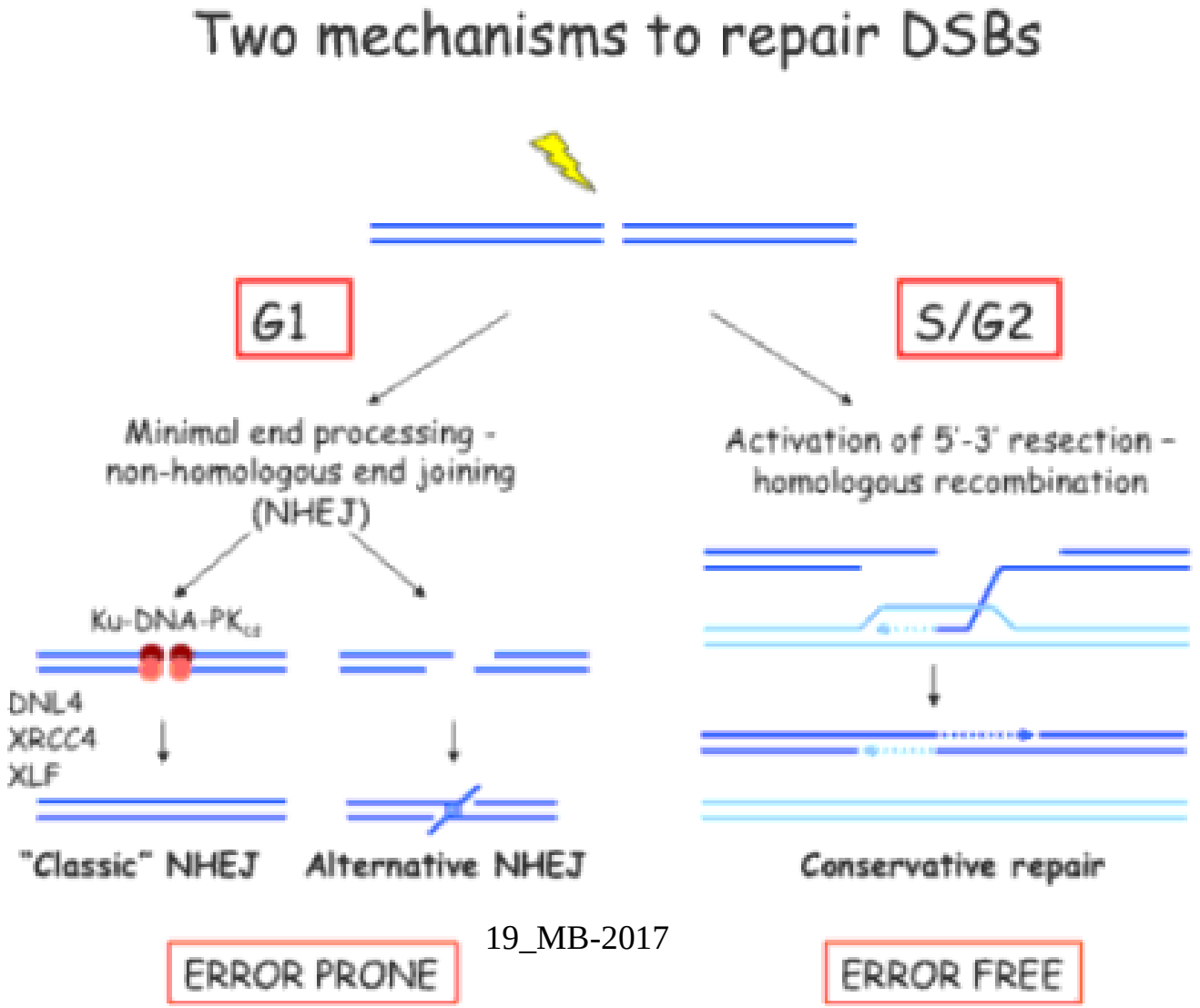
TEST

DNA repair



DNA repair

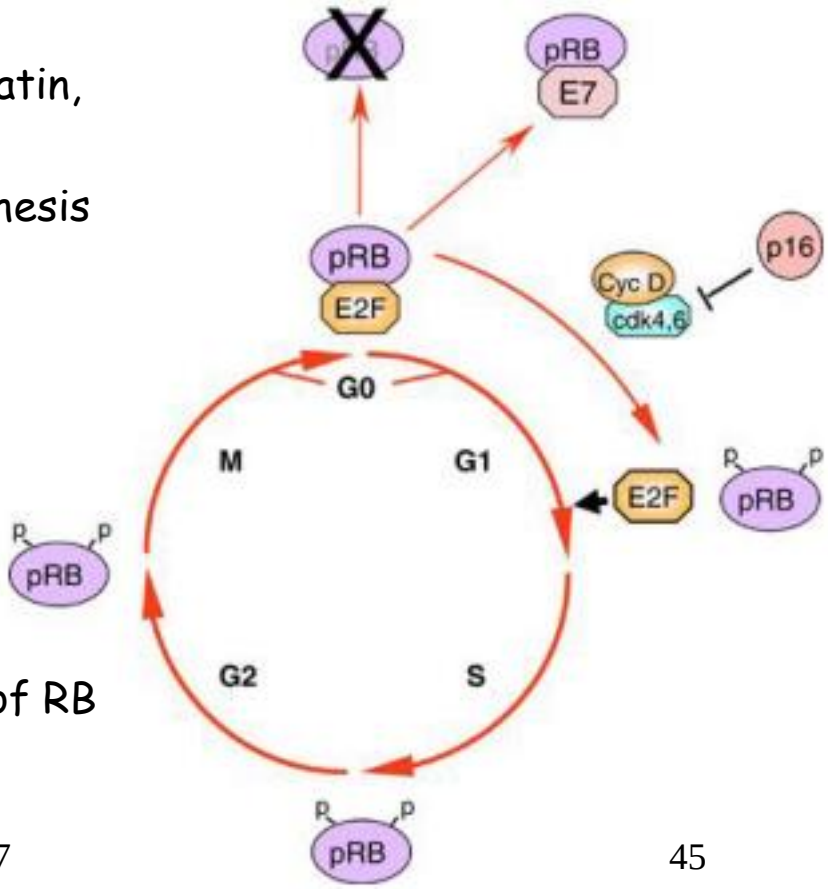
- Homologous recombination (HR) is more accurate than the non-homologous end joining (NHEJ), but requires the presence of template DNA chain of sister chromatids appears to the S / G2 phase, less often used as a template in a second chromosome in G1 – non-sister chromatid)



Retinoblastoma protein- tumor suppressor RB

- Retinoblastoma protein (pRB) inhibit excessive cell division (proliferation) by cell cycle arrest

- prevents the transition to the S phase of the cell cycle **by binding and inhibition of the transcription factors E2F family**
- until Rb bound E2F, the cell remains in the early G1 or G0 phase
- In proliferating cells complex CycD + CDK4,6 **pRB is phosphorylated, thereby releasing E2F** → entry into S-phase
- Rb-E2F complex also attracts HDAC to the chromatin, which reduce transcription factors supporting transition into S-phase → suppression of DNA synthesis



In tumor cells, pRB often does not work and E2F is still free → unregulated proliferation

a) mutations in the RB gene - not bind to E2F

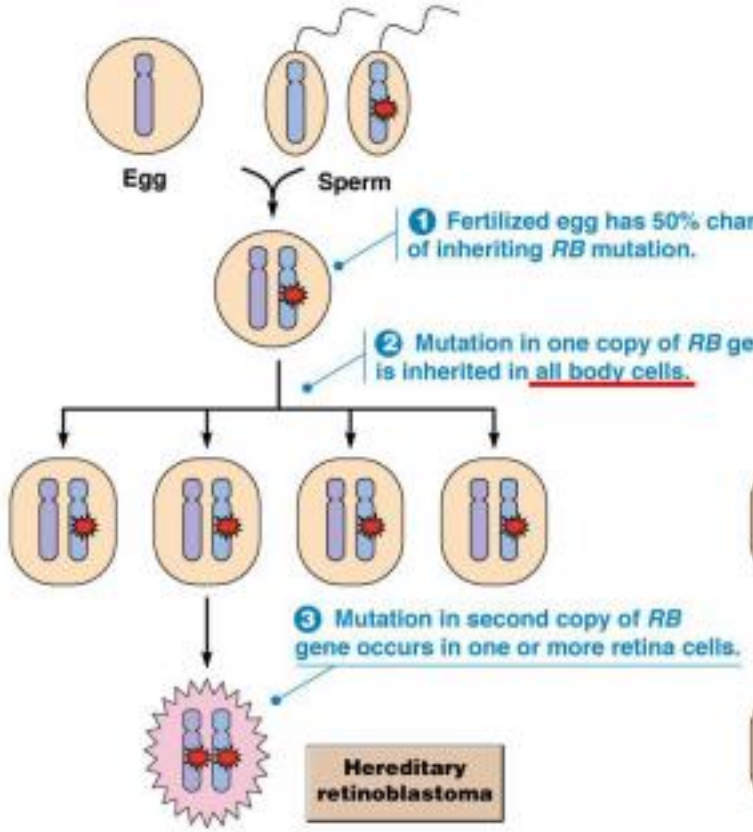
b) viral protein E7 displaces pRB

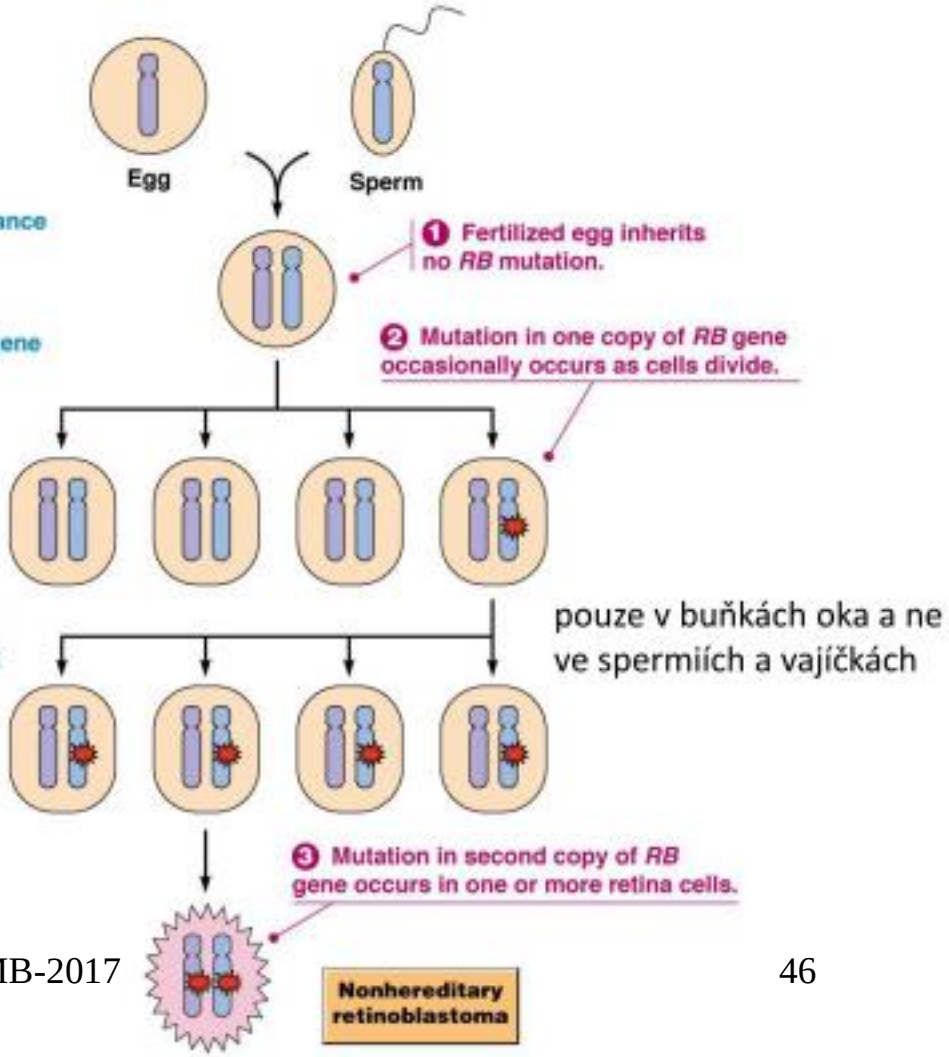
c) the overexpression of cyclin D or CDK 4.6 or loss of p16 inhibitory → excessive phosphorylation of RB

HDAC - Histone deacetylases -
suppress the expression (chromatin wraps) - HDAC1, HDAC2, H

Hereditary cancers – RB mutation

- mutation in one allele is already in sexual cell → in all somatic cells of a descendant
- mutation in the second allele can occur during life
- non-functional RB protein was first described in connection with eye tumors (retinoblastoma), plays a role in various types of tumors





Pro vznik nádoru v oku je potřeba mutace v obou alelách - často u dětí (two-hit model)

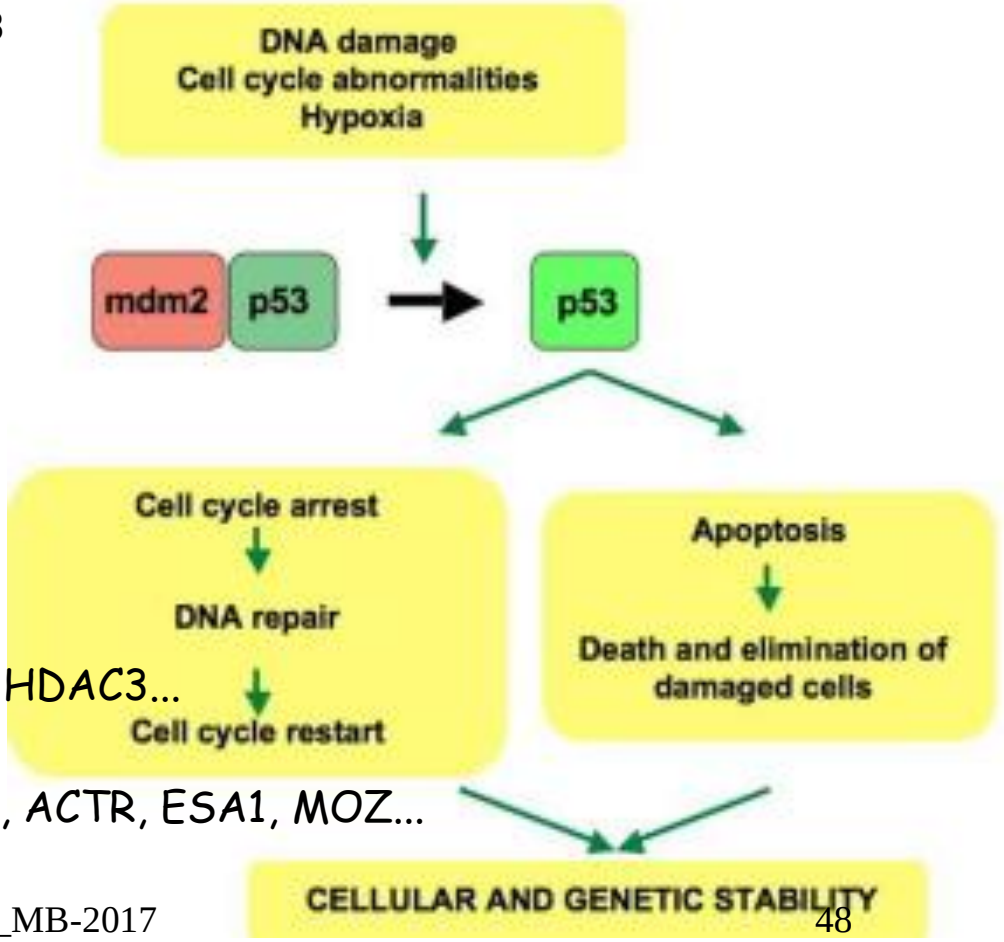
Two-hit model

For the formation of retinoblastoma two genetic changes are needed

- in 1971 Alfred Knudson defined "Two-hit" theory based on a comparison of hereditary and sporadic forms of retinoblastoma
- researchers in the field of cancer initially paid no attention to this theory, because hereditary cancer is very rare
- This theory, however, was behind the discovery of tumor suppressor genes in all types of cancer

Tumor-suppressor p53
The guardian of the genome

- p53 induces transcription of p21 that binds to CDK2 - inhibits the transition to the S phase
- Binding with Mdm2 inhibits its activity
- HATS (eg. P300 / CBP, PCAF) may in response to stress acetylated p53 → increase in activity
- HDAC1, 2 and 3 can reduce the p53 activity by deacetylation
- 50% f tumors have a mutation or deletion of p53
- p53 mutation is mostly negative prognosis for cancer patients



HDAC - histone deacetylase
suppress the expression of HDAC1, HDAC2, HDAC3...
HAT - Histone acetyl transferase activated
Expression of Gcn5, p300/CBP, PCAF, SRC-1, ACTR, ESA1, MOZ...

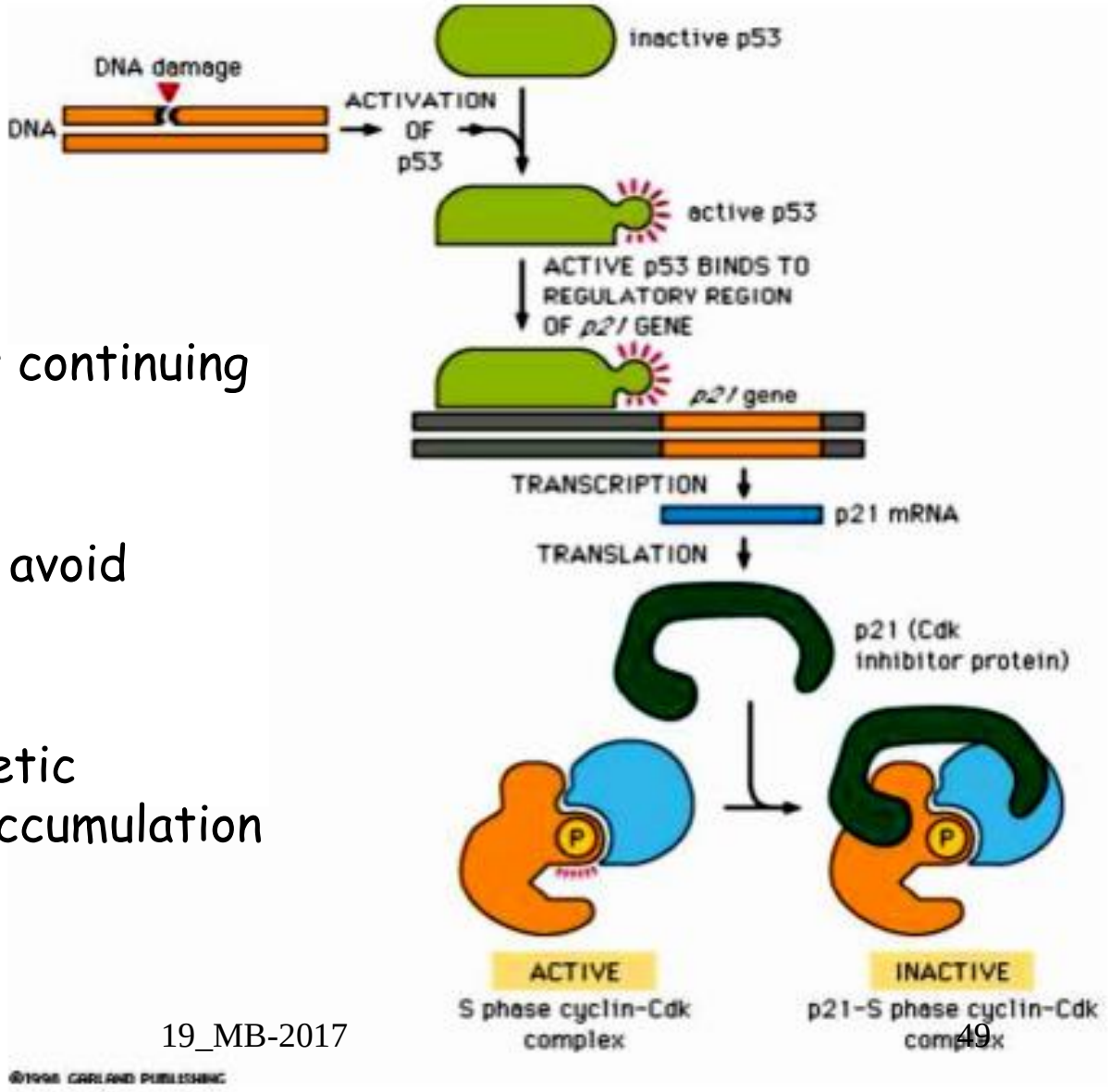
Tumor-suppressor p53

Brake entry into S-phase cell cycle arrest in the G1 phase enables break necessary to DNA repair

1) damaged mutated cells continuing the cell cycle

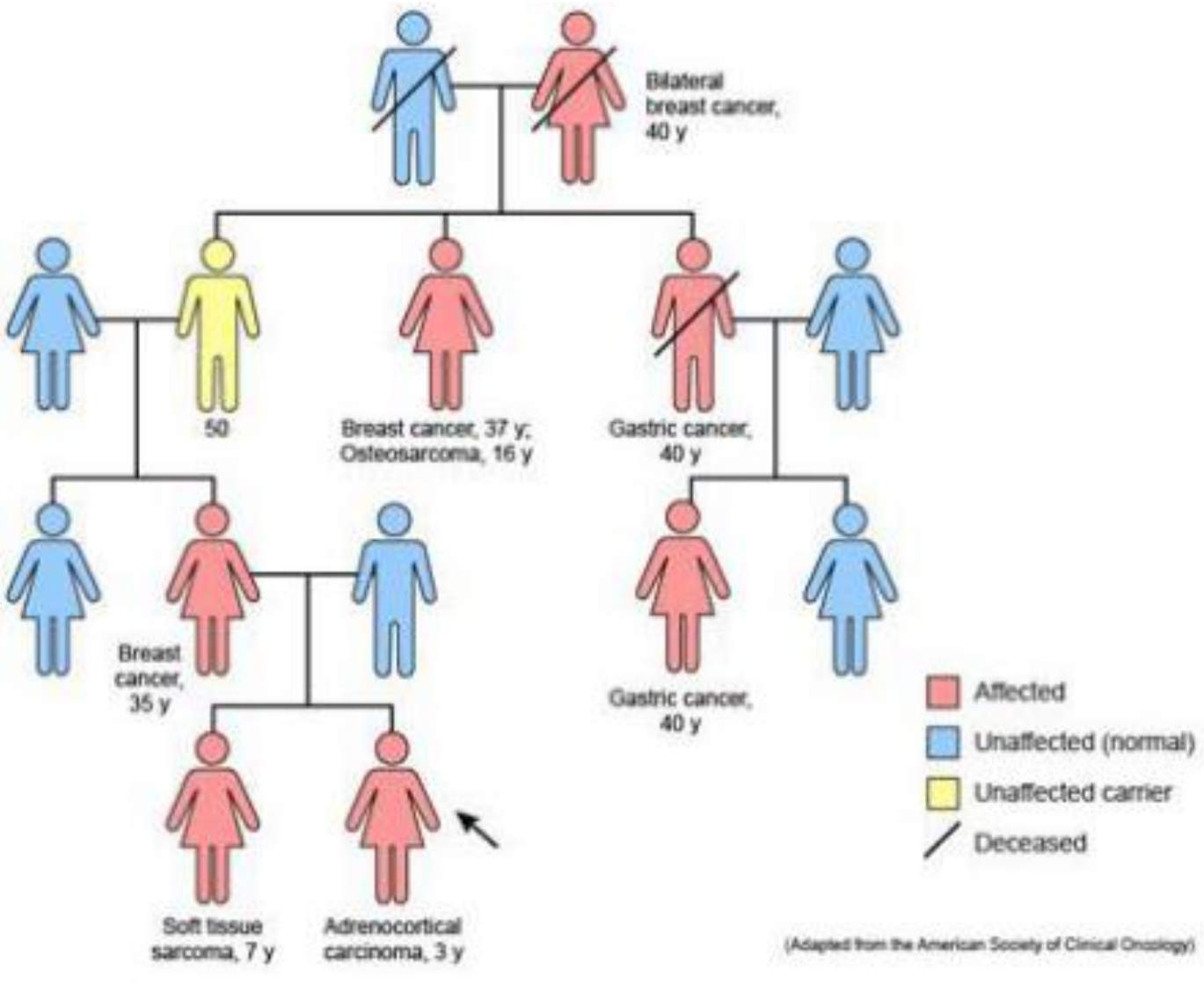
2) allow damaged cells to avoid apoptosis

3) the emergence of genetic instability, allowing the accumulation of mutations



Li-Fraumeni syndrome

- hereditary disease
- **Mutations or deletions in one allele of the p53 gene** causing a hereditary predisposition to cancer
- increased incidence of cancers of different tissues in early age in the family



Coordination tumor-suppressors RB and p53

- Two main pathways ensuring cellular response on potential oncogenic stimuli

- Signals (e.g. DNA damage, oncogene activation

1. pathway p53

induction of ARF, that separates the MDM2 - p53

- active p53 regulates a number of genes, eg..:

- WAF1 → CDK inhibition → cell-cycle arrest

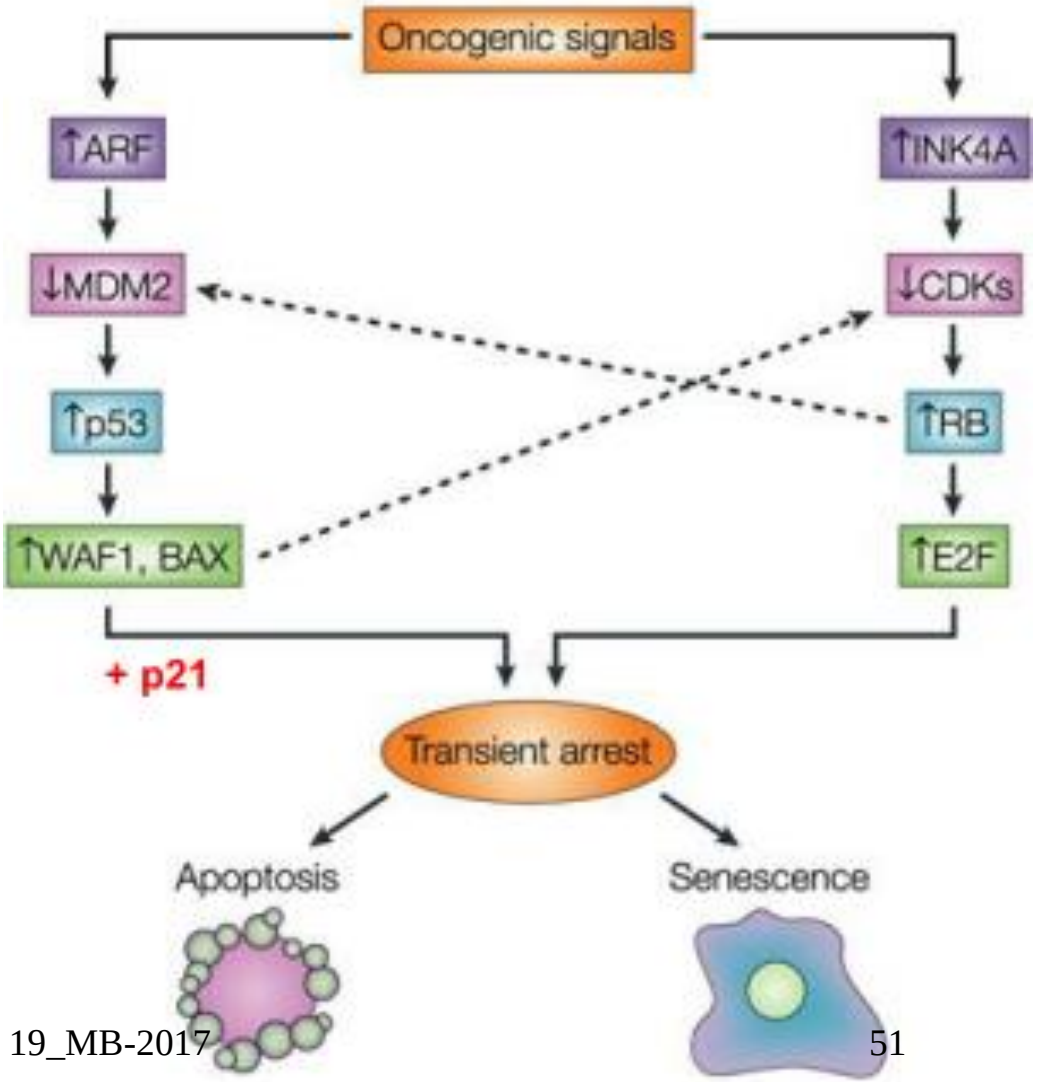
- BAX → induction of apoptosis

- p21 → CDK inhibition

2. pathway RB

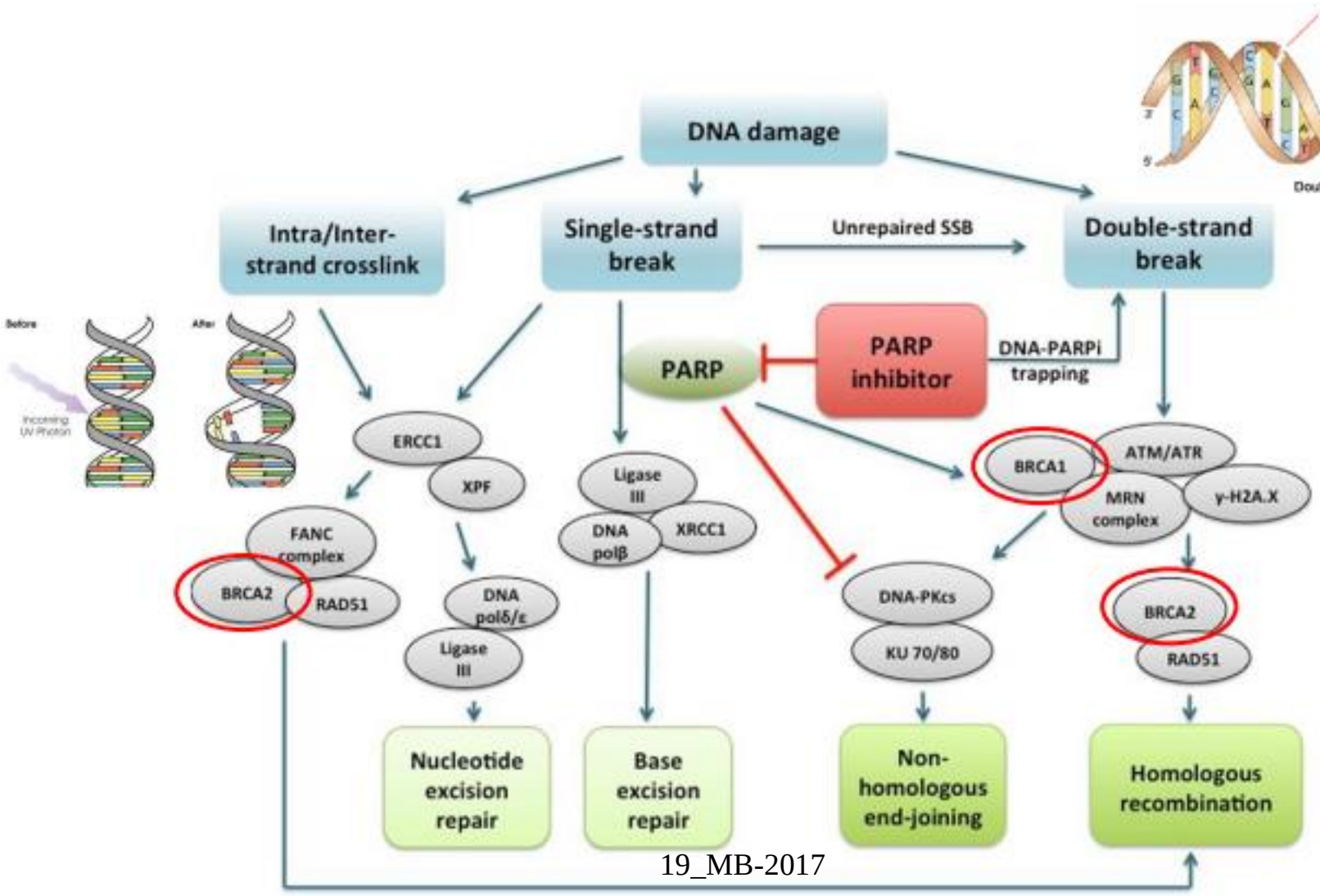
induction of INK4A → CDK inhibition (4,6) → inhibition of RB phosphorylation→ complex RB+E2F arrest cell cycle

RB may also bind to MDM2-p53 and regulate the activity of p53



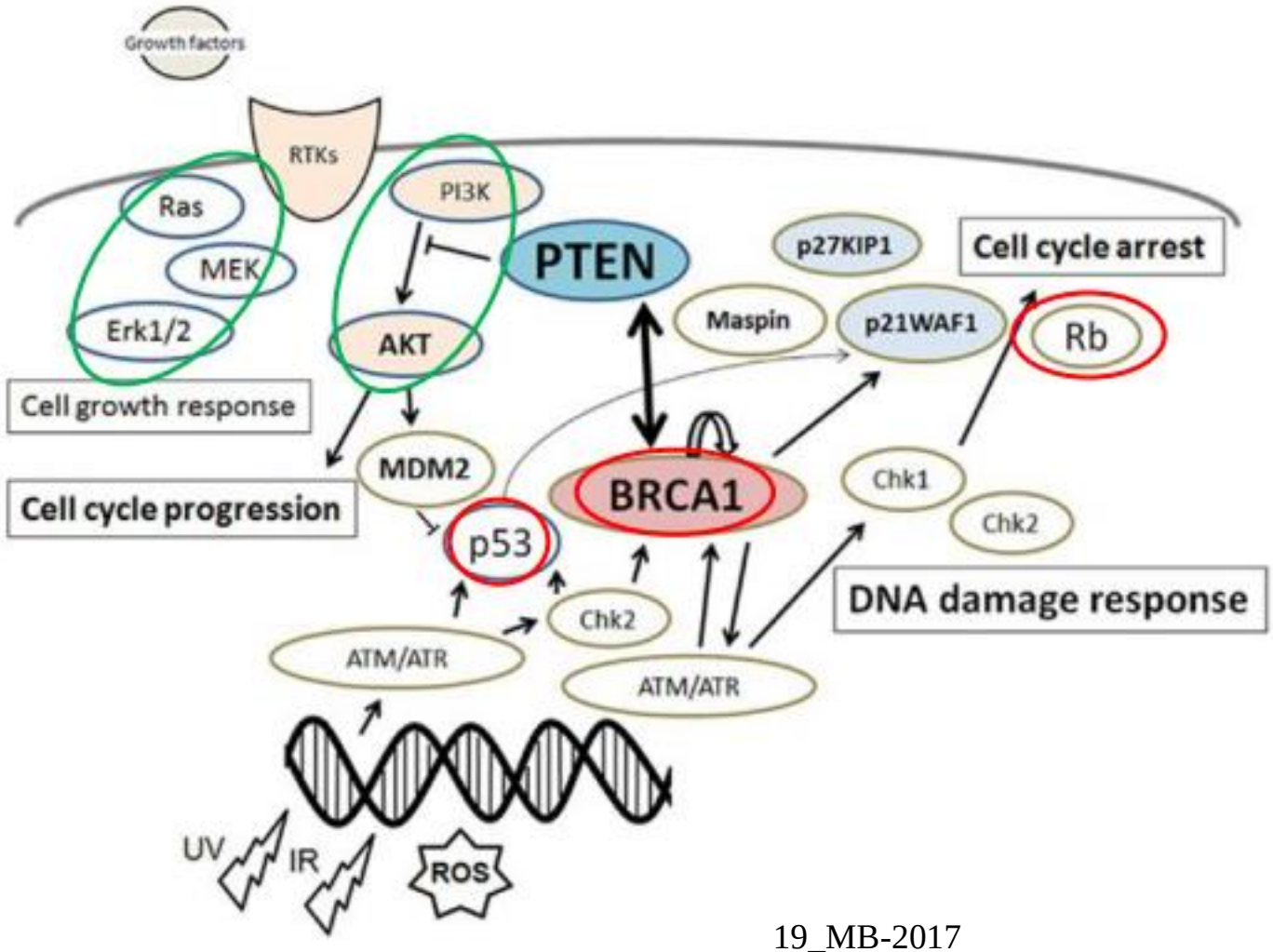
Tumor-suppressors BRCA1 a BRCA2

BReast CAncer 1 and 2 genes - It helps to repair DNA damage, in particular DSBs (double breaks)



Tumor-suppressors BRCA1 a BRCA2

- only 5-10% of breast cancer is caused by a mutation in the BRCA
- dangerous mutations in BRCA increases breast cancer and ovarian cancer (not all mutations are dangerous)



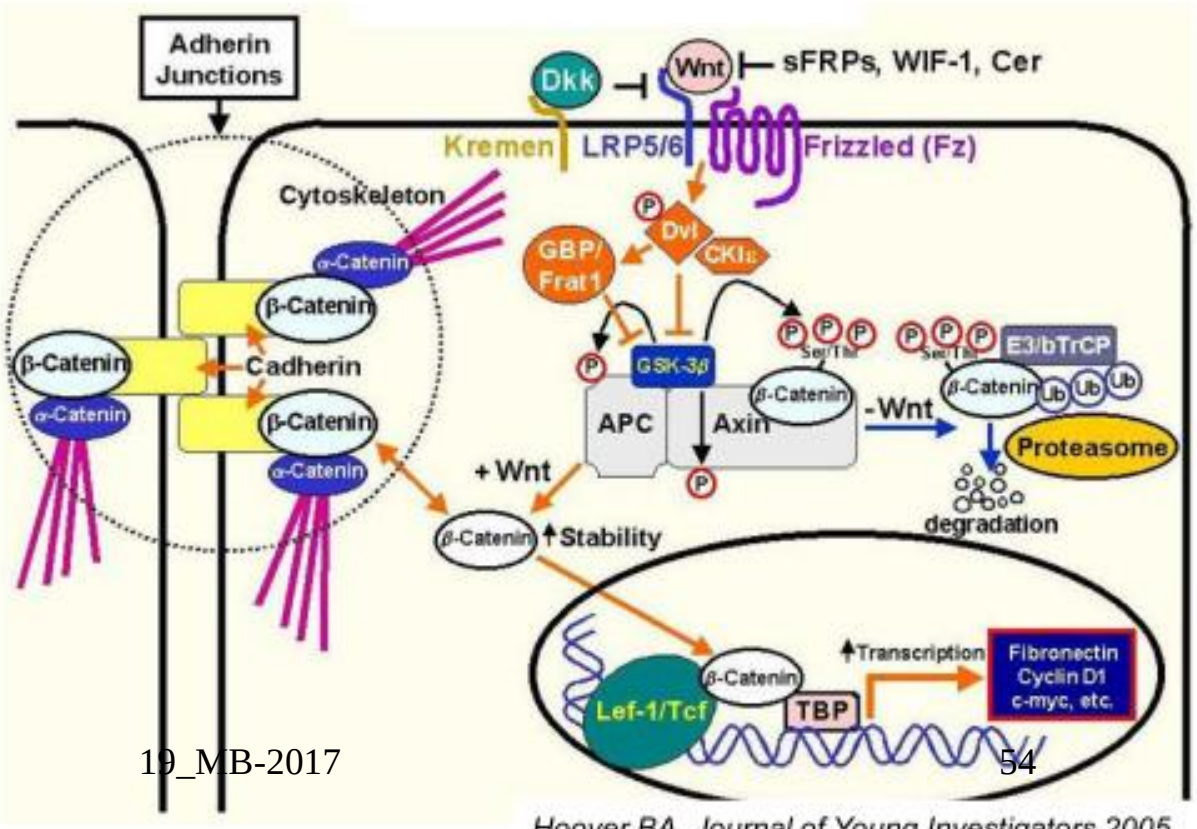
Proto-oncogenes - signal pathway Wnt

the gradual transformation of healthy cells of colon cancer

1. **The loss of the tumor suppressor *APC*** → stabilization of β-catenin → polyp formation
   a) transcription change (increased gene transcription promoting proliferation: cyclin D, c-myc...)
   b) increased cell adhesion (β-catenin links E-cadherin and a-catenin)
2. "**Gain-of-function" mutation of Ras** → benign adenoma
3. "**Loss-of-function" mutation of p53** → carcinoma

Colon cancer:
p53 mutation in 70%
APC mutation in 70%

APC is negative regulator of β-catenin



Hoover BA, Journal of Young Investigators 2005

Proto-oncogenes - receptors for growth factors (GFR)

1. Constitutive activity

- Demonstrate kinase activity in the absence of ligand

2. Overexpression

- multiplication of receptors number

EGFR - breast carcinoma, stomach, colorectum

Her2 – breast carcinoma

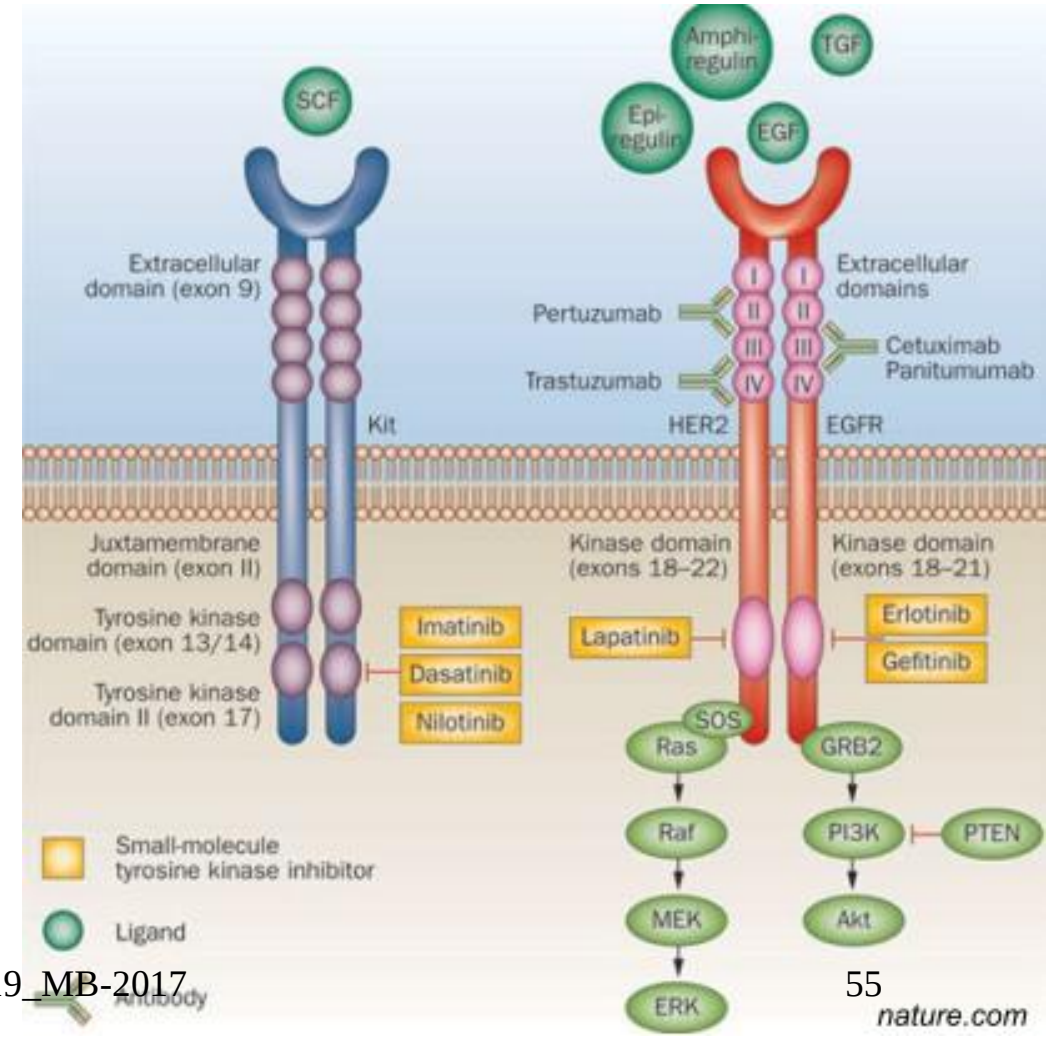
c-Kit- role in hematopoiesis

- physiologically expressed mainly on immature blood progenitors
- skin cancer

Treatment with antibodies or tyrosin-kinase inhibitors

Diagnosis receptors can predict treatment response

- SCF - stem cell factor; steel factor
- Trastuzumab = Herceptin
- Epidermal growth factor receptor (EGFR; ErbB-1; HER1)

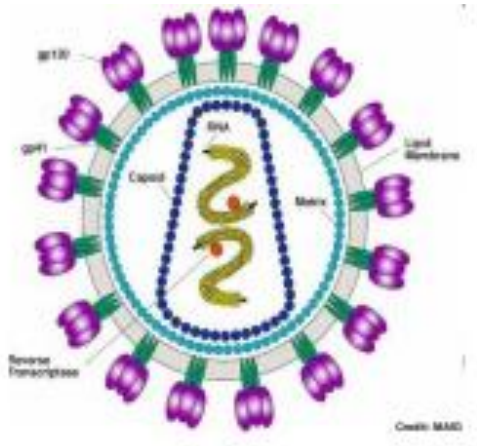


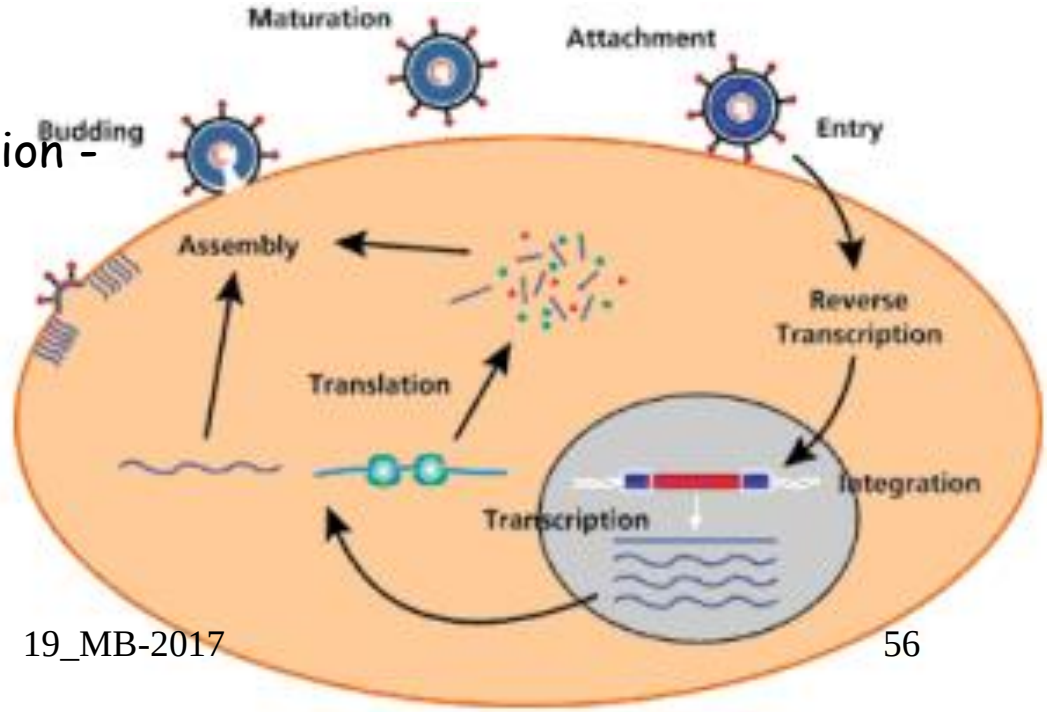
3. Biological carcinogenes: oncogenic (tumor) viruses

a) Retroviruses (RNA viruses): *single stranded RNA –*
uses reverse transcription
- Contain oncogene in their genome (acutely transforming viruses)
- Activate the protooncogene, next to which are integrated (slowly transforming)

Oncoviruses
human lymphotropic virus type I (HTLV-1)
- Adult T-leukemia (lymphoma) (ATTL), latency period of about 30 years
- high proliferative activity of infected cells, mutations are more likely

Lentiviruses
viruses HIV-1 and HIV-2
-tumors associated with their infection -
lymphomas and sarcomas





3. Biological carcinogenes: oncogenic (tumor) viruses

b) DNA tumor viruses

- not contain oncogenes, but encode proteins that interact with tumor suppressor in host cells
- Pushing the host cell into the S phase → cell cycle acceleration

Inactivation of p53 is one of the key events in the transformation of cells by DNA viruses

Hepatitis B virus (HBV)

- chronical infection - integration into the chromosome
- hepatocellular carcinoma (HCC) –20-30 years after infection

Herpes viruses - EB (Epstein Barr virus)

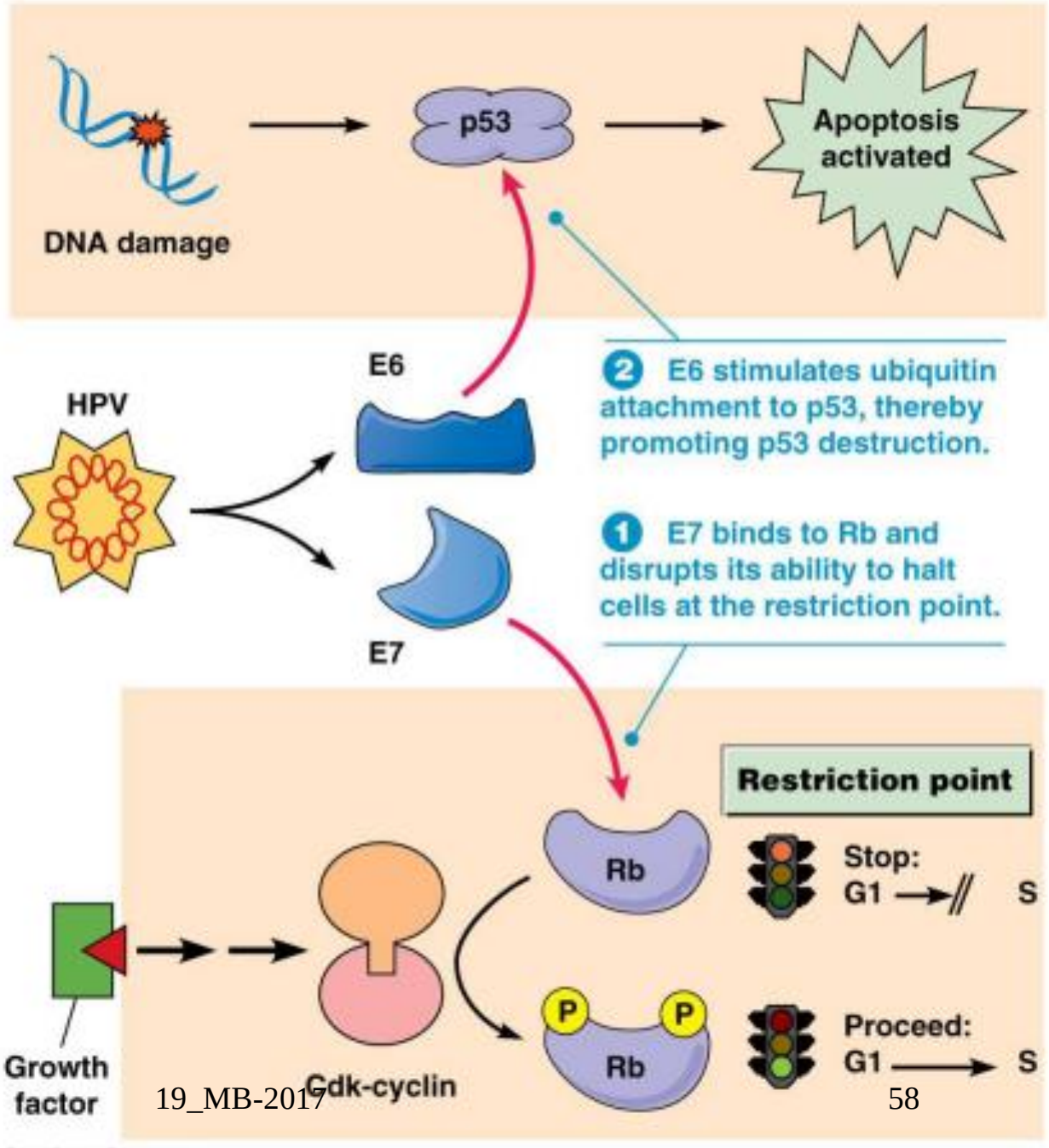
- in the cell nucleus in an episomal state (extrachromosomal)
- Lymphomas and carcinomas

Papillomaviruses (HPV xx)

- causes cervical cancer
- in benign tumors - in the form of episomes in malignant integration into the genome
- Described about 100 different types of papillomaviruses - is divided into "high-risk" and "low-risk" types according to prognosis

Human papilloma virus influences RB and p53

- virus produces proteins that inhibits tumor suppressors:
  - E6 → p53
  - E7 → RB

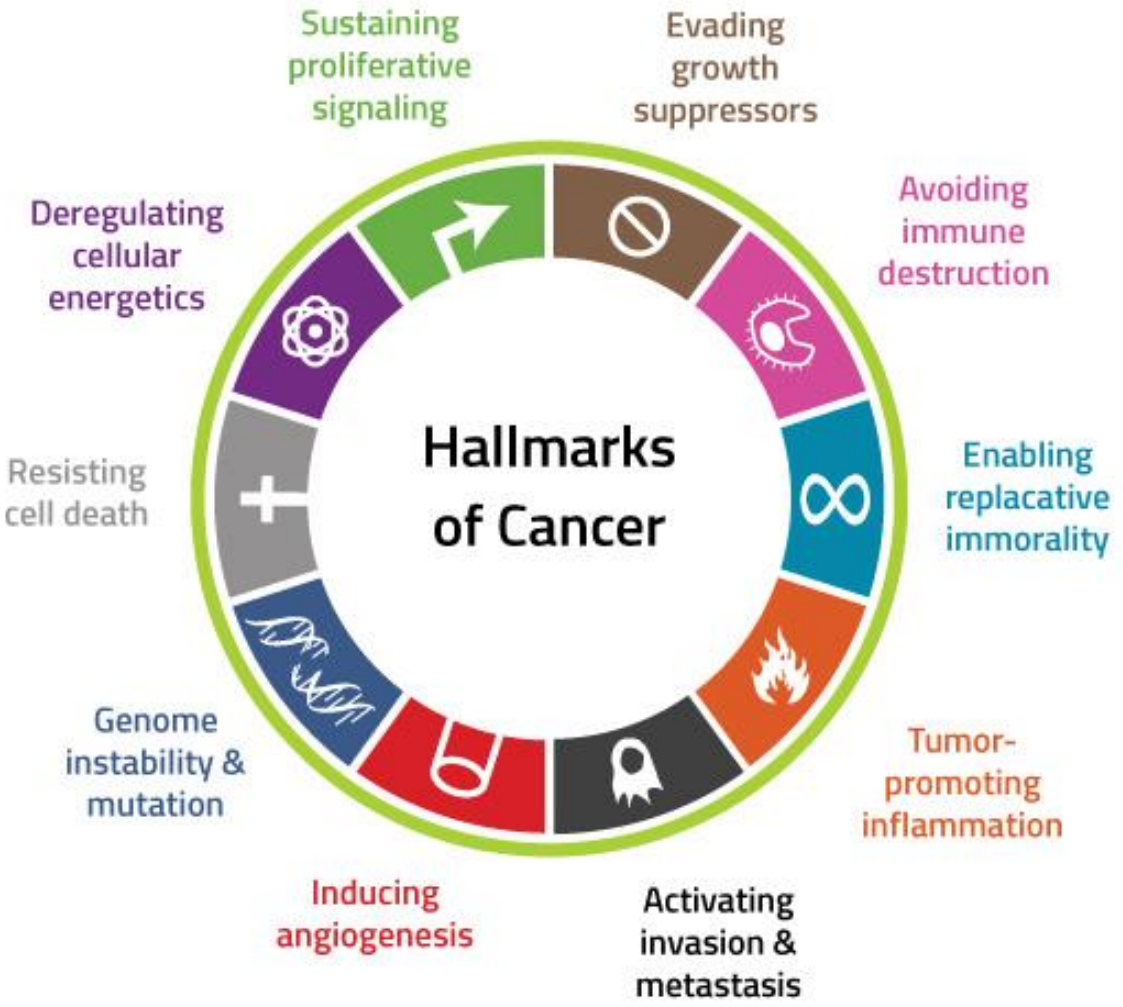


Selected mutations in tumor diseases:

Ras - 25% of all cancers
active telomerase - 90% tumors
K-Ras - 80% pancreas carcinoma
p53 - the most frequently inactivated in tumors - various tumors, Li-Fraumeni
p16 - melanoma
Rb - retinoblastoma
t(8;14) active Myc - B-cell CLL, ALL, Burkitt lymphoma
N-Myc amplif. - 30% neuroblastoma
β-catenin (WNT) - colorectal carcinoma (mutant catenin insensitive to the APC, transcription of genes cc)
TGF-β, SMAD4 - resistance against antiproliferative signals
Fas receptor - tumor
Bax - tumors of the digestive tract and leukemia
Bcl-2 translocation - follicular lymphoma
loss chr10, inactive PTEN - glioblastoma
gain chr7, dupl MET - kidney carcinoma
t(9;22) Bcr-Abl - CML, ALL (30%), rarely AML
transl. RAR - acute PML
autocrine TGF - sarcoma
autocrine PDGF - glioblastoma
overexpr EGFR/ERBB – breast carcinoma, stomach, colorectum
overepr HER2 - breast carcinoma (prediction - herceptin Ab against HER2 receptor)
PML/RARA - binds histone deacetylase, which prevent transcription of target genes ATRA

Marker: CD20, CD30, CD33, CD52, CD90

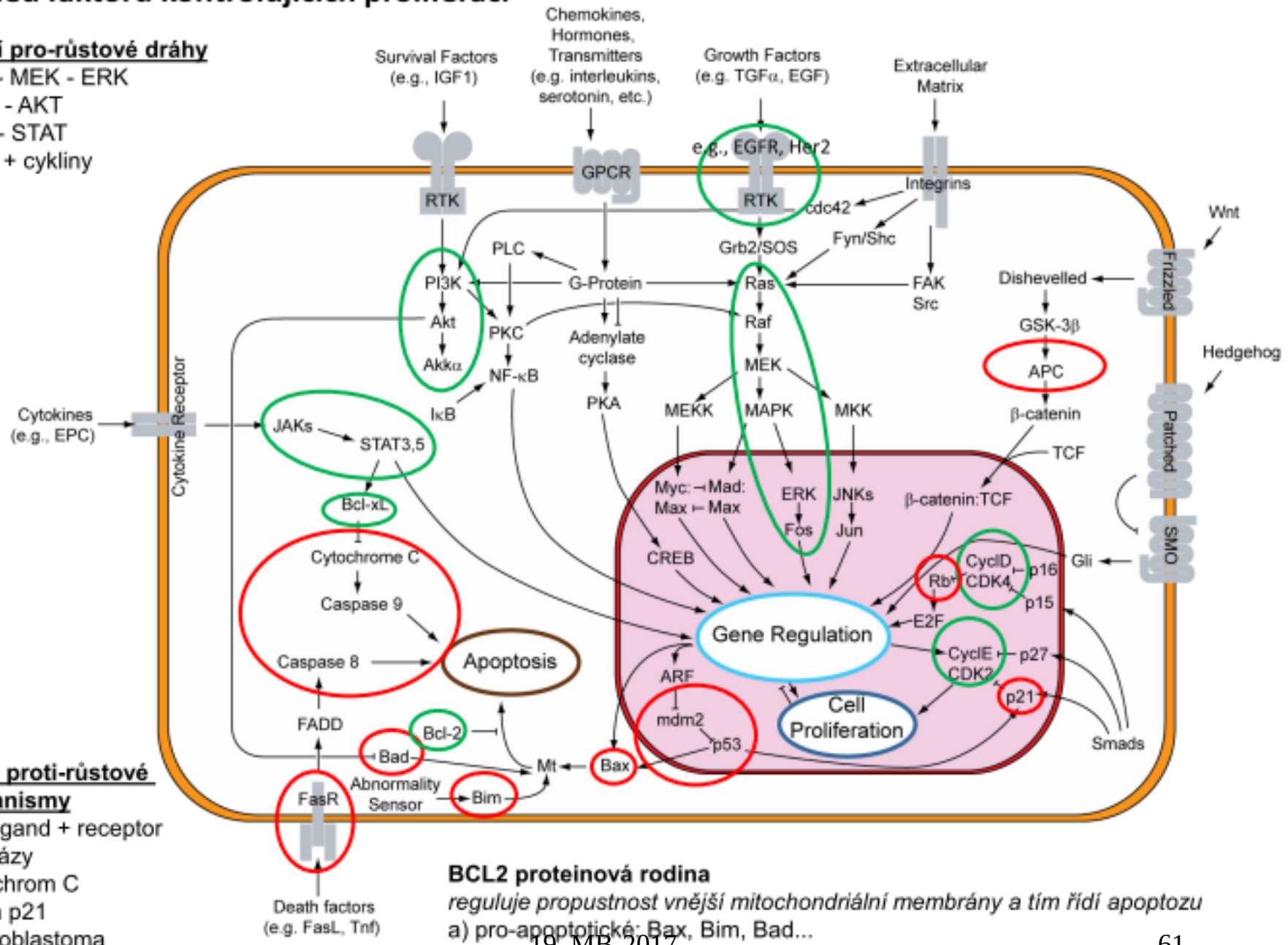
Hallmarks of cancer



⇒ multistage carcinogenesis ⇒ models

Přehled faktorů kontrolujících proliferaci

Hlavní pro-růstové dráhy
- Ras - MEK - ERK
- PI3K - AKT
- JAK - STAT
- CDK + cykliny



Hlavní proti-růstové mechanismy
- Fas ligand + receptor
- Kaspázy
- Cytochrom C
- p53 a p21
- Retinoblastoma
- APC

BCL2 proteinová rodina

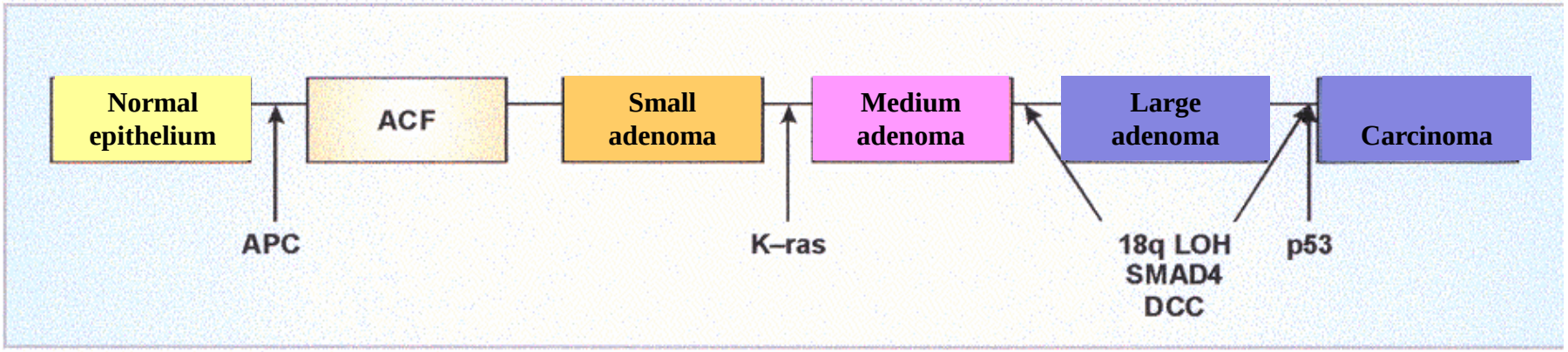
reguluje propustnost vnější mitochondriální membrány a tím řídí apoptozu

a) pro-apoptotické: Bax, Bim, Bad...

b) anti-apoptotické: Bcl-2, Bcl-xL...

Models of multistep carcinogenesis

- The development of colorectal cancer

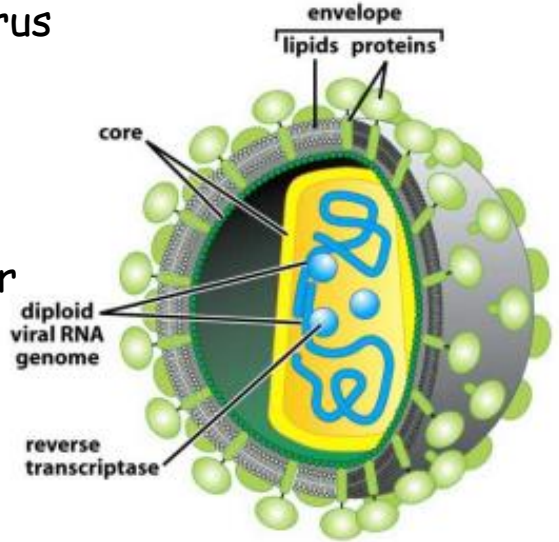


APC is classified as a tumor suppressor gene. The APC protein is a negative regulator that controls beta-catenin concentrations and interacts with E-cadherin, which are involved in cell adhesion. Mutations in the *APC* gene may result in colorectal cancer.

Transforming of DNA by oncogenic viruses

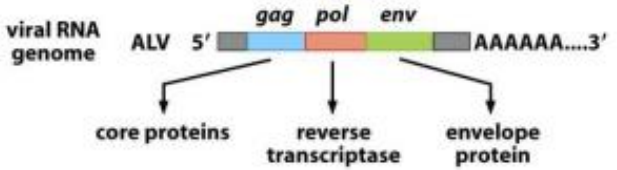
Oncogenic (tumor) viruses

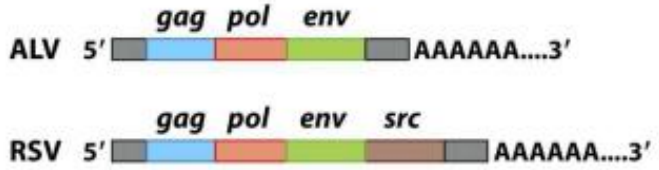
Rous sarcoma virus is a retrovirus derived from the avian leukosis virus ALV
- contains ***src gene***, applies to development of cancer
- does not have any function for viruses

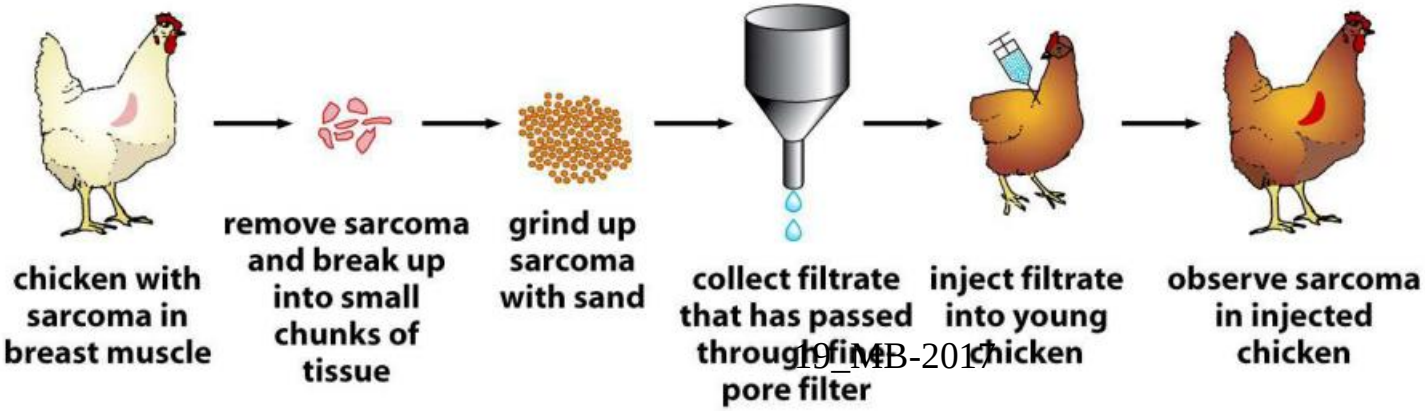


Induction of sarkomas in chickens

Peyton Rous was awarded the Nobel Prize in 1966
evidence of involvement of viruses in some types of cancers







Oncogenic (tumor) viruses

- **Retroviruses** (RNA viruses): contain in their genome oncogene (acutely transforming viruses) or activate the protooncogenes, next to whicg is intergrated (slowly transforming)

- **DNA tumor viruses** use another transformation strategy: do not contain oncogenes, but encode proteins, that can interact with the tumor suppressors (RB, p53, p300/CBP) of the host cell and the host cell thus pushed into S-phase:

  SV40: large T antigen with different domains interacts with p53, Rb, p300/CBP

  Adenoviruses: E1A interacts with RB and p300/CBP; E1B interacts with p53

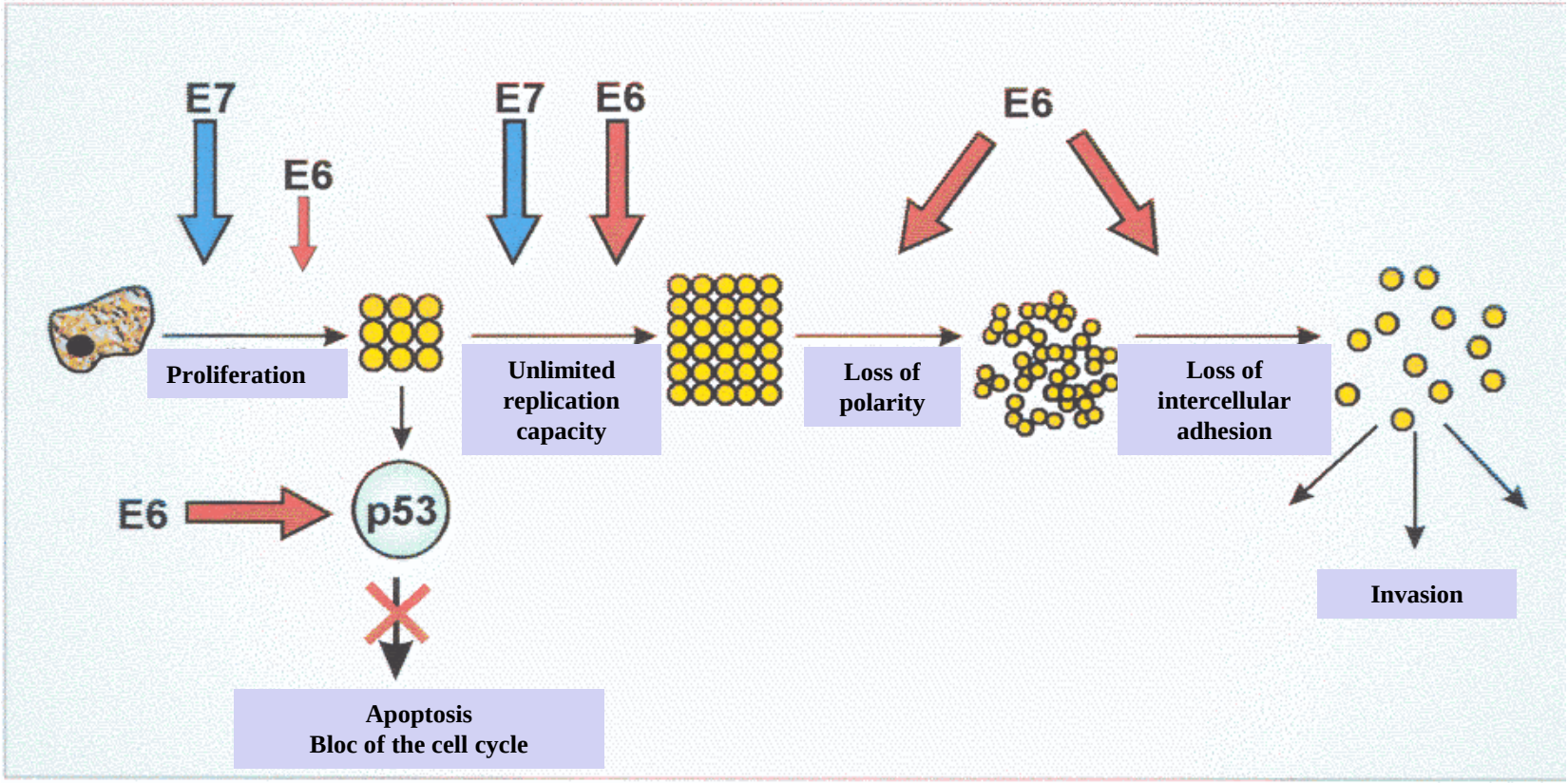
  papilomaviruses HPV-16, HPV-18: E6 interacts with p53, p300/CBP; E7 interacts with RB

Some methods of inactivation of p53 by viral oncoproteins

Inactivation of p53 is one of the key events in the transformation of cells DNA viruses.

- **LT** (SV40) – binds to the DNA binding domain of p53 and prevents binding of p53 to DNA
- **E1B** (adenoviruses) – binds to the transactivation domain of p53 and prevents transactivation of target genes
- **E4orf6** (adenoviruses) – causes degraduation of p53
- HBV **X** (hepatitis B virus) – retains p53 in cytoplasm
- **E6** (papillomavirus) – induces degradation of p53 using ubiquitin ligase E6AP; E6 also directly inhibits the transactivation ability of p53

The proportion of proteins E6 and E7 of papillomaviruses to transformation of the cells



Viral oncoproteins: stimulate proliferation, inhibit apoptosis, increase the replicative potential, change the morphology of the cells, induce the malignant phenotype

The proportion of E6 protein of papillomaviruses to cell transformation

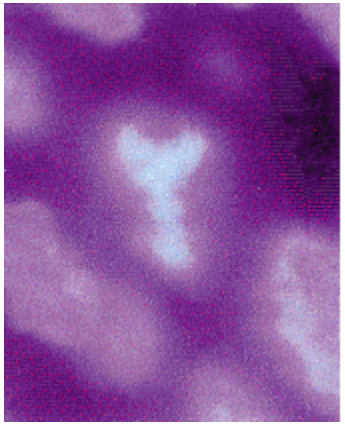
E6 – inactivate **p53** (G_1 block, dependent apptosis, genetic stability)

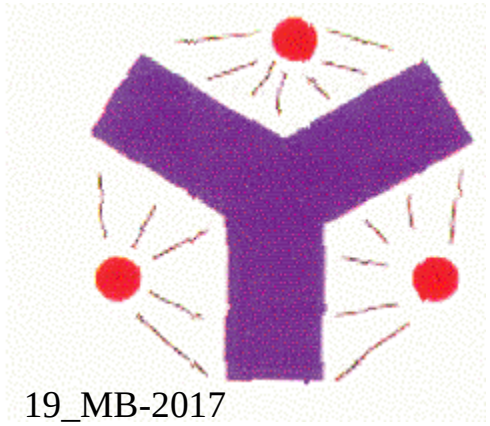
- interact with **p300/CBP** (disruption of homeostasis)
- activate the expression of **hTERT** (telomerase activation)
- inactivate **p16^{ink}** (disruption of maintain rest stage)
- interact with **Bak** (inhibition of apoptosis)
- interact with **E6BP/ERC-55** (inhibition of terminal differentiation)
- induce degradation of **hDlg** (and other interactions with proteins containing PDZ binding structure) (change in morphology, obtaining of invasive nature)

The proportion of E7 protein of papillomaviruses to cell transformation

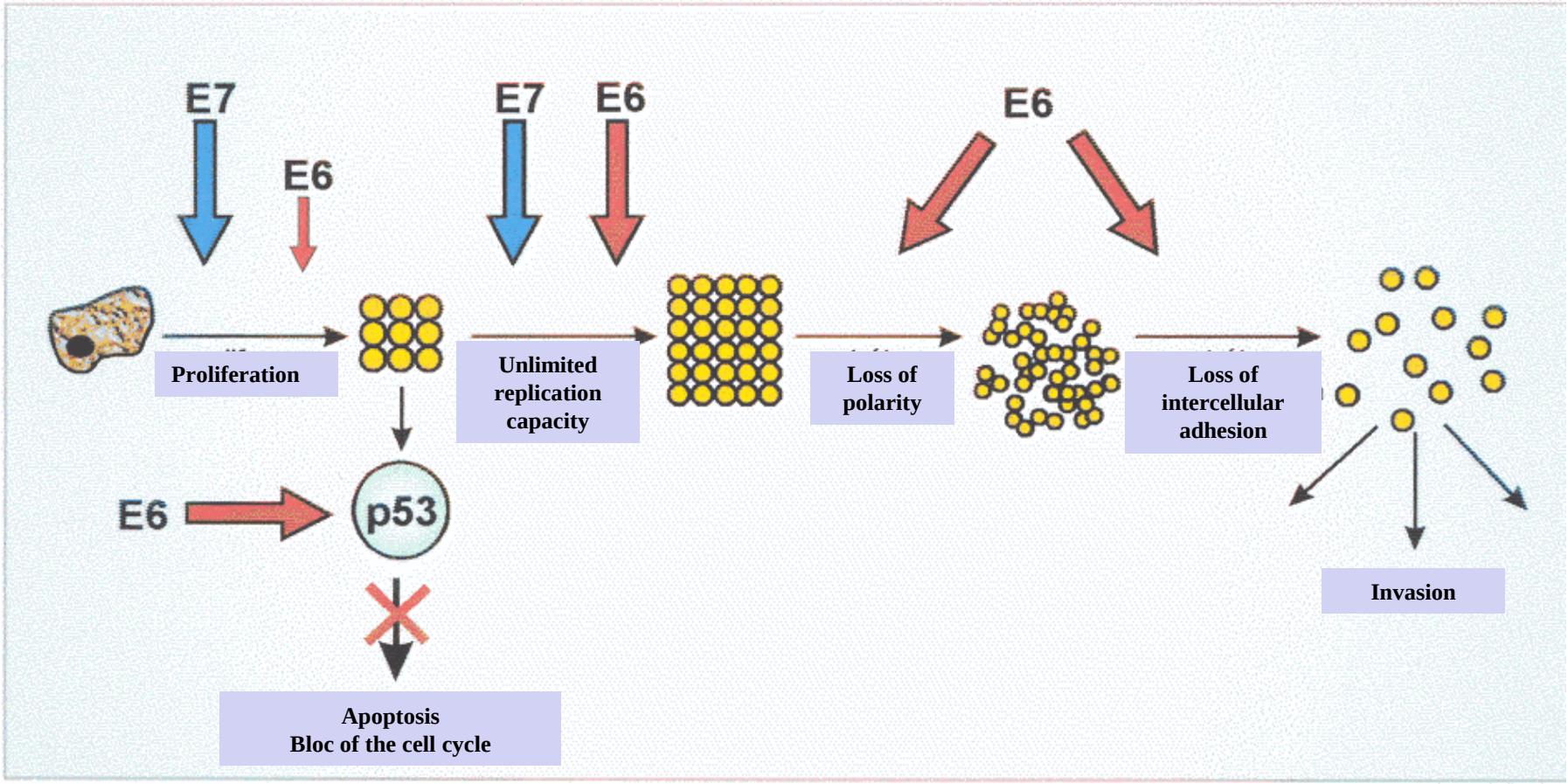
E7 – bind to **RB** (release TF E2F)

- inactivation of **p21^{Cip}** and **p27^{Kip}** (disconnection: differentiation - proliferation)
- cancel the inhibitory effect of **TGF-β** on cell growth
- lead to the formation of multiple centrosome





The proportion of proteins E6 and E7 of papillomaviruses to transformation of the cells



The clinical significance of papillomaviruses

- Described more than 100 different types of papillomaviruses.
- They are divided into „high-risk" and „low-risk" types according to the prognosis which they are associated with. „Low-risk" viruses induce the formation of bening tumors, „high-risk" viruses are associated with malignant progression.
- About 30 types of HPV preferentially infect the anogenital area, infection with „high-risk" viruses are associated with almost all cervical cancer.
- About 20 % of all cancers of the oral cavity, which are not associated with a history of smoking and alcohol consumption is associated with HPV infection.

Oncogenic viruses and human tumors

RNA viruses:

- human lymphotropic virus type I (HTLV-1) - T-leukemia (lymphoma) adults (ATTL)

DNA viruses:

- Epstein-Barr virus (EBV) - Burkitt lymphoma (BL), Hodgkin´s lymphoma (HD), lymphomas, nasopharyngeal carcinomas (NPC)
- Hepatitis B virus (HBV) – hepatocellular carcinoma (HCC)
- Human papillomaviruses (HPV 16, 18,..) – anogenital tumors, tumors of oral cavity, verruca
- Human herpes virus type 8 (HHV8) – Kaposi´s sarcoma (KS)

Cancer treatment

1. Conventional chemotherapy

Target is proliferating cells, non-specific, always the same % of proliferating cells

Target:

- damage tumor DNA
- stop of the proliferation
- apoptosis induction of p53 or massive damage (p53 independent)

tumor more susceptible to general pro-apoptotic stimulus (genotoxic substanceslátky, mitotic poisons, antimetabolites)

Disadvantages: huge side effects (removal of healthy tissues – can lead to the formation of secondary cancers)

2. Target therapy

Selective for tumor cells (specific for particular cell process), low toxicity toward healthy cells

Disadvantages:

- is not 100% specific for molecules
- target molecule is larger and also fill up the physiological function (partial exception for fusion gene)
- requires the identification of the molecular basis - individualized medicine (tailored medicine)

In oncology, chemotherapy = cytostatic drug with a cytotoxic effect (synthetic or plant/fungi)

cytostatic: lsubstance moderating growth and cell reproduction epecially the tumor tissue

Mechanism of action of conventional cytostatics

1. Alkylating agents

Attacking the negative charge of the DNA and cause breaks in DNA - prevent replication

- can induce formation of secondary leukemia
- ***Chlorambucil (lymphoma, CLL)***
- ***Cyclophosphamide – the most common***
- ***Busulfan – pre-transplantation myeloablation, CML***
- ***Cisplatina - DNA damage, intercalation, active intracellularly, nephrotoxicity***

2. Antimetabolites

- interfere with synthesis of nucleic acids
- Targeting mainly on proliferating cells

- Methotrexate - block of purine synthesis with inhibition of dihydrofolatereductase (osteosarkoma)

- Fludarabine - block purines – substitution of adenosine – DNA fragmentation, (AML, CLL)

- 5-fluoruracil – integration into RNA

- Hydroxyurea - block of ribonucleotide reductase, inhibition of pyrimidine, CML

Mechanism of action of conventional cytostatics

3. Antitumor antibiotics*

- *Doxorubicin*
- *intercalates between DNA strands*
- *induce formation of free radicals*
- *blocking topoisomerase II*

*antitumor antibiotics in this context do not indicate antibacterial substances
topoisomerase: unwinding the DNA during replication

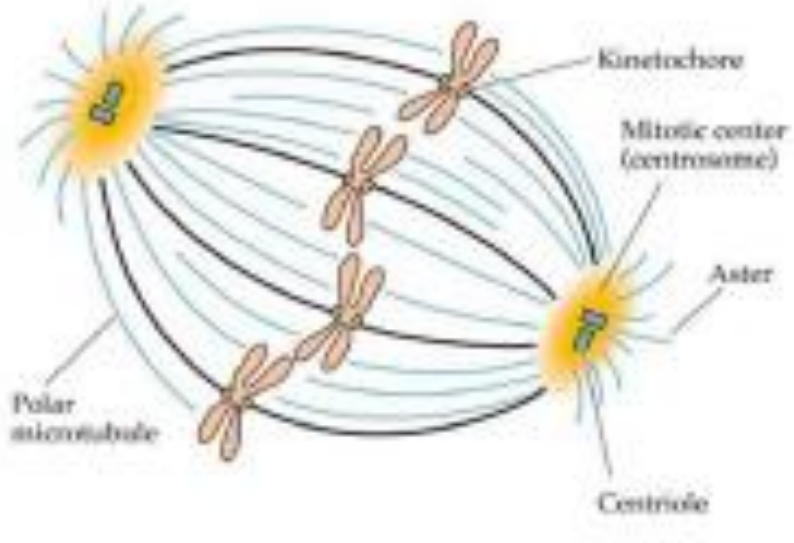
4. Herbal alkaloids

Block the formation of the mitotic spindle by binding to microtubules

- Vinca alkaloids (from Vinca rosea) – depolymerization of microtubules – desintegration of the spindle

Camphothecin- block of topoisomerase I

- Taxanes - (yew needles),
- Paclitaxel - blokc of depolymerization microtubules (breast carcinoma or ovarial)



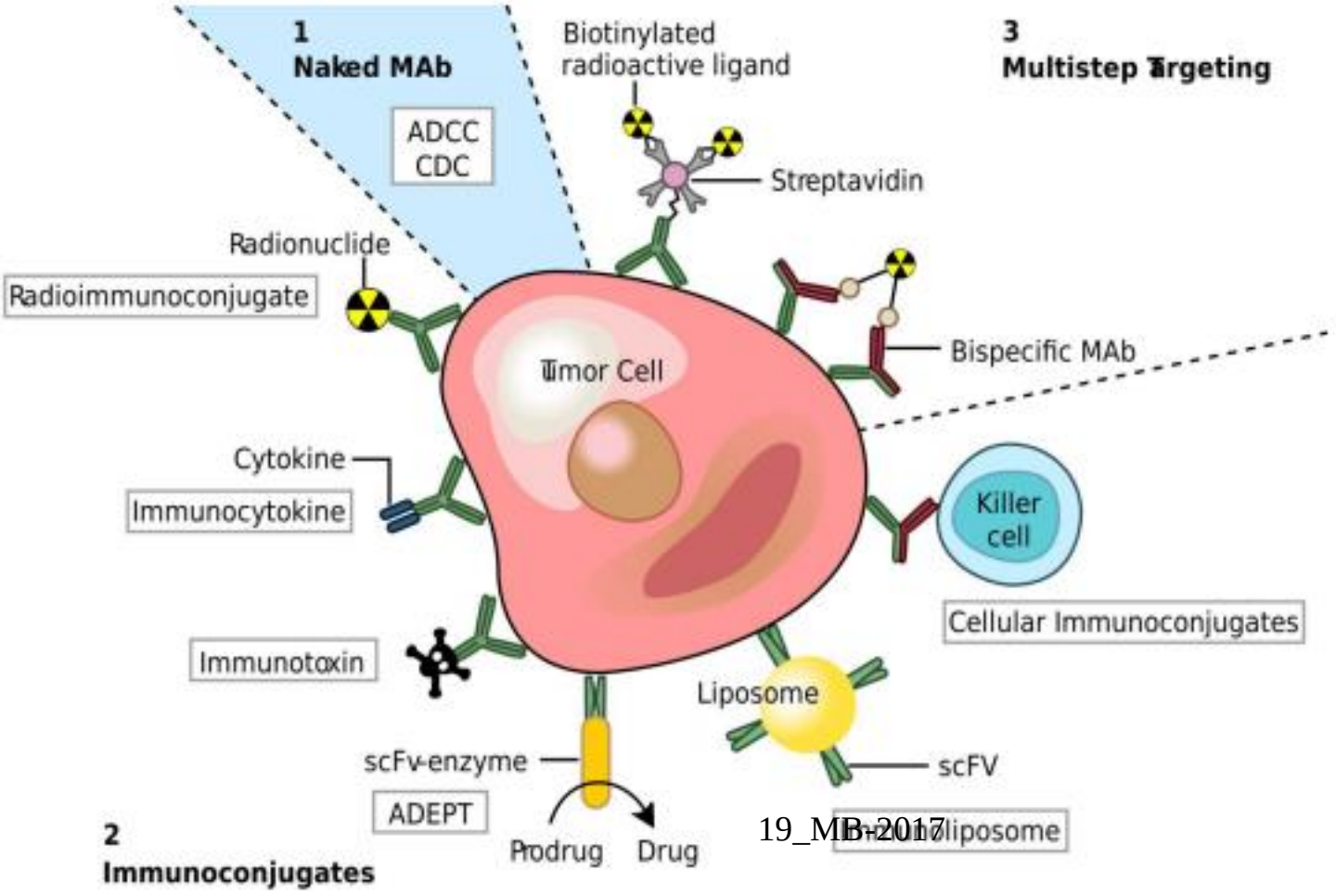
Targeted therapy - examples

Monoclonal antibodies

- Specific antibody (Ab) agains selected antigens on the cell surface

a) Naked: after binding can block the receptor, or activate immune cells

b) Conjugated: with a toxin, radioisotope, cytokine

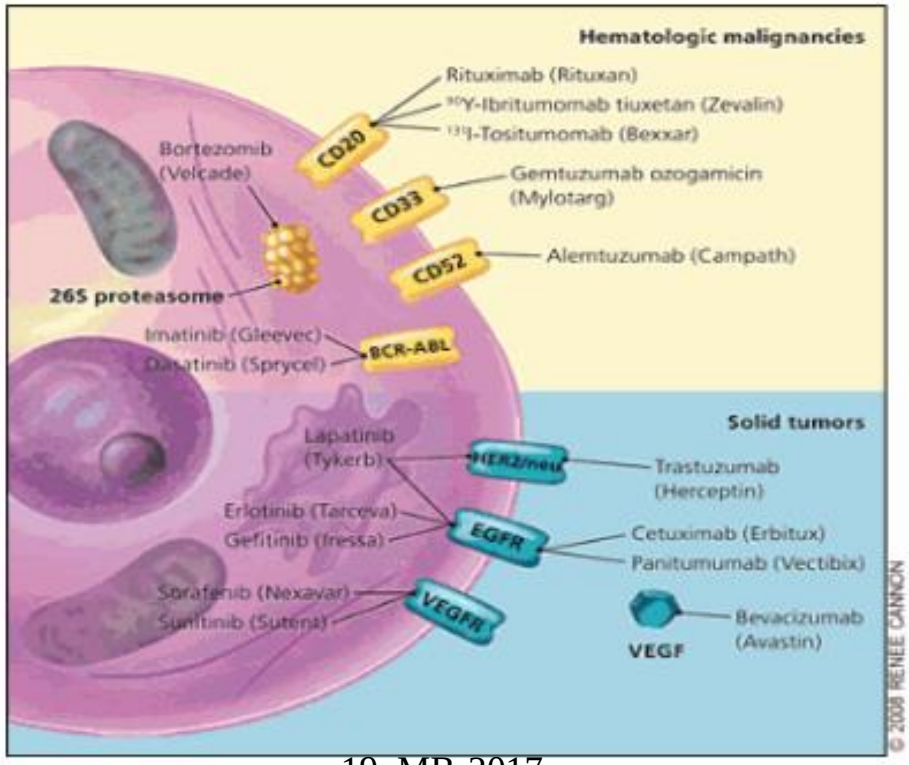


MAb - monoclonal antibody
ADCC - antibody-dependent cell-mediated cytotoxicity
CDC - complement-dependent cytotoxicity

Targeted therapy - examples

Monoclonal antibodies

- **Herceptin** - anti-HER-2 (breast cancer 30% amplification of the gene for the receptor HER-2)
- **Rituximab** - anti-CD20, malignant B-cell lymphomas, B-lymphatic CLL, follicular lymphoma
- **Gemtuzimab** - anti-CD33 (on larger leukemia cells), AML, conjugation with ATB colcheamicine
- **Cetuximab** - anti-EGFR, conjugation with toxin, internalization into cells, colorectal carcinoma



Targeted therapy - examplexs

Tyrosine kinase inhibitors (TKIs)

- occupying the ATP binding site
- high structural variability allows for the specific binding
- Do not lead to complete cure :(
  - Gefitinib – lung and kidney carcinoma, solid tumors
  - Erlotinib – ovary carcinoma
  - Imatinib, Dasatinib, Nilotinib – cure of CML

Farnezyltransferase inhibitors (FTIs)

Inhibition of Ras function (permanently switched on in tumors)

- Lonafarnib

Targeted therapy of chronic Myelogenous Leukemia (CML)

Over-expression of tyrosine kinase Bcr-Abl in CML caused:

• cytokine-independent growth and survival of the cells demonstrated oncogenic adiction

• protects cells from apoptosis in response to growth factors, or DNA damage * ,

Tipifarnib, BMS-214662

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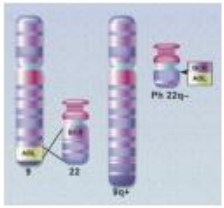
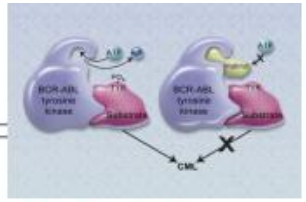
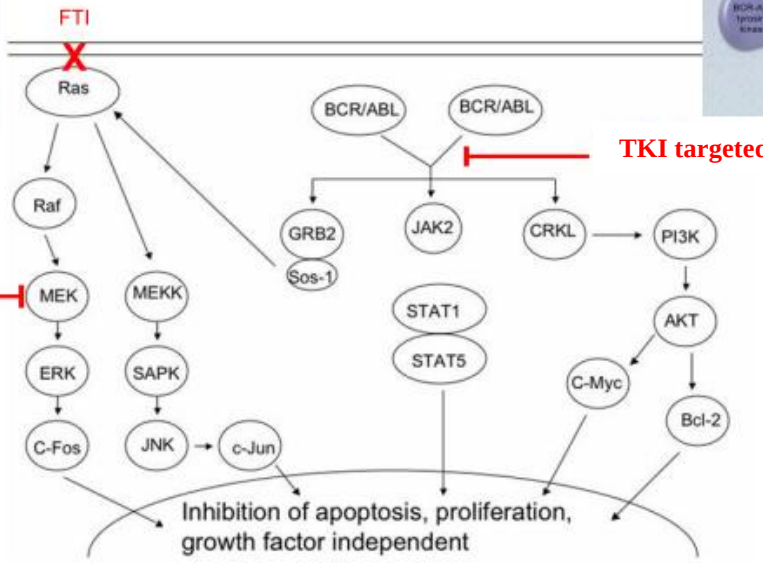
Tipifarnib, BMS-214662







Evans et al. Cancer Res 1993; Druker, Blood 112, 2008, p 4810