Academic lectures for general medicine students – 3rd Year
2004 - 2015

GENERAL PATHOPHYSIOLOGY

NEOPLASMS 2
Molecular pathology

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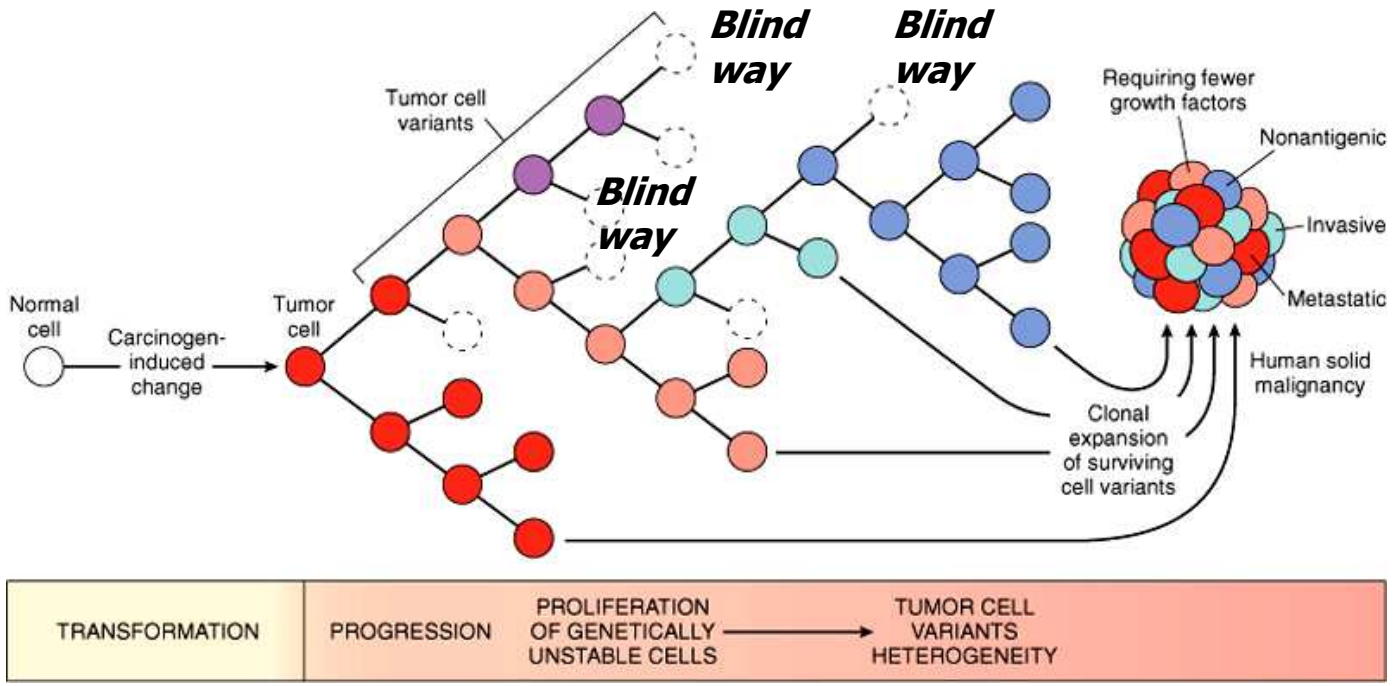
Tumorigenesis

- Oncogenes
- Tumor supressor genes
- Apoptotic genes
- Stability genes (DNA repair genes)

Principles of tumor formation (cont)

- **Carcinogenesis is a multistep process** at both the phenotypic and the genetic levels. A malignant neoplasm has several phenotypic attributes, **excessive growth, local invasiveness, make stroma and vessels, ability to move, ability to get in & out of vessels to form distant metastases.** These features are acquired **not continuosly** and overnight but by **in stepwise way**; tumor can wait for suitable mutation-adaptation years.
- Even been risen from 1 mother cell **(initial trasformation)** , clones try to evolve in Darwinian „trial & error“ way and **accumulate new advantagous mutations**. Many tumor cells die due to **disadvantageous /incapabling mutations** and/or in competition for nutrition, resources (population reduction). From once **homogenous pack** selection makes up small undernumbered mutlicor population where every cell has „speciality“ = **tumour heterogenity.**
- to grow independently, parasitically, increasing malignancy for a body - referred to as **tumor progression.**

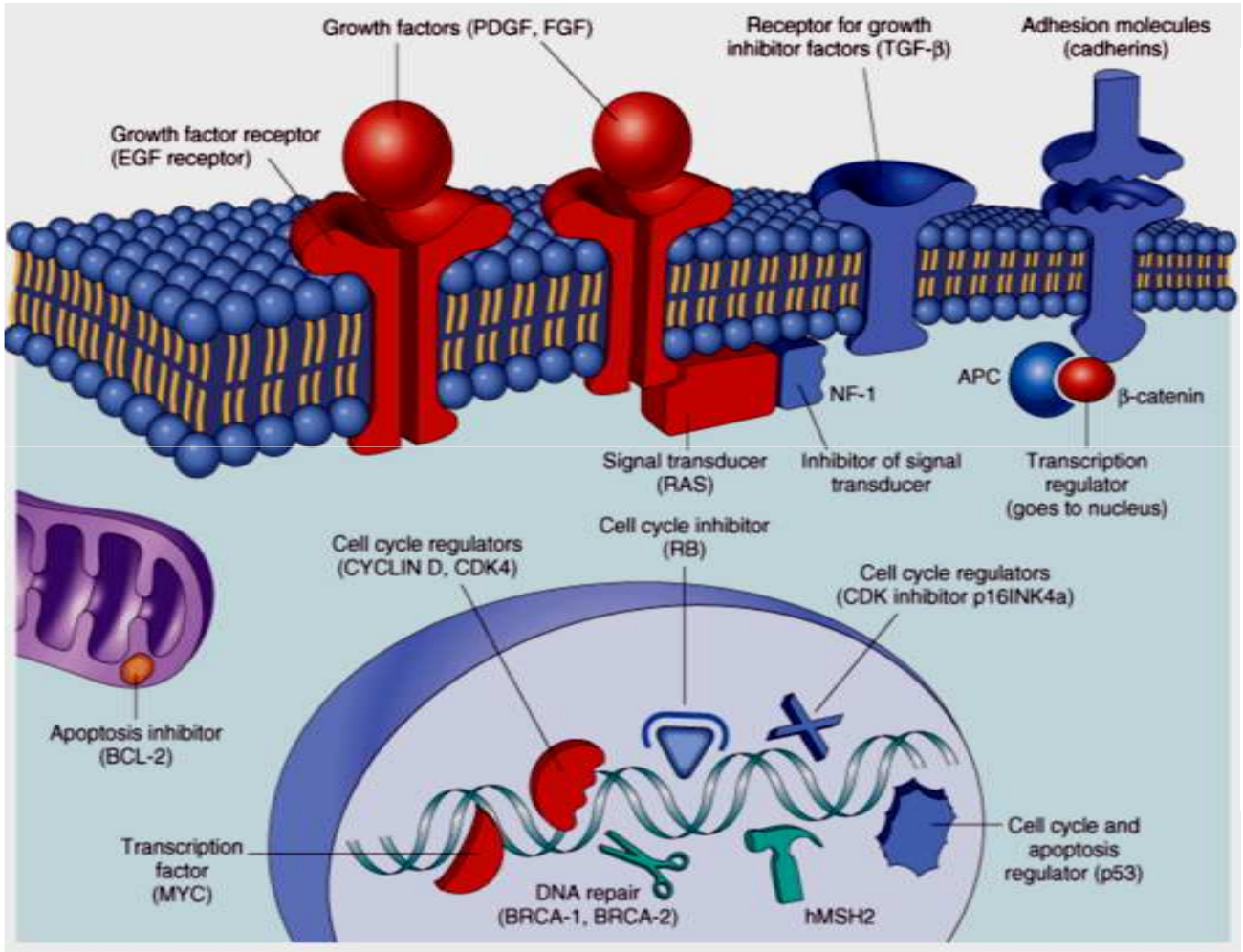


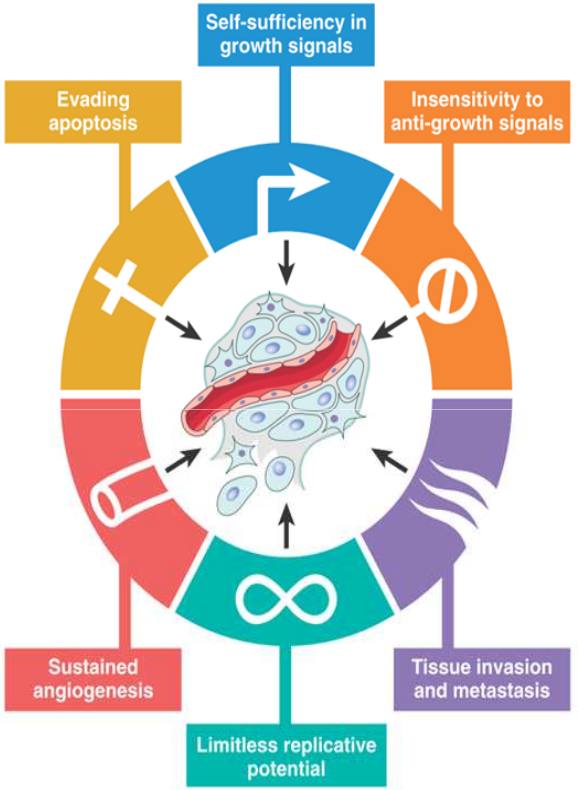
Principles of tumor formation

- Tumors arise due to nonlethal **genetic damage** (external factors - chemicals, radiation, viruses, or inherited in the germ line), i.e. **genetic mutation** (single *gene mutations* (mostly missence), and *chromsomal abberations*).
- Tumor is formed up by the **clonal expansion** of a **„single precursor cell“** that has incurred the genetic damage (i.e., tumors are monoclonal).
- **Tumorigenic process**. Acquired tumours are formed due to unrepaired genetic mutations in somatic cells; carcinogenic alterations start due to defective DNA- repair mechanisms. Such cells shoul not be allowed to multiply and mostly die. Control over this checkout process is done by tumor supressor genes. If damage is inreparable apoptosis id executed. Certain stress or mutagenic stimuli trigger apopostis via mitochondrial mechanism directly. Oncogenes and likely other genes confer the tumor mass high proliferative advantage. Process involves **alterations in 3-4 classes of genes:**
  - **Oncogenes** are considered **dominant**; one mutant allele is enough to promote neoplasia despite the presence of a normal counterpart
  - **Tumor suppressor genes (recessive** oncogenes) both normal alleles must be damaged for transformation to occur, loss of function of a recessive gene caused by damage of a single allele is called haploinsufficiency.
  - **Genes that regulate apoptosis** - may be **dominant,** as are protooncogenes, or they may behave as tumor suppressor genes
  - **Genes involved in DNA repair** behave in **recessive** way

- **Local invasivenes and metastasis process** is second chapter after tumorigenessis. In addition to enormous competition for resources, oxygen, acidosis, small living space, hostile tumor cells clones should show certain cooperativity and specilisation for survival. Because of large tumor mass progression is a product of tumor cells + tumor stroma + vascular supply. Cells must pass through connective tissue barriers.

Genes associated with cancer





Subcellular localization and functions of major classes of cancer-associated genes. The **protooncogenes (red)**, **cancer suppressor genes (blue)**, **DNA repair genes (green)**, and **genes that regulate apoptosis (purple).**

Another classification of cancer genes

Kinzler Vogelstein (1997): cancer susceptibility genes into 2 classes: **"caretakers"** and **"gatekeepers".**
Michor, Iwasa, Nowak (2004) :another group **"landscaper" genes.**

1. Caretaker genes encode products that **stabilize the genome**; mutations herein lead to **genomic instability**. Tumor cells arise from 2 distinct classes of genomic instability: **mutational instability** arising from changes in the nucleotide sequence of DNA and **chromosomal instability** arising from improper rearrangement of chromosomes. DNA maintenance operations encoded by caretaker genes include **nucleotide excision repair, base excision repair, non-homologous end joining recombination pathways, mismatch repair pathways, and telomere metabolism.**

2. Gatekeeper genes encode gene products that act to prevent growth of potential cancer cells and **prevent accumulation of mutations** that directly lead to increased cellular proliferation.

3. Landscaper genes = encode products that, when mutated, **contribute to the neoplastic growth of cells by fostering a stromal environment** conducive to unregulated cell proliferation.

ONCOGENES

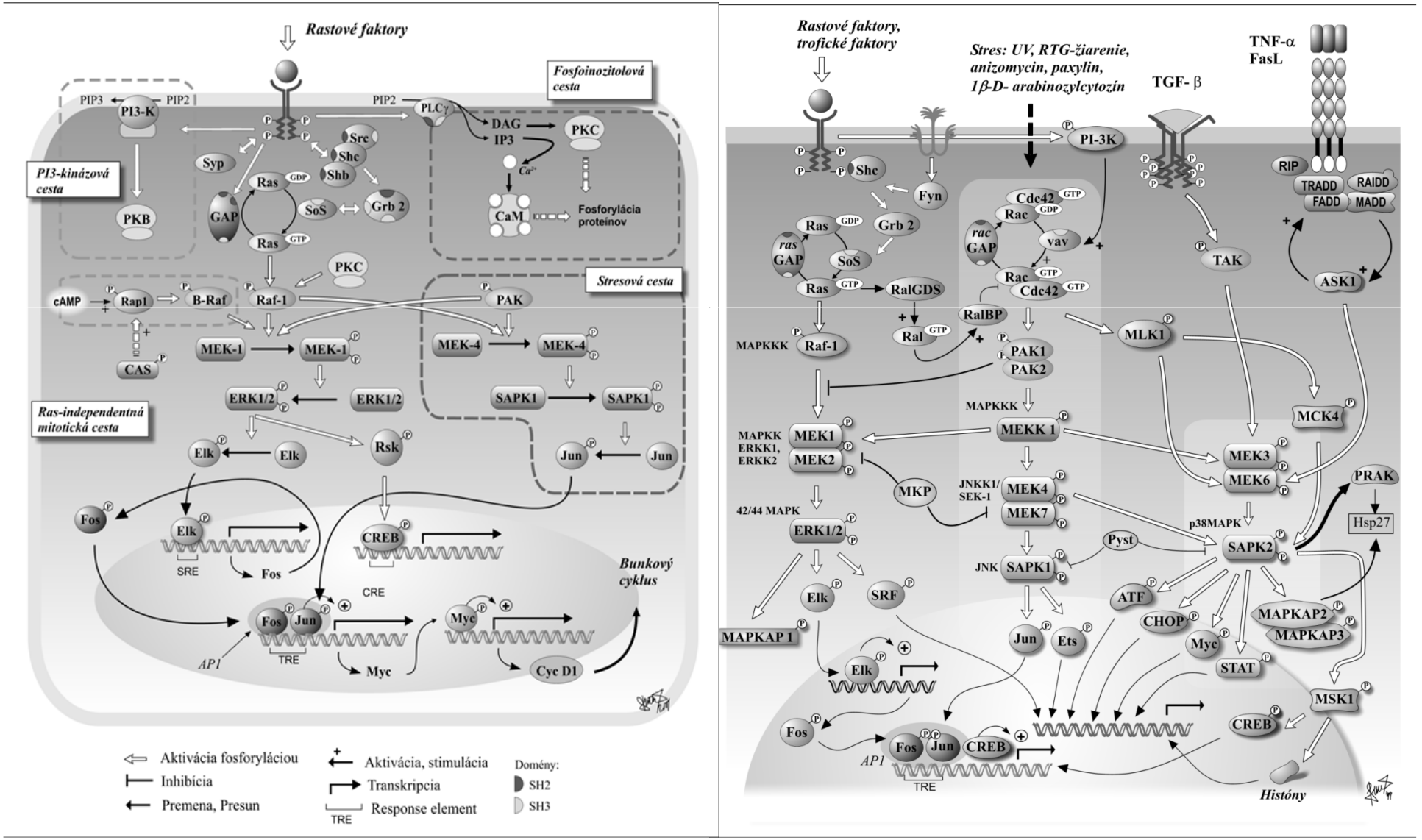
1. Oncogenes

- **Oncogenes** and **oncoproteins** = are special tumor – producing (proliferative) mutational variants of otherwise normally present cellular **protoncogenes** and their products - **protoncoproteins.**
- Oncogene mutations are considered **dominant** because one mutant allele is enough to promote neoplasia despite the presence of a normal counterpart; they are considered **gain in function mut.**
- Loss in function mutations of protoncoges **are more common than oncogenic** and lead to opposite effect – **cell and tissue atrophy.** During embryogenesis this may lead to **abortion**.
- Proto-oncoproteins normally serve in **various intracellular signalling pathways**, which have important **mitotic or growth promoting effects** e.g. classical Ras dependent mitotic pathway or Ras-independent mitotic pathway, Wnt-catenin pathway, PI-3K dependent pathway, IP3/DG dependent – pathway.
- Proto-oncoproteins serve as **growth factors /mitogens, membrane receptors for growth factors, submembrane adaptor proteins** and switchers as monomeric G-proteins, **intracell. mitotic kinases,** other parts of signaling cascades, **transcription factors**, transcription products important as **cell cycle regulators** (cyclin-dependent kinases, cyclins). Formally, several cancer- related gene products which show dominant pattern of expression were transferred into oncogenic group.
- In the multistep process of tumor evolution oncogenes are **not mutations to start tumorigenesis**, yet not those to start malignity nor those to start metastasis; **they confer cell populations enormous growth and clonal mutiplication.**
- **Oncoproteins** work in different signalling pathways.

Classical a stress- induced mitogenic pathway

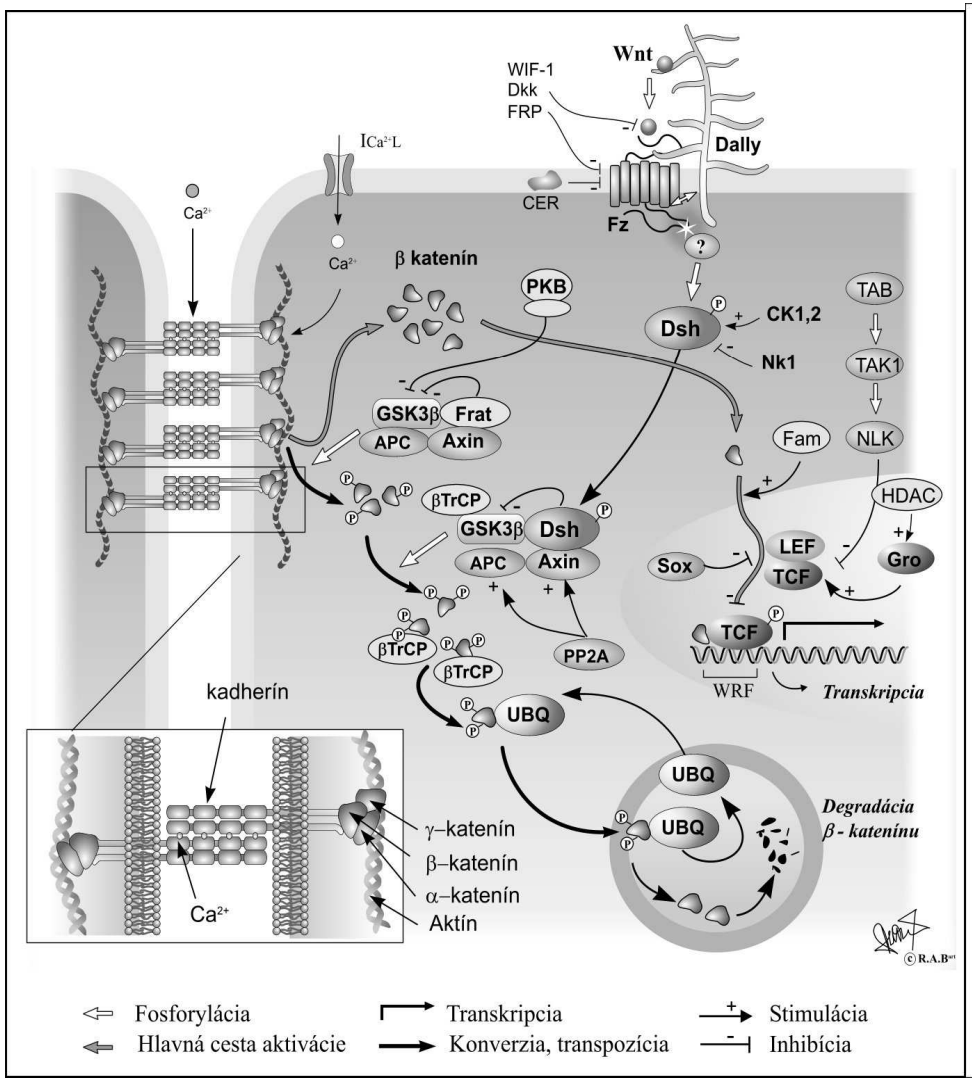
Ras – dependent pathway

Ras – independent stress pathway

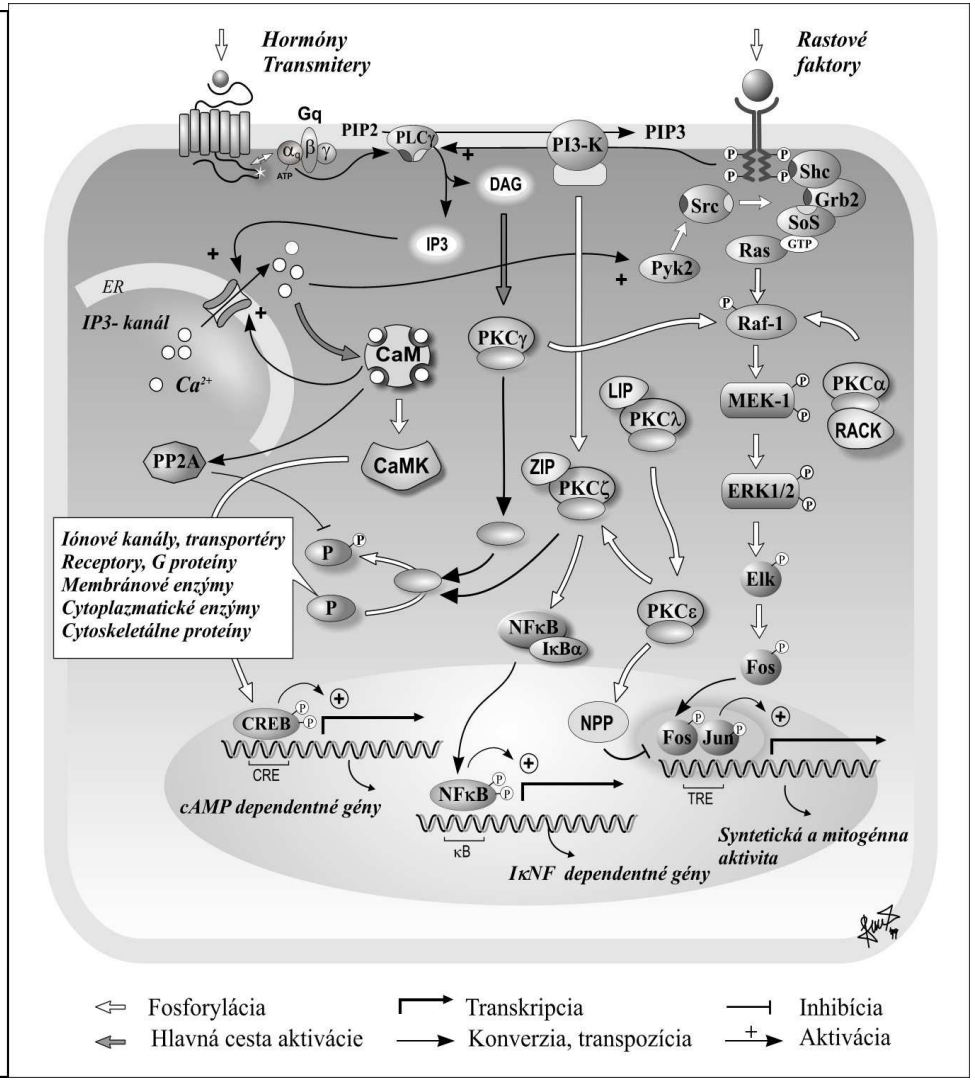


Signaling cascades in carcinogenesis

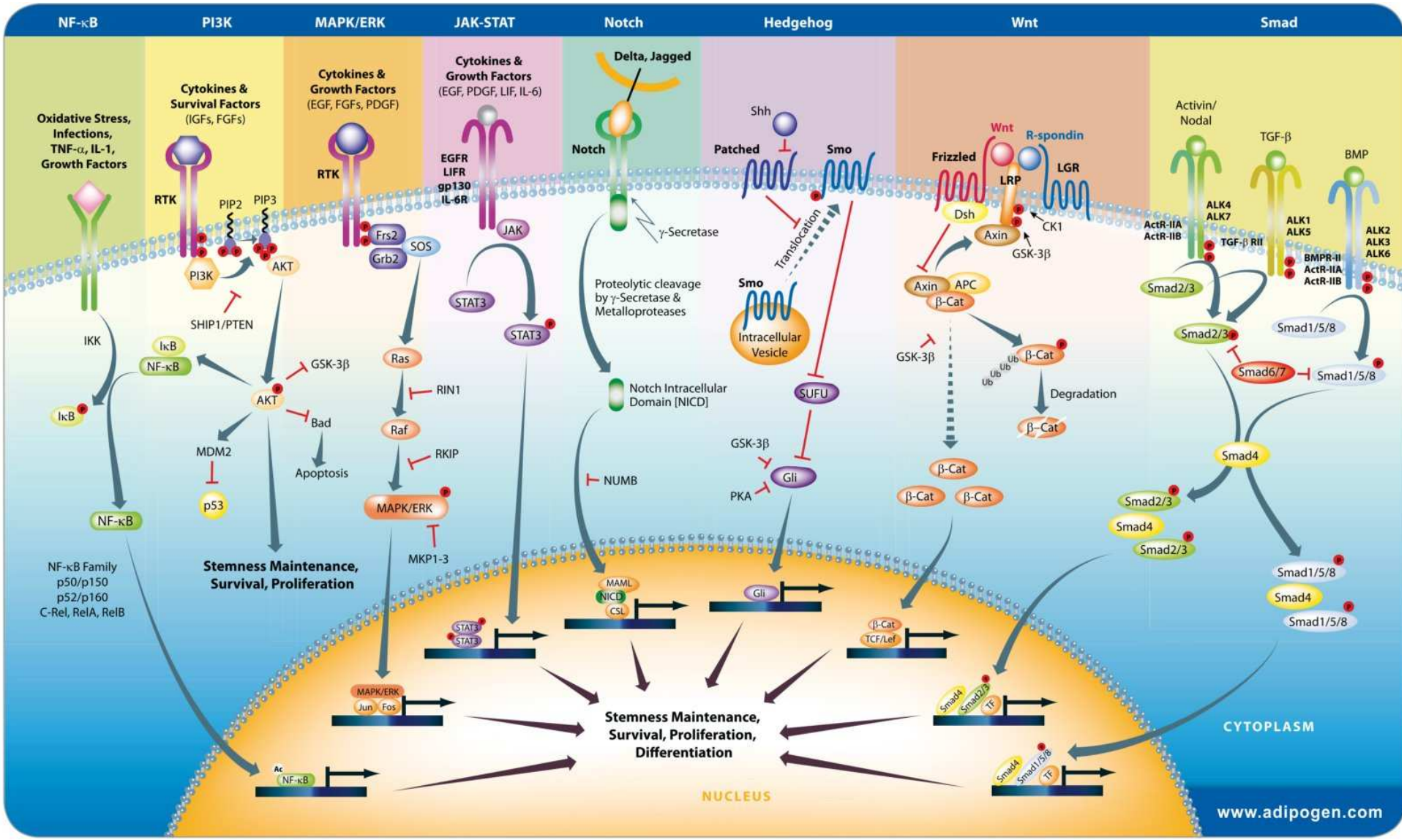
Wnt/Frizzlet – β catenin pathway



IP3/DG dependent pathway



Signaling cascades in carcinogenesis



Signaling cascades in carcinogenesis

PI-3K - dependent pathway

DSL/Notch pathway

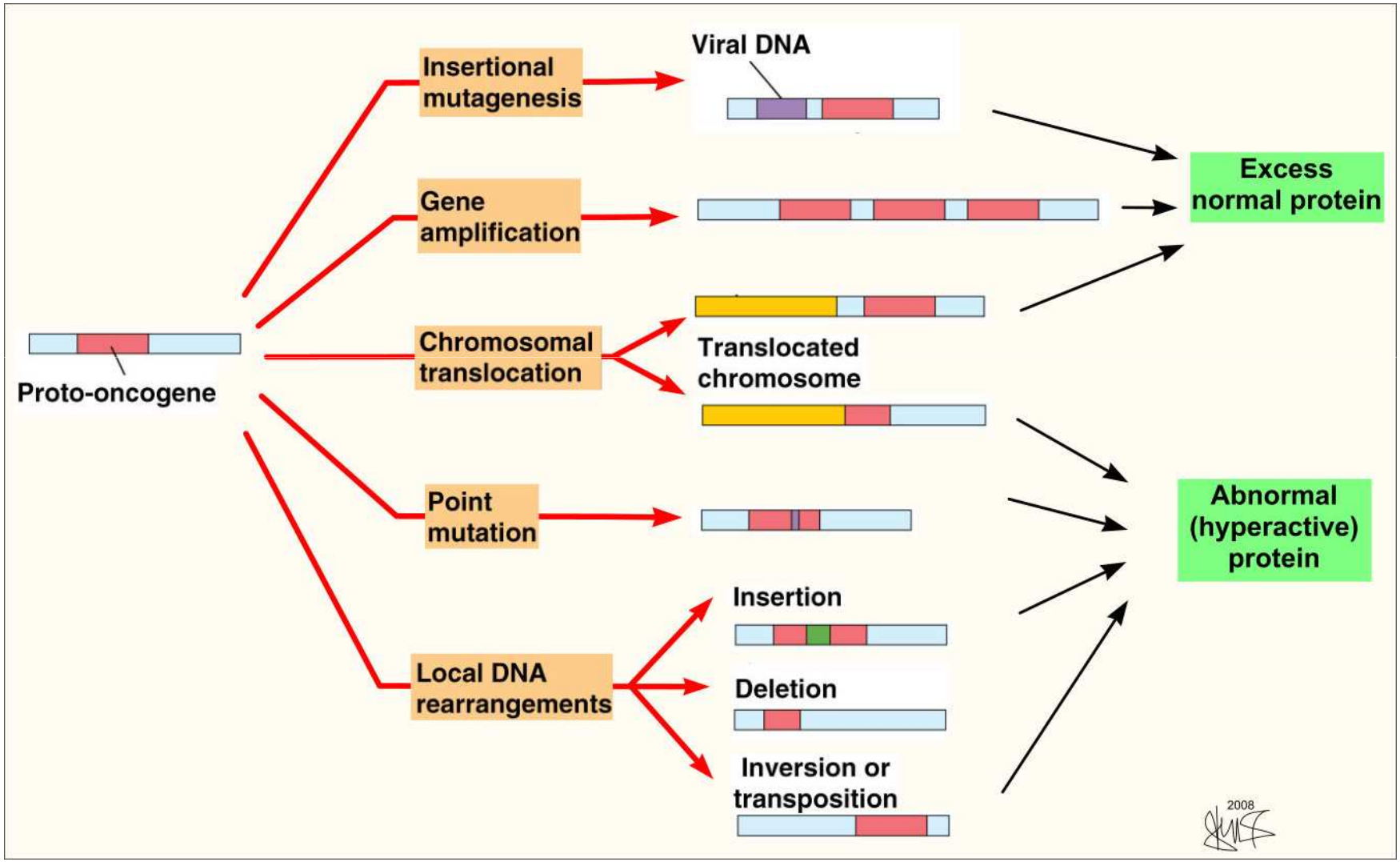
Common oncogenes types

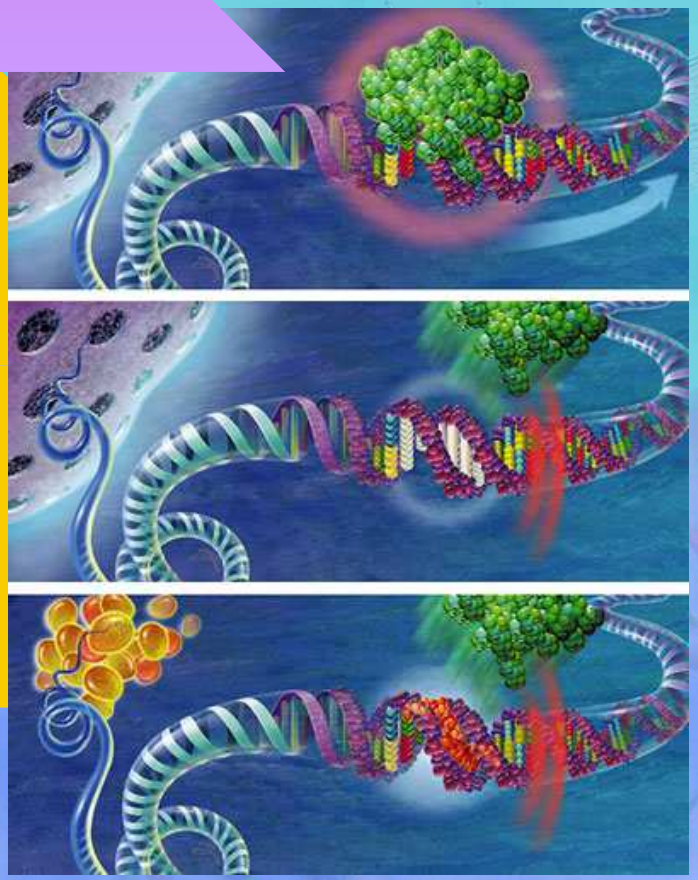
Category	Proto-oncogene	Mode of Activation	Associated Human Tumor
1. Growth Factors			
PDGF-ß chain	SIS	Overexpression	Astrocytome Osteosarcoma
Fibroblast growth factors	HST-1 INT-2	Overexpression Amplification	Stomach cancer Bladder cancer Breast cancer Melanoma
TGFalfa	TGFalfa	Overexpression	Astrocytomas Hepatocellular carcinomas
HGF	HGF	Overexpression	Thyroid cancer
2. Growth Factor Receptors			
EGF-receptor family	ERB-B1 ERB-B2	Overexpression Amplification	Squamous cell carcinomas of lung, Breast and ovarian cancers
CSF-1 receptor	FMS	Point mutation	Leukemia
Receptor for neurotrophic factors	RET	Point mutation	Multiple endocrineneoplasia 2A and B, familial medullary thyroidcarcinomas
PDGF receptor	PDGF-R	Overexpression	Gliomas
Receptor for stem cell (steel) factor	KIT	Point mutation	Gastrointestinal stromal tumors and other soft tissue tumors

Common oncogenes (con't)

3. Proteins Involved in Signal Transduction			
GTP-binding proteins (G proteins monomeric)	K-RAS	Point mutation	Colon, lung, and pancreatic tumors
	H-RAS	Point mutation	Bladder and kidney tumors
	N-RAS	Point mutation	Melanomas, hematologic malignancies
Nonreceptor tyrosine kinase	ABL	Translocation	Chronic myeloid leukemia Acute lymphoblastic leukemia
RAS signaling	BRAF	Point mutation	Melanomas
WNT signal transduction	ß-catenin	Point mutation Overexpression	Hepatoblastomas, hepatocellular carcinoma
4. Nuclear Regulatory Proteins			
Transcriptional activators	C-MYC N-MYC L-MYC	Translocation Amplification Amplification	Burkitt lymphoma Neuroblastoma Small cell carcinoma of lung
5. Cell-Cycle Regulators			
Cyclins	CYCLIN D	Translocation Amplification	Mantle cell lymphoma, Breast and esophageal cancers
	CYCLIN E	Overexpression	Breast cancer
Cyclin-dependent kinase	CDK4	Amplification or point mutation	Glioblastoma, melanoma, sarcoma

Mechanisms of proto-oncogenic activation





TUMOR SUPRESSORS

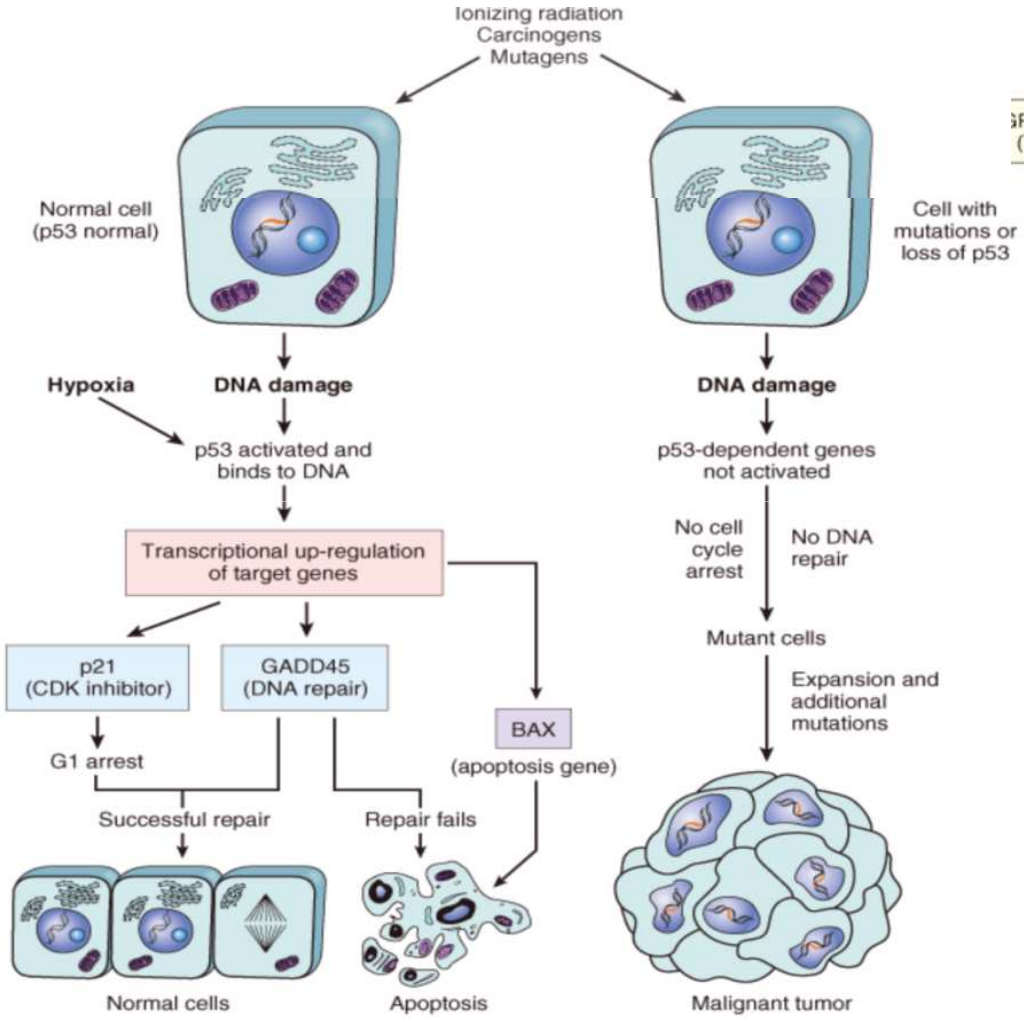
2. Tumor suppressor genes

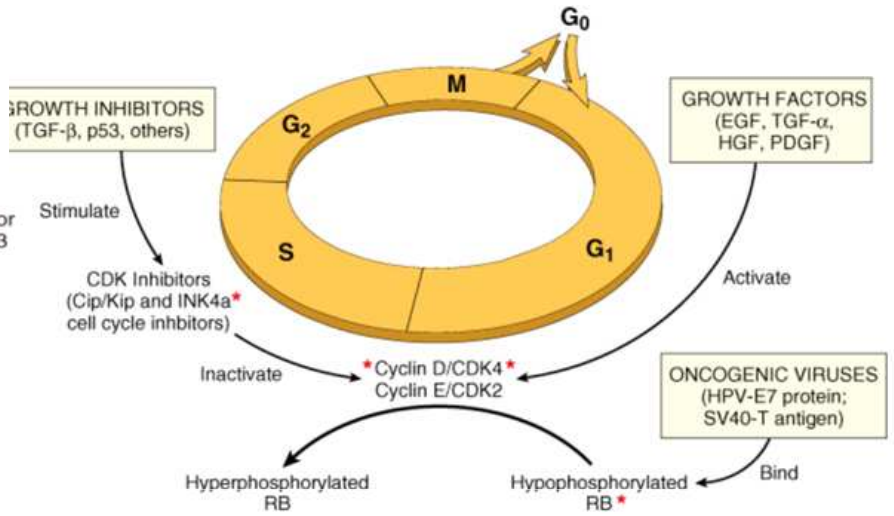
- Tumor suppressor genes are **normally present in cells**. Working actively in main cell cycle checkpoints, they **control the correct execution of the cell cycle and mitotic activity,** being involved in checkout of many preparatory steps (G1 stage) (rediness of resources -lipids, peptides, nucleotides, energy, oxygen, etc.), DNA control before and after replication, contact (anchorage) dependent cell growth. If not correct → **switch on cell death (apoptosis)** (However they are not a part of apoptosis execution). Through these processes, they can also suppress tumor development.
- TSGs mutations **behave "recessively"** at the cellular level - **both copies of TSG need to be mutated** order to cause a change in cell growth and tumor formation to occur.
- TSGs mutations are **loss of function mutations**
- Mutations are **usually acquired** as the result of aging and/or environmental exposures. A mutation in TSG is **often inherited.** In these cases, a mutation in **one copy of the tumor suppressor gene pair is inherited** (germline mutation) from a parent (present in all cells of a body) . The **mutation in the second copy of the gene**) is acquired and usually occurs only in a single cell or a handful of cells.
- Inactivation of TSG is commonlty one of the **first hit in line in multistep genetic alterations.**

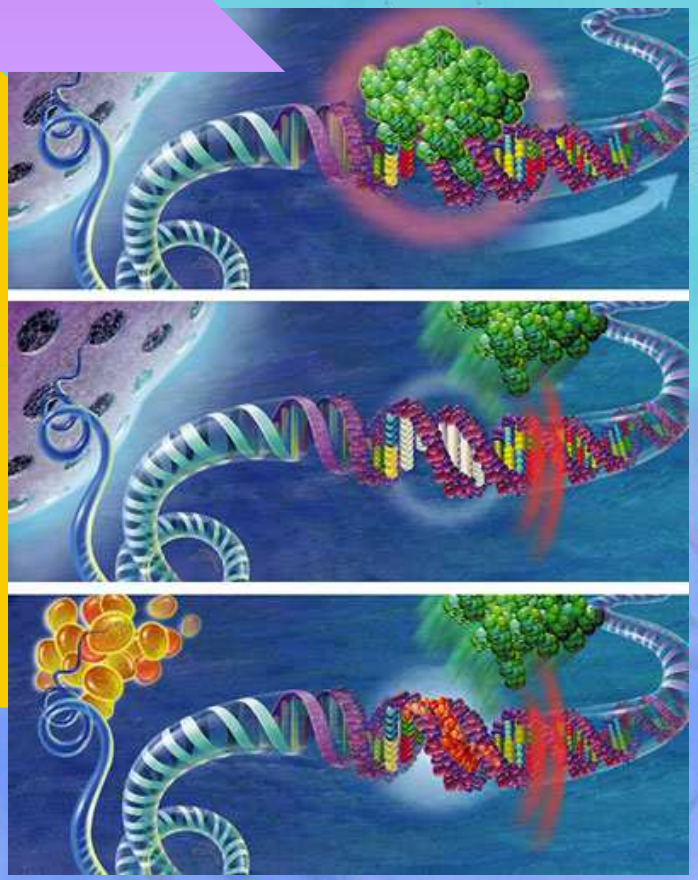
Tumor supressor genes

Location	Gene	Function	Somatitic mutation	Germinative mutation
Cell surface	**TGF-beta receptor**	Growth inhibition	Ca colon	Unknown
	E-cadherin	Cell adhesion	Ca stomach	Familial gastric cancer
Inner plasma membrane	**NF-1**	Inhibition of RAS sign. p21 cell-cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	**NF-2**	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromatosis type 2, acoustic schwannomas
Cytosol	**beta-catenin/ APC**	Inhibition of signal transduction	Carcinomas of stomach, colon, pancreas; melanoma	Familial adenomatous polyposis coli/ Ca colon
	PTEN	PI-3 kinase signal transduction	Endometrial and prostate cancers	Unknown
	SMAD 2,SMAD 4	TGF-ß signal transd.	Colon, pancreas tumors	Unknown
Nucleus	**RB**	Regulation of cell cycle	Retinoblastoma; osteosarcoma	Retinoblastomas, Ca breast, colon, lung
	p53	Cell-cycle arrest; apoptosis	Most human cancers	Li-Fraumeni sy., multiple Ca
	WT-1	Nuclear transcription	Wilms tumor	Wilms tumor
	p16 (INK4a)	Regulation of cell cycle by inhibition of CDK	Pancreatic, breast, and esophageal cancers	Malignant melanoma
	BRCA-1, BRCA-2	DNA repair	---	Ca breast, ovary, male breast
	KLF6	Transcription factor	Prostate	--

Factors controlling cell cycle







DNA- REPAIR GENES + APOPTOTIC GENES

3. DNA repair genes (DRGs)

- DNA repair process is constantly active as environmental factors and normal metabolic processes insidethe cell cause permanent DNA damage at a rate **of 10,000 to 1,000,000 molecular lesions per cell per day.** Even if this represents only **0,001 - 0,01% from 6 billion bases** (3 billion base pairs), when normal repair processes fail, and when cellular apoptosis (safety bit) does not occur, irreparable DNA damage may occur including double-strand breaks and DNA crosslinkages (interstrand crosslinks or ICLs
- **DNA repair genes of (DRGs)** are normally present in cells. There were identified more than 100 human DRGs which create several categories. **Mismatch-repair genes (MMR)** produce proteis correcting naturally occurring DNA errors in replication. **Base excision repair (BER)** or **Nucleotide excision repair (NER)** code products involved in repair errors in DNA that occur from mutagenic agents**), Homologous recombination,**
- When DRGs are altered or mutated and the **mistakes remain unrepaired** in genes such as **tumor suppressor genes or proto-oncogenes** likelihood of uncontrolled cell growth and tumor formation extremely increase and in existing tumors contribute to tumour heterogeneity.
- Mutations in DNA repair genes
  - are invovlved in very early stages of tumorigenesis; do not cause cancer directly
  - behave in **"recessive" way -** both alelles must be altered (homoyzgote) to be effective
  - Both mutations in DRG **can be acquired** (most cases of cancer; aging and environmental exposure) **one mutation may be inherited** from the parent the remainder are acquired
  - **Epigenetic mutations (DNA-hypermethylation or hypomethylation; histone modification,)** seems to play critical role in mutagenesis of DNA- repair genes
- Because of inherent limitations in the DNA repair mechanisms, if humans lived long enough, they would all eventually develop cancer

DNA-methylation mutations in DNA repair genes

Repair system	Genes	Cancer
Base excision repair (BER)	MBD4	Colorectal cancer (cell lines), ovarian cancer, multiple myeloma (cell lines)
	TDG	Multiple myeloma (cell lines)
	OGG1	Thyroid cancer (cell lines and tumors)
Direct reversal of DNA damage	MGMT	Colon cancer,gastric carcinoma, glioblastoma, non-small cell lung cancer
Nucleotide excision repair (NER)	XPC	Bladder cancer
	RAD23A	Multiple myeloma (cell lines)
	ERCC1	Glioma (cell lines and tumors)
Mismatch excision repair (MMR)	MLH1	Acute myeloid leukemia, gastric ca,neck squamous cell ca, non-small cell lung ca, oral squamous cell ca, ovarian cancer, sporadic colorectal cancer
	MSH2	Colorectal ca, non-small cell lung ca, oral squamous cell ca, ovarian ca
	MSH3	Gastric ca, sporadic colorectal ca
	MSH6	Colorectal cancer
Homologous recombination	BRCA1	Breast ca, ovarian ca, gastric ca, non-small cell lung ca, uterine bladder ca
Non-homologous end-joining	XRCC5	Non-small cell lung cancer
Editing and processing nucleases	FEN1	Breast cancer (hypomethylated)
Genes defective in diseases associated with sensitivity to DNA damaging agents	WRN	Breast ca, colon ca, colorectal ca, gastric ca, non-small cell lung ca, prostate ca, thyroid ca
	ATM	Colorectal cancer (cell lines) head and neck squamous cell carcinoma
Fanconi anemia	FANCC	Sporadic leukemia (0.7–3.1%)
	FANCF	Neck squamous cell ca, non-small cell lung cancer ovarian
	FANCL	Sporadic leukemia (~1%)
Other conserved DNA damage response genes	CHK2	Glioma, non-small cell lung carcinoma

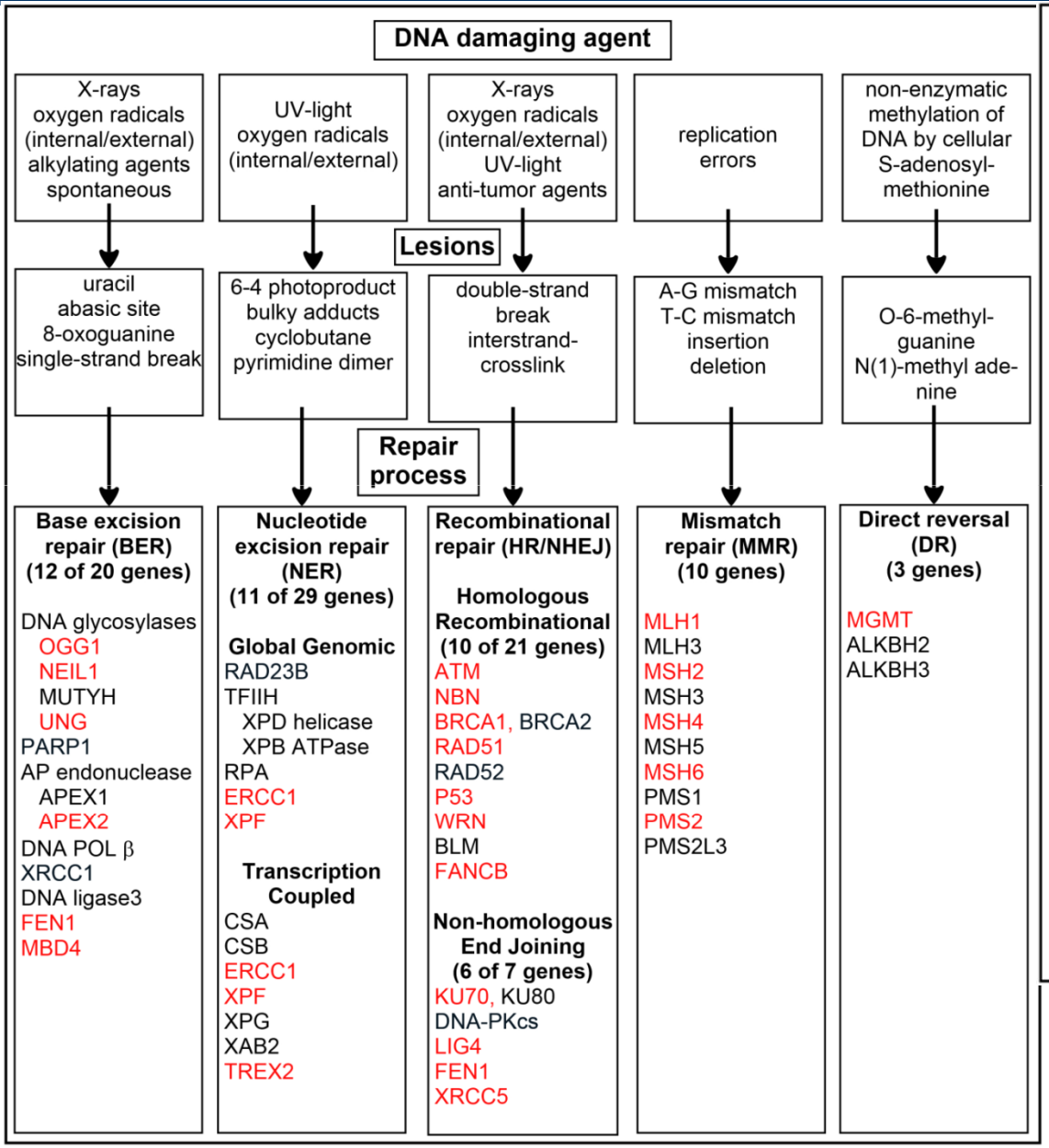
DNA repair genes (DRGs) Causes of DNA damage

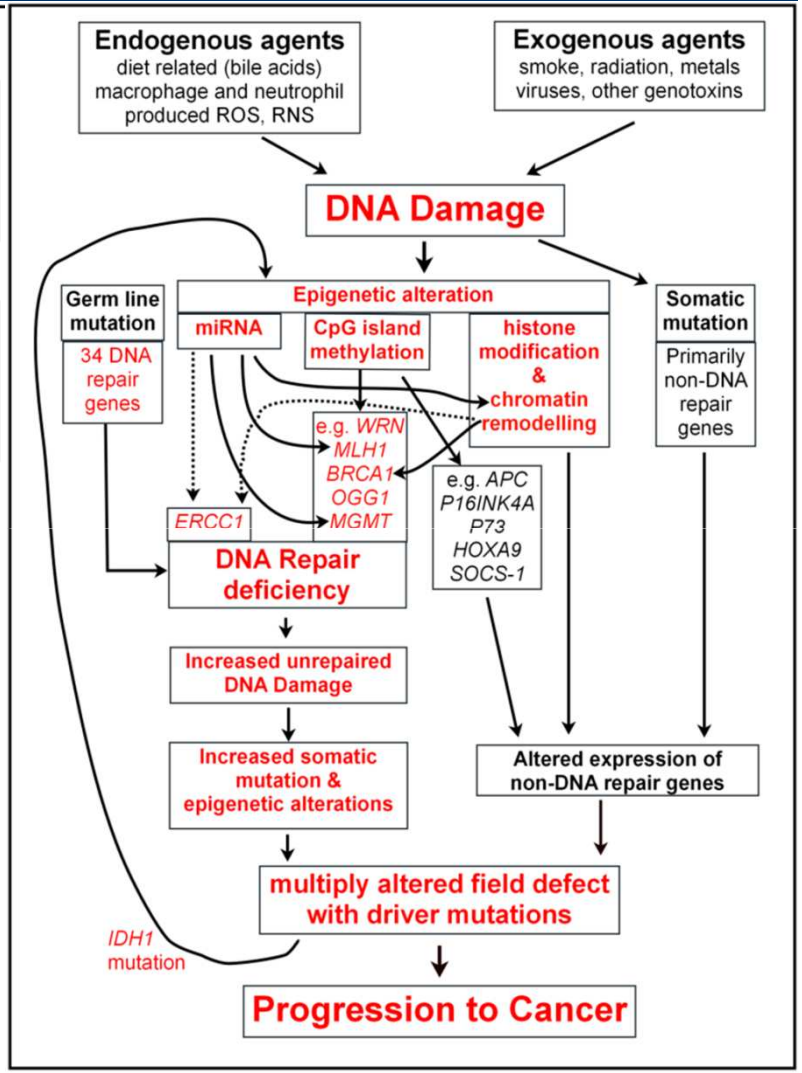
- ***endogenous (spontaneous) mutation***
  - mismatch of bases in DNA replication - DNA base is skipped over or mistakenly inserted
  - ROS (reactive oxygen species) produced from normal metabolic byproducts cause **oxidation of bases** [e.g. 8-oxo-7,8-dihydroguanine (8-oxoG)], DNA strand interruptions, **alkylation of bases** (formation of 7-methylguanosine, 1-methyladenine, 6-O-Methylguanine **hydrolysis of bases**, such as **deamination, depurination, and depyrimidination**.
- ***exogenous damage***
  - ***ultraviolet rays*** (200-400 nm), UV-A light free radicals - indirect DNA damage; .UV-B light makes direct DNA damage (pyrimidine dimers = crosslinking between adjacent cytosine and thymine bases)
  - ***x-rays and gamma rays***;
  - ***certain plant toxins; viruses***
  - ***elevated temperature*** increases the rate of **depurination** (loss of purine bases from the DNA backbone) and **single-strand breaks.**
  - ***chemicals*** (vinyl chloride and hydrogen peroxide, polycyclic aromatic hydrocarbons found in smoke, soot and tar create a **huge diversity of DNA adducts**- ethenobases, oxidized bases, alkylated phosphotriesters and crosslinking of DNA
- **mitochondria**, posses a highly oxidative environment containing reactive oxygen species (ROS) that are known to damage mtDNA. A critical enzyme in counteracting the toxicity **superoxide dismutase,** which is present in both the mitochondria and cytoplasm of eukaryotic cells.
- Because it takes more than a single mutation to cause cancer, not all people who inherit a mutation in a tumor suppressor gene, proto-oncogene, or DNA repair gene will develop cancer.

Examples od DNA- repair genes dysfunction

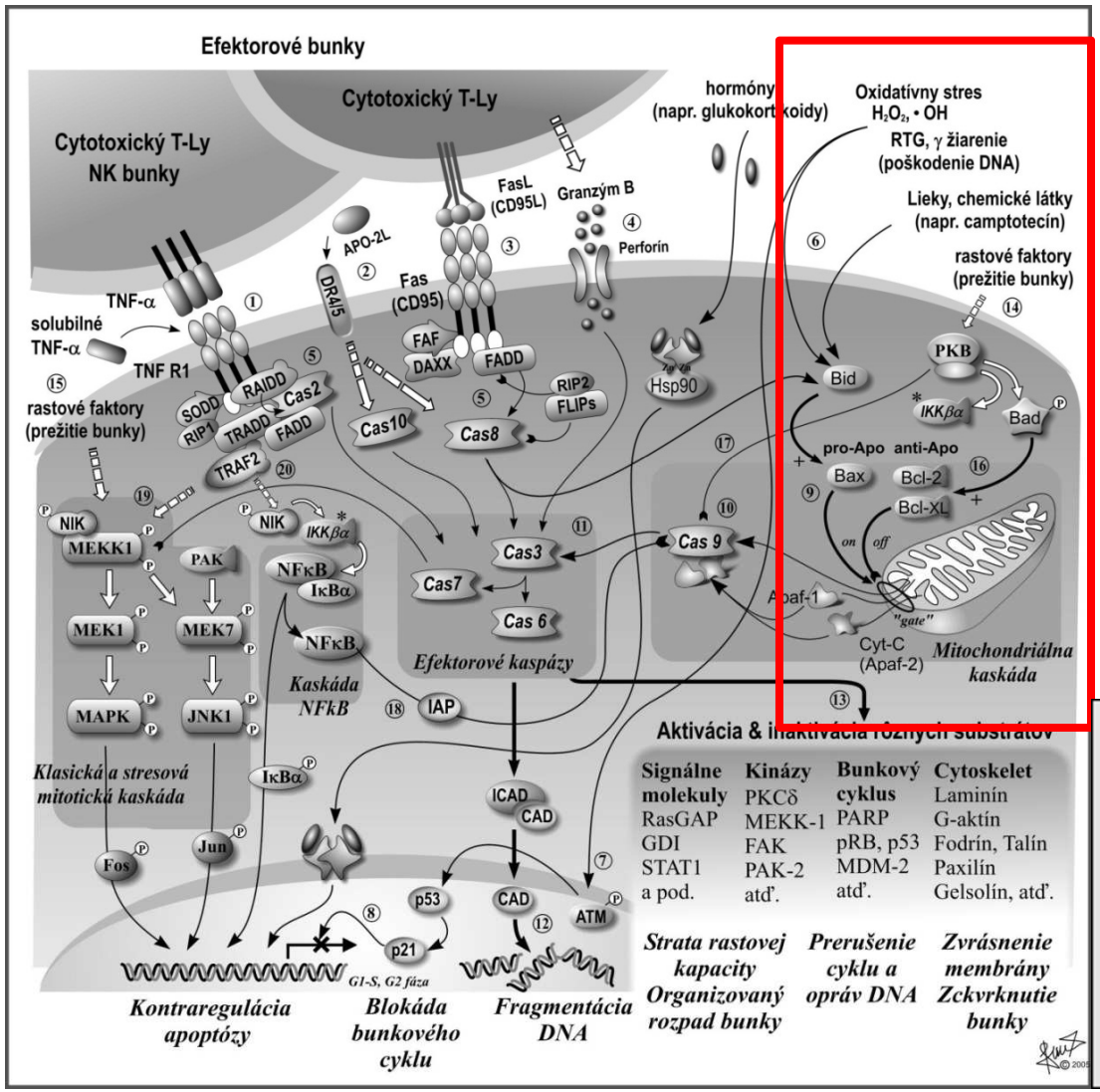
- **BRCA.** If it is not possible to repair the DNA damage before replication (S phase) , the DNA may be repaired by homologous pairing. Because of DNA polymerase-blocking damage, DNA strand breaks will be generated, which can be repaired by the **homologous recombination repair system.** BRCA1 and BRCA2 (Breast Cancer 1 and 2) proteins are involved in this repair pathway. **BRCA1 and BRCA2** genes are tumor suppressor genes and the proteins, together with RAD51, form a complex to repair DNA strand breaks. BRCA1 is most **often methylated** in **breast and gastric cancer, uterine cancer and bladder cancer**.
- **MLH.** Function of **mismatch mutation repair system (MMR) is** associated with DNA replication, to correct for deficiencies in DNA polymerase proofreading function. Missing gene or mutations of MMR protein and other MMR genes (MSH2, MSH6, or PMS2) lead to microsatellite instability (MSI) and this dysfunction is highly associated with **hereditary non-polyposis colon cancer.** Gene is epigenetically inactivated also in other types of cancer, for example, in sporadic endometrial carcinoma, gastric cancers , ovarian tumors, oral squamous cell carcinoma, acute myeloid leukemia (AML).
- The **nucleotide excision repair (NER)** system consists of two sub-pathways. The global genome repair (GGR) mechanism repairs DNA damage in transcriptionally inactive parts of the genome .The second NER component is responsible for repair of transcribed DNA and is referred to as transcription-coupled repair (TCR). These two NER functions differ in the damage recognition step. The protein encoded by the **xeroderma pigmentosum** group C (XPC) is essential for GGR. Alteration in XPC was found in **bladder** cancer. Two other genes, which are part of the NER system, are methylated in human tumors. The RAD23A gene is methylated in the **multiple myeloma** and ERCC1 is methylation-silenced in glioma **glioma tumors** .

Role of DNA repair genes mutation in cancer

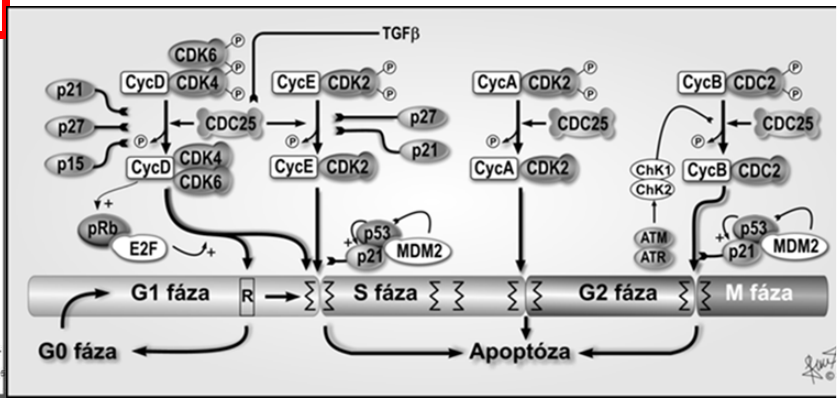




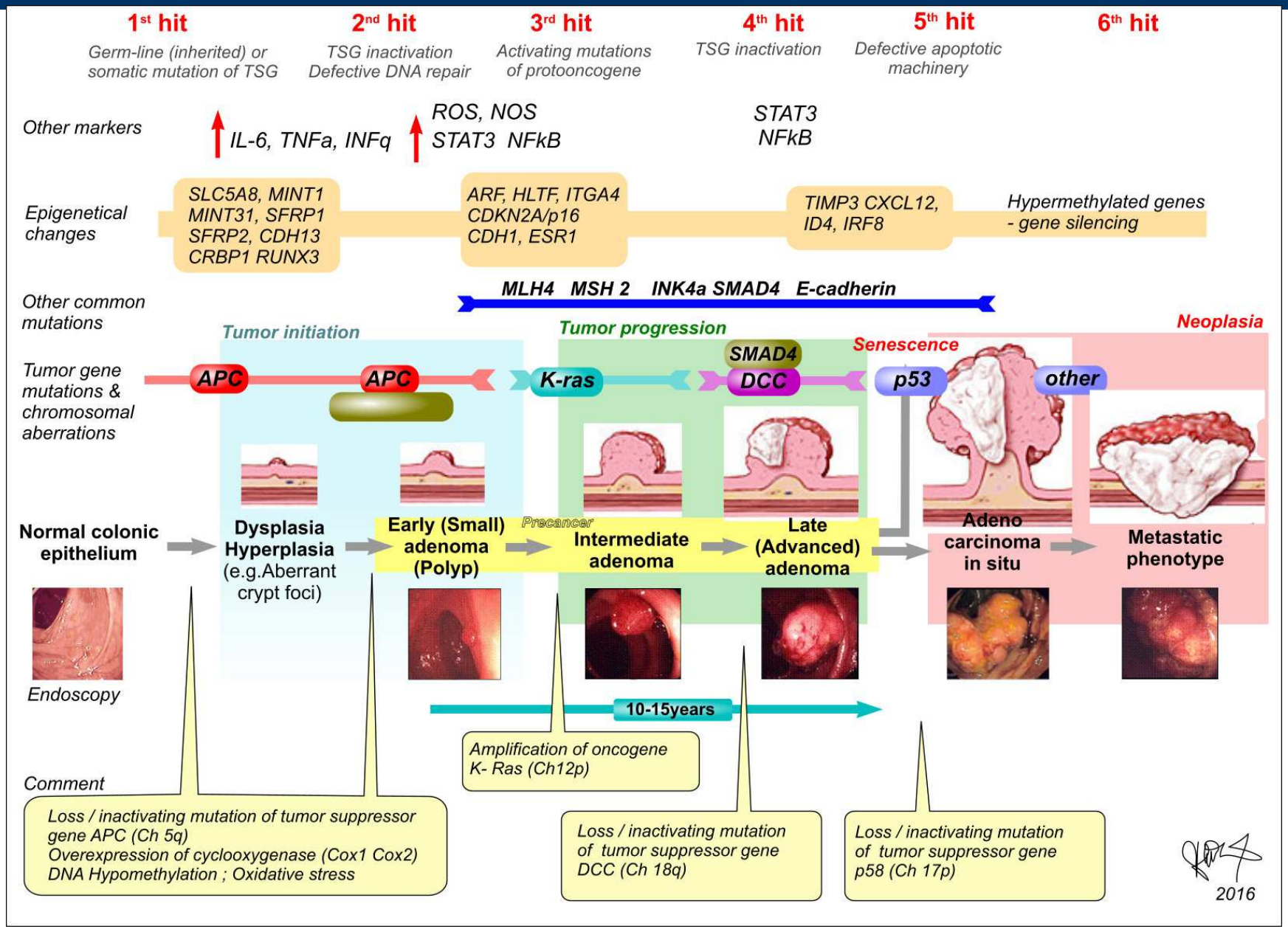
4. Endogenously triggered apoptotic machinery



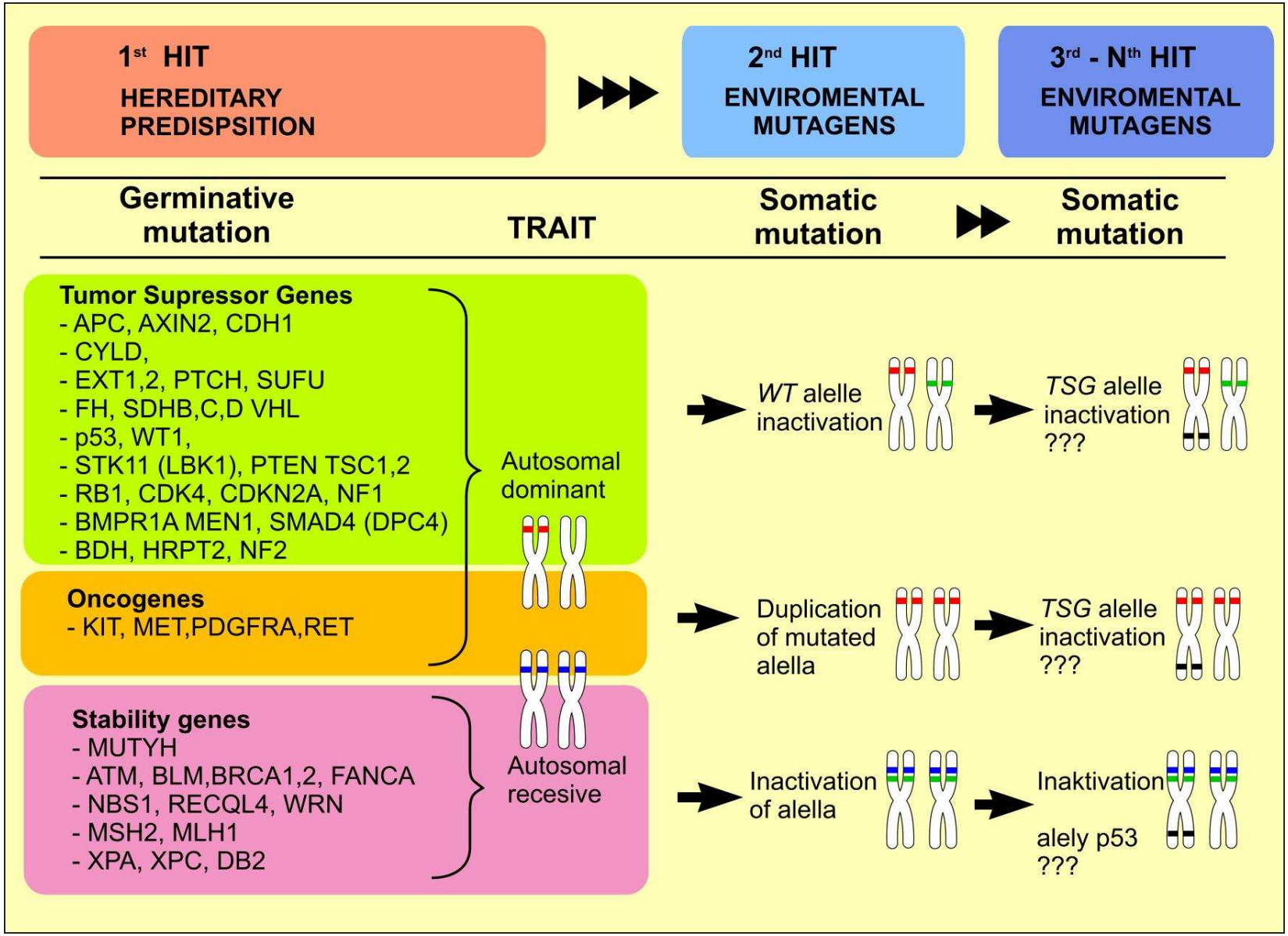
- Mutation in apoptotic execution machinery allows cell with critical mutations to survive and mutiply
- Altered apoptotic genes: **Bax, Blc-2, Bad,Bcl-XL, Apaf**
- Suppression of **Caspase 8** prevents apoptosis and promote invasion



Mutistep mutational accumulation in colon cancer

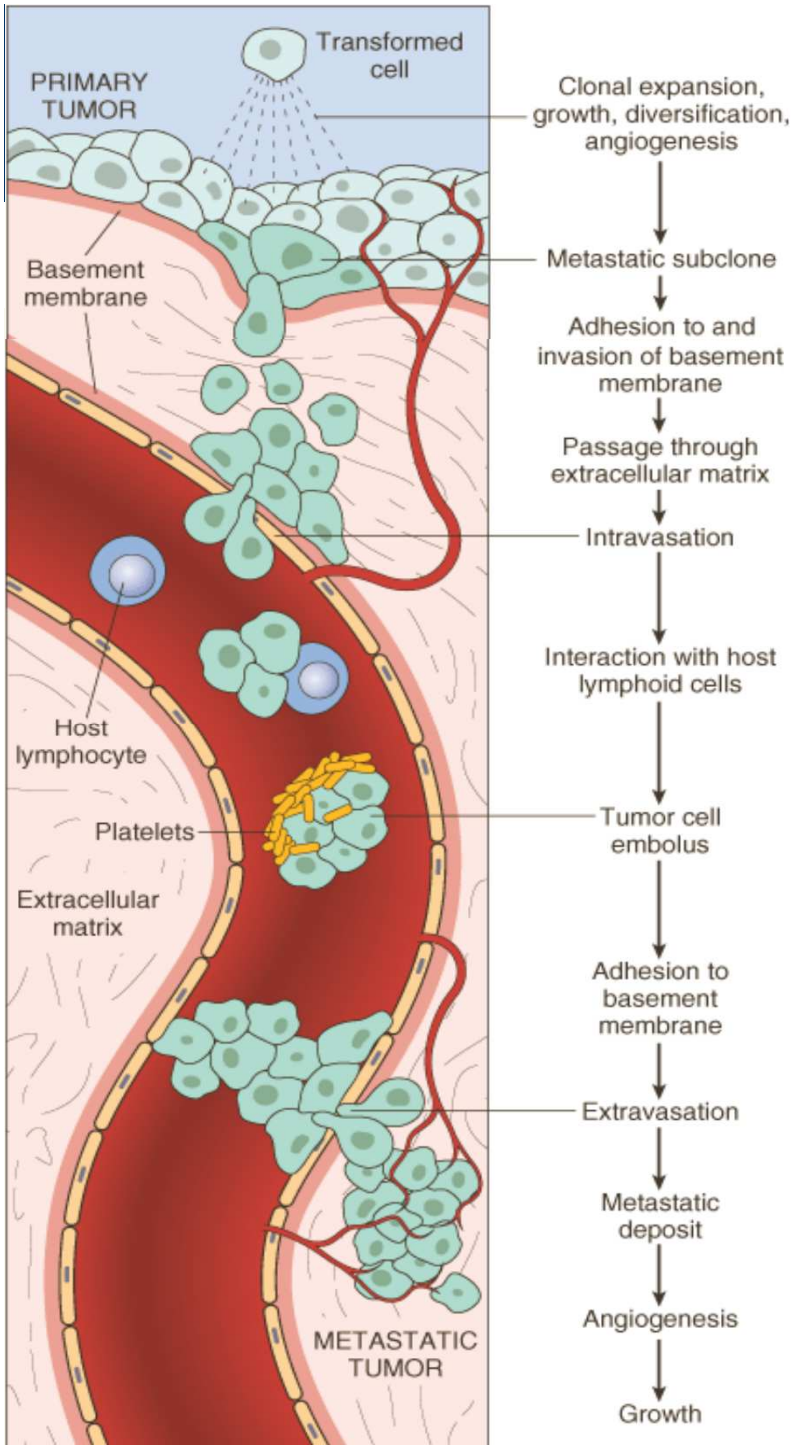


Mutihit concept of tumor genetic mistakes



METASTASING

- Oncogenes
- Tumor supressor genes
- Apoptotic genes
- Stability genes (DNA repair genes)

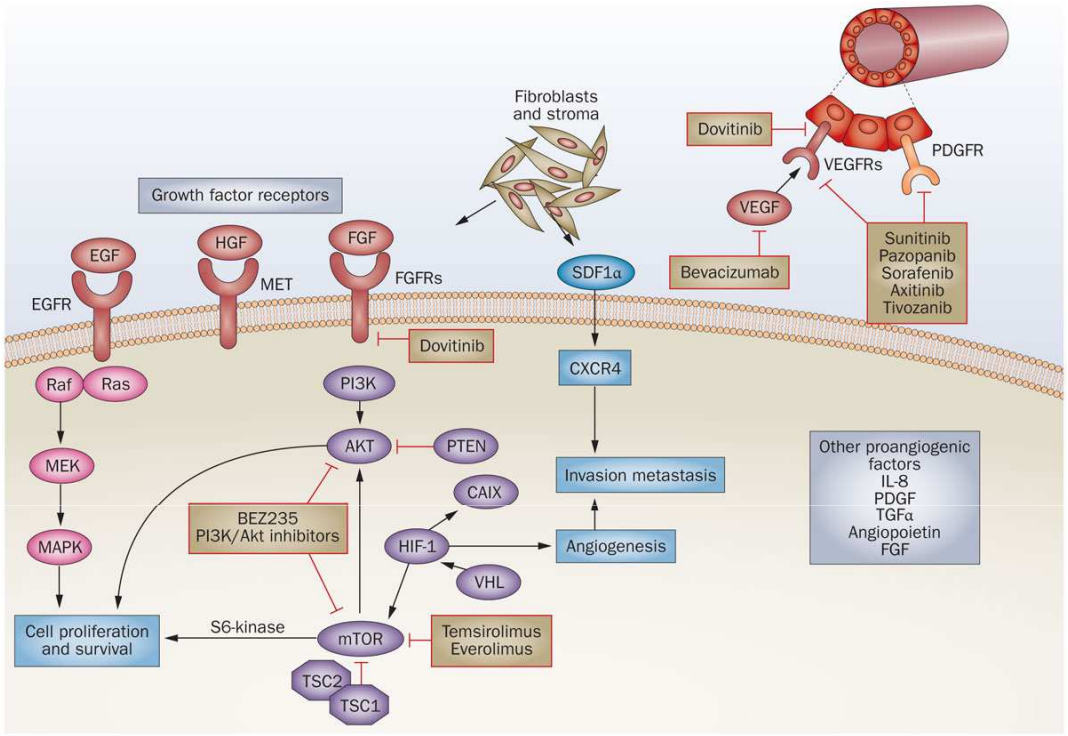


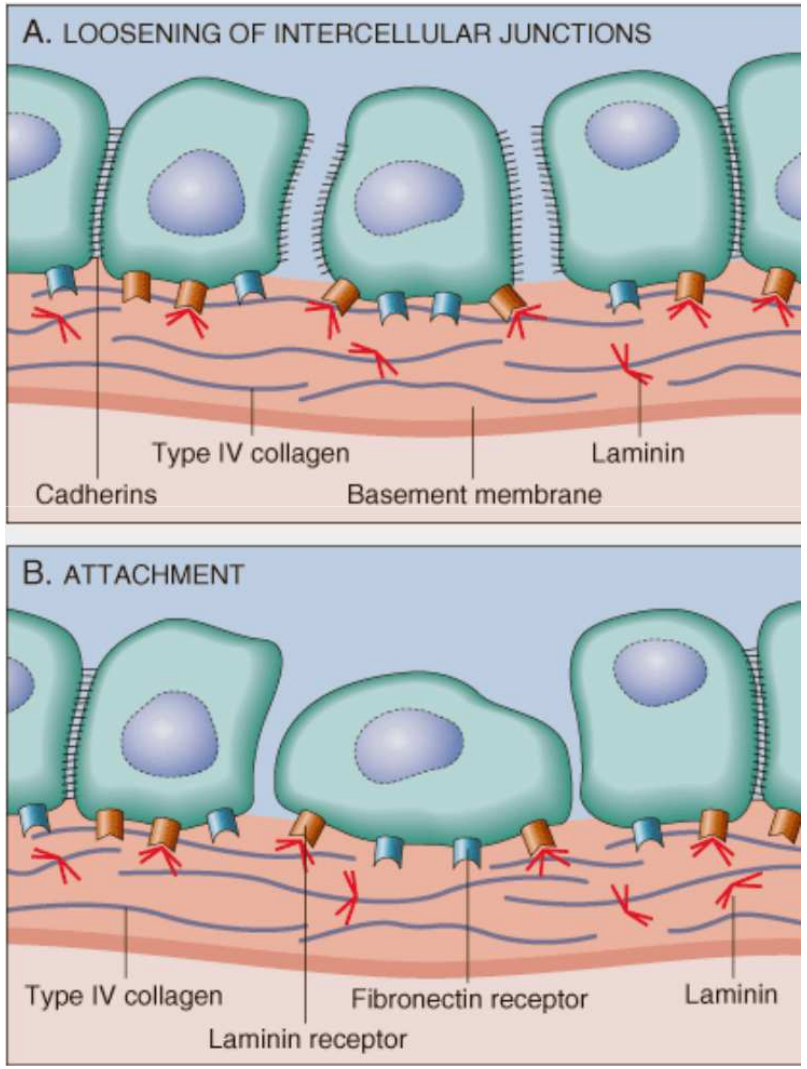
Steps in metastasis

Metastasis is one of the most lethal attributes of cancer (90% of cancer deaths). Complex process in which primary tumors disseminate to secondary organs via activation of metastasis-promoting genetic programs and the inhibition of metastasis-suppressing programs. It is a cascade with multiple steps.

1. **Epithelial to mesenchymal transition (EMT),** break through basal membrane require abolishion of all cell-cell/cell-matrix adhesions, rebuilding cytoskeleton to allow ameboid movement,
2. **Movement through loose connective tissue** – escape from resident protective macroph., excessive release of matrix metalloproteases dissolve all tissue around, cells crawl
3. **Intravasation** – getting into vessel presume reverse diapedesis + reqire expression of leukocyte adhesion molecules
   - Lymph vesels
   - Blood vessels
4. **Survival in circulation** – cell in vasculature must survive attack by NK, T-Ly, Ig-complement.
5. Adhesion to endothelium and extravasation
6. Building up the colony - neovascularisation

Signalling pathways involved in angiogenesis and metastasis





Tumor cells detach from each due to **abolishion of cell-cell adhe sion (gap junction, tigh junctions, belt desmosome).** Cells attach to basement membrane via the laminin receptors, secrete proteolytic enzymes, incl. collagenase IV and plasminogen activator. Degradation of the basement membrane and tumor cell migration follow.

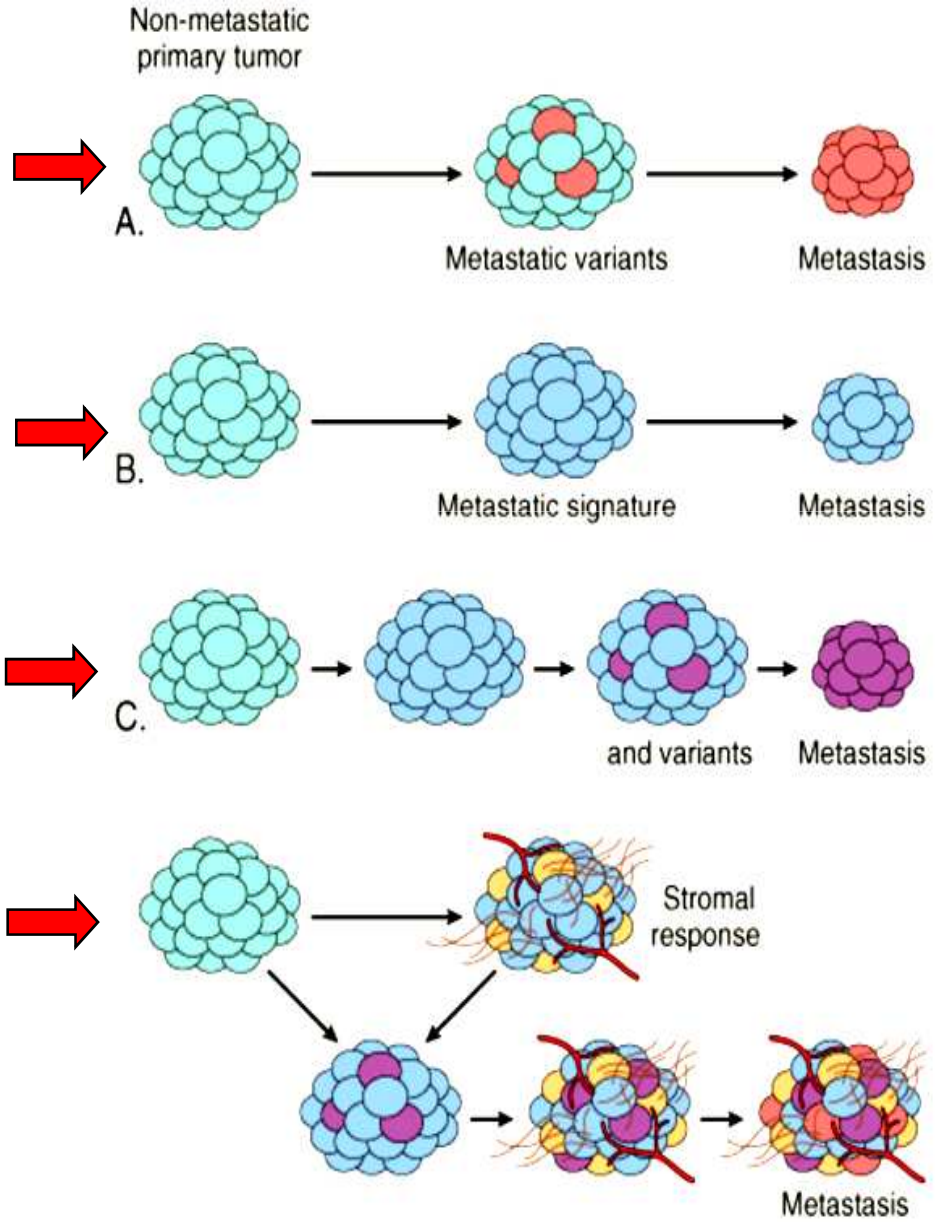
Models of metastasis within the primary tumor

1. Metastasis ability develops in **rare variant clone** that develop in the primary tumor (top of the pyramid)

2. Metastasis is caused by the gene expression pattern of **most cells of the primary tumor** (metastatic signature)

3. M**etastatic variants appear in a tumor with a metastatic gene signature** (combination of A and B)

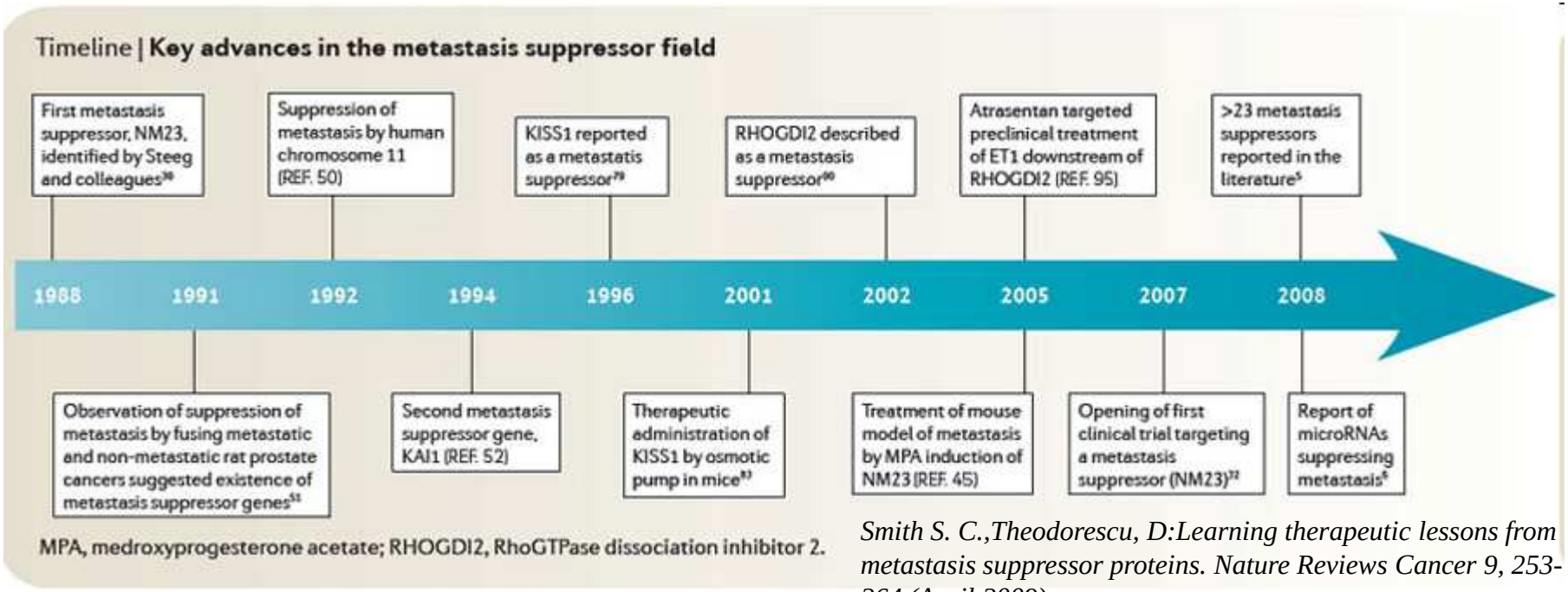
4. Metastasis development is greatly influenced by the **tumor stroma** (regulate angiogenesis, local invasiveness and resistance to immune elimination) allowing cells of the primary tumor



Metastasis supressor genes

Metastasis supressor genes (MSG)

- **Metastasis suppressors (MS)** = proteins that acts to slow or prevent metastases by altering of signal transduction; encoded by **metastasis suppressor genes (MSG)**
- by definition MSG have no effect on primary tumors or tumorigenesis and malignany turn; they act by different mechanisms than tumor (growth) suppressors
- 1988 - first MSG was identified (**NM23**); in 1994 - 1996 second and third **(KAI1 and KISS1**) ; dozen such proteins are known in humans and animals; 2007 first clinical trial targetting MSG
- Because increasing data suggest that tumours are widely disseminated on an individual cellular basis even at the time of diagnosis of localized disease, the role of metastasis suppressors in preventing outgrowth of isolated single or cellular clusters (micrometastases) is principal.



Smith S. C.,Theodorescu, D:Learning therapeutic lessons from metastasis suppressor proteins. Nature Reviews Cancer 9, 253-264 (April 2009)

Metastasis supressor genes

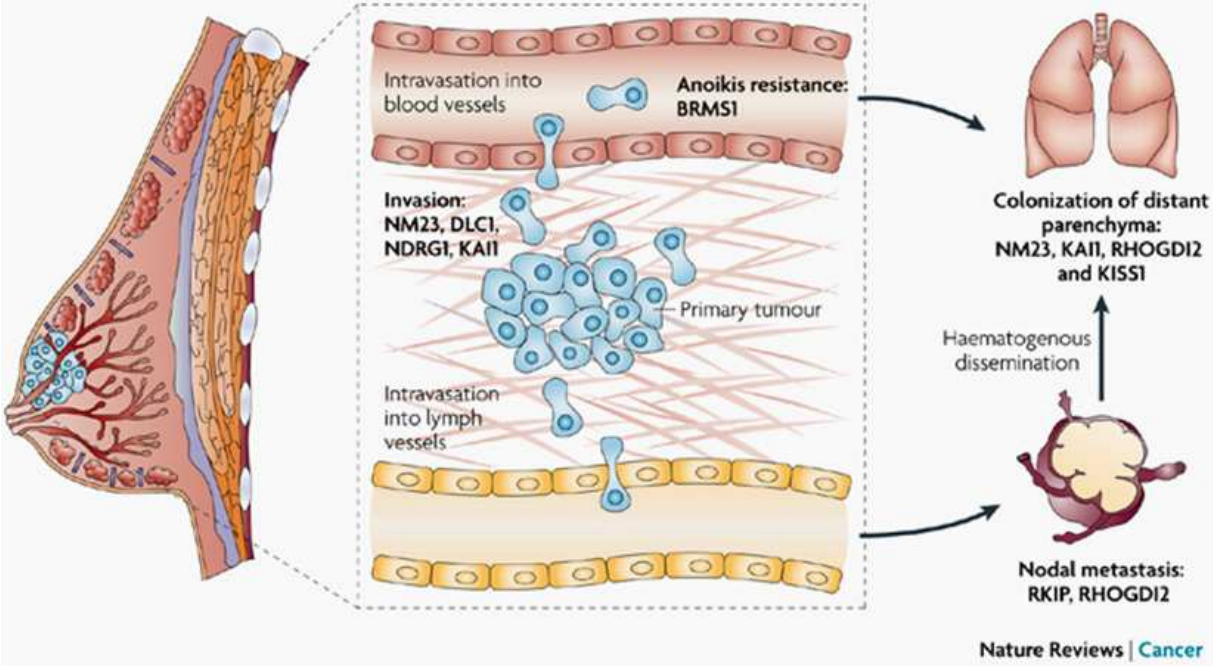
Symbol	Alias(es)	Function(s)	Potential targeting strategy
BMP4	BMP2B	Soluble cytokine	Direct therapeutic administration of suppressor protein*
BRMS1	None	Chromatin and transcriptional regulation; regulation of gap junctions	None published at present
CTGF	CCN2, IGFBP8	Soluble cytokine	None published at present
DLC1	ARHGAP7	Regulation of RhoGTPase signalling	Re-induction of endogenous gene through HDAC inhibition[69]
KAI1	CD82, kangai 1	Inhibition of EGFR signaling; induction of senescence through interaction with DARC	Therapeutic re-induction of endogenous gene by plant extracts[60]; viral[62] and non-viral[61] gene therapy
KISS1	KiSS-1, metastin	Soluble ligand for G-protein-coupled receptor	Direct therapeutic administration of suppressor protein[83]; possibly small molecule mimetics[84]
MKK4	MAP2K4	Signal transduction	Antibody-mediated activation pathway upstream of MKK4 (REF. 122)
NDRG1	CAP43, DRG1, RTP	Unknown	Induced by iron chelators[123], p53 (REF. 124) and PTEN expression[125]
NM23	NME1, NM23-H1	Histidine kinase activity to KSR1, decreasing Ras signalling; regulation of downstream gene expression	Re-induction of endogenous gene[42,47,48]; viral gene therapy[49]; inhibition of downstream genes[40]
RHOGDI2	ARHGDIB, LyGDI, GDID4	Regulation of Rho family member activation; regulation of downstream gene expression	Inhibition of downstream genes[95]
RKIP	PEBP1	Binds to and inhibits Raf kinase activity and downstream signalling	Epigenetic re-induction of endogenous gene[97]

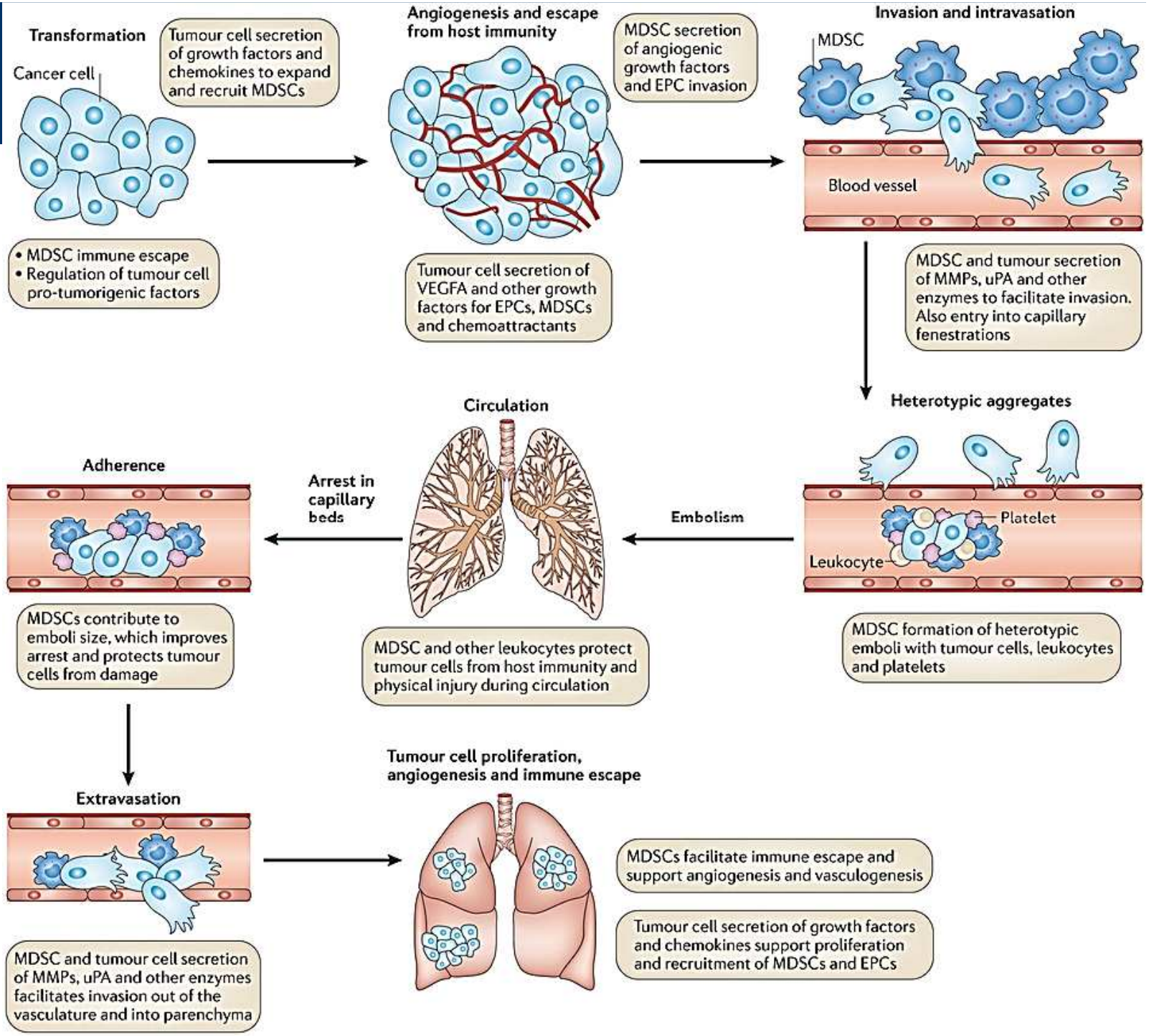
*R. Anderson, unpublished data, also presented at the 2008 Meeting of the American Association for Cancer Research-Metastasis Research Society, Vancouver, Canada. *BMP4*, bone morphogenetic protein 4; *BRMS1*, breast cancer metastasis suppressor 1; DARC, Duffy chemokine receptor; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; *MKK4*, MAPK kinase 4; *RHOGDI2*, RhoGTPase dissociation inhibitor 2; *RKIP*, Raf kinase inhibitory protein.

Model of invasion and metastasis in breast cancer

- In the **primary tumour,** deregulation of oncogenes and tumour suppressor genes mediates the conversion of normal cells to a neoplastic phenotype.
- **Invasion of the basement membrane, stroma and vasculature** constitutes one key, negative prognostic turning point in the natural history of breast and other cancer types. Many metastasis suppressor genes, including **NM23**, **DLC1**, **KAI1** and **NDRG1** have been shown to function in in vitro and in vivo surrogates of invasion in breast cancer cells.
- **Nodal metastasis** **i**s also a key prognostic stage for cancer patients, Loss of the metastasis suppressors Raf kinase inhibitor protein (**RKIP**)66 and RhoGTPase dissociation inhibitor 2 (**RHOGDI2)** are associated with nodal metastasis in breast cancer.

- **Survival during circulatory dissemination** is another step of the metastatic cascade; **resistance to anoikis** is important for metastasis. M**etastasis suppressor gene BRMS1** (breast cancer metastasis suppressor 1) which does not work in certain breast cancers increases anoikis.
- **First colonization** with isolated single or cellular clusters (micrometastases), is prevented by **NM23, KAI1, RHOGDI2, KISS1.**

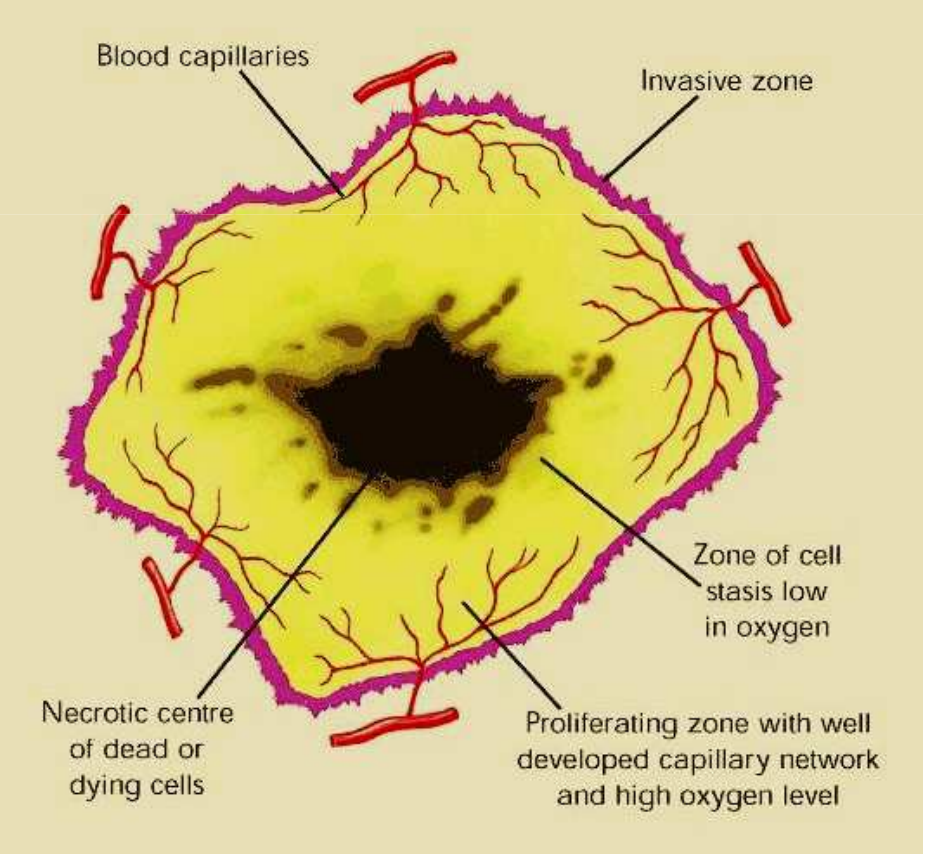



Transformation
Cancer cell
Tumour cell secretion of growth factors and chemokines to expand and recruit MDSCs
• MDSC immune escape
• Regulation of tumour cell pro-tumorigenic factors
Angiogenesis and escape from host immunity
MDSC secretion of angiogenic growth factors and EPC invasion
Tumour cell secretion of VEGFA and other growth factors for EPCs, MDSCs and chemoattractants
Invasion and intravasation
MDSC
Blood vessel
MDSC and tumour secretion of MMPs, uPA and other enzymes to facilitate invasion. Also entry into capillary fenestrations
Heterotypic aggregates
Platelet
Leukocyte
MDSC formation of heterotypic emboli with tumour cells, leukocytes and platelets
Circulation
Embolism
MDSC and other leukocytes protect tumour cells from host immunity and physical injury during circulation
Arrest in capillary beds
Adherence
MDSCs contribute to emboli size, which improves arrest and protects tumour cells from damage
Extravasation
MDSC and tumour cell secretion of MMPs, uPA and other enzymes facilitates invasion out of the vasculature and into parenchyma
Tumour cell proliferation, angiogenesis and immune escape
MDSCs facilitate immune escape and support angiogenesis and vasculogenesis
Tumour cell secretion of growth factors and chemokines support proliferation and recruitment of MDSCs and EPCs

Angiogenesis

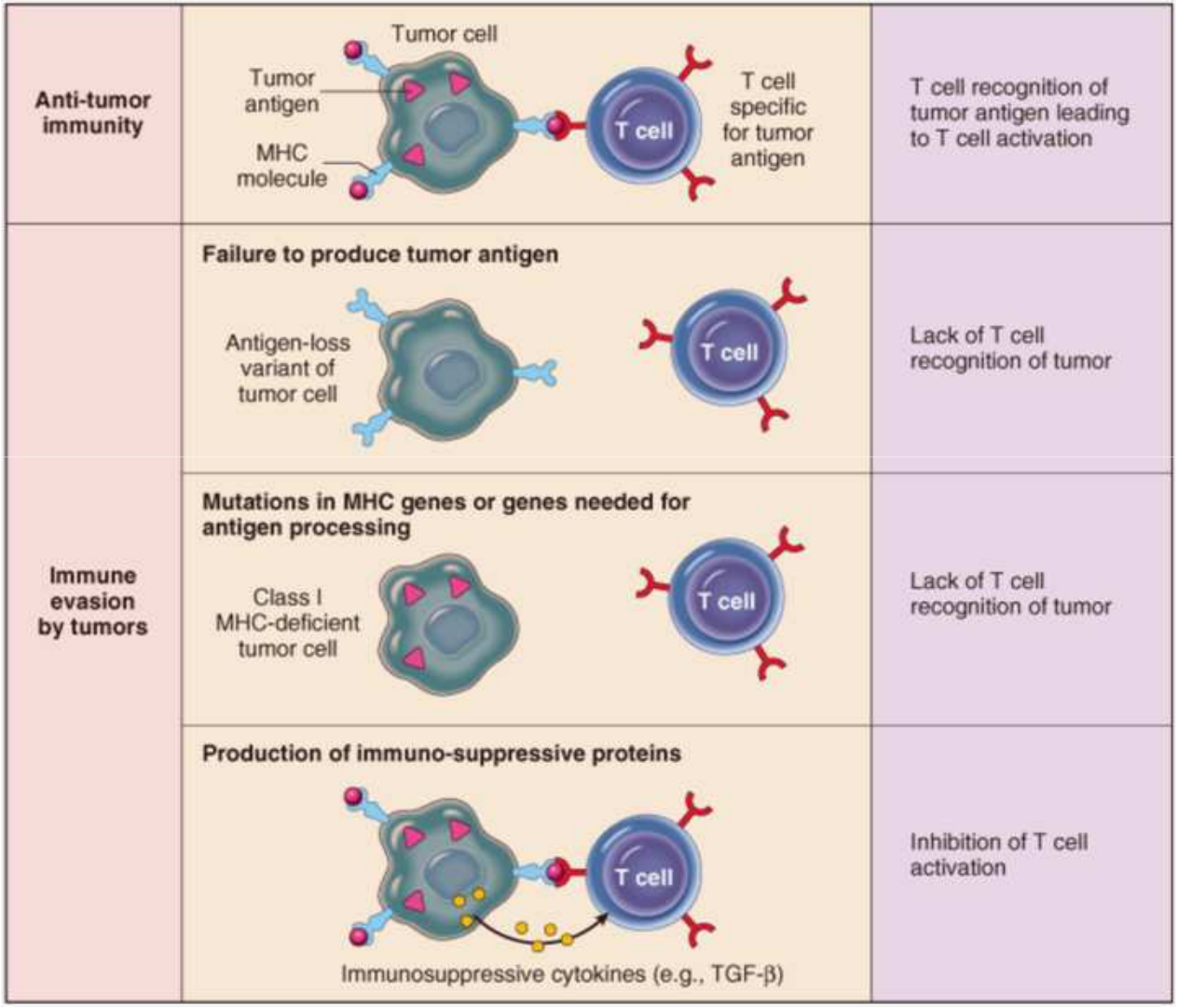
- Angiogenesis is the **principal step in the tumorigenesis**
  - Endothelial cells are the most quiescent cells of the body (turnover hundreds of days) ; endothelial proliferation 6 billion cell divisions/ hour
  - Degradation of basement membrane, sprouting from preexisting microvessels,
  - Invasion into extracel. matrix, forming of tubes, cubs; nutrification of tumour from **outside-in**

Protein	MW
FGF-b	18
FGF-a	16.4
Angiogenin	14.1
Transforming growth factor-a	5.5
Transforming growth factor-b	25
Tumor necrosis factor-a	17
Vascular endothelial growth factor(VPF/VEGF)	40 45
Platelet-derived endothelial growth factor	45
Granulocyte colony-stimulating factor	17
Placental growth factor	25
Interleukin-8	40
Hepatocyte growth factor	92
Proliferin	35
Angiopoietin-1	70
Leptin	16



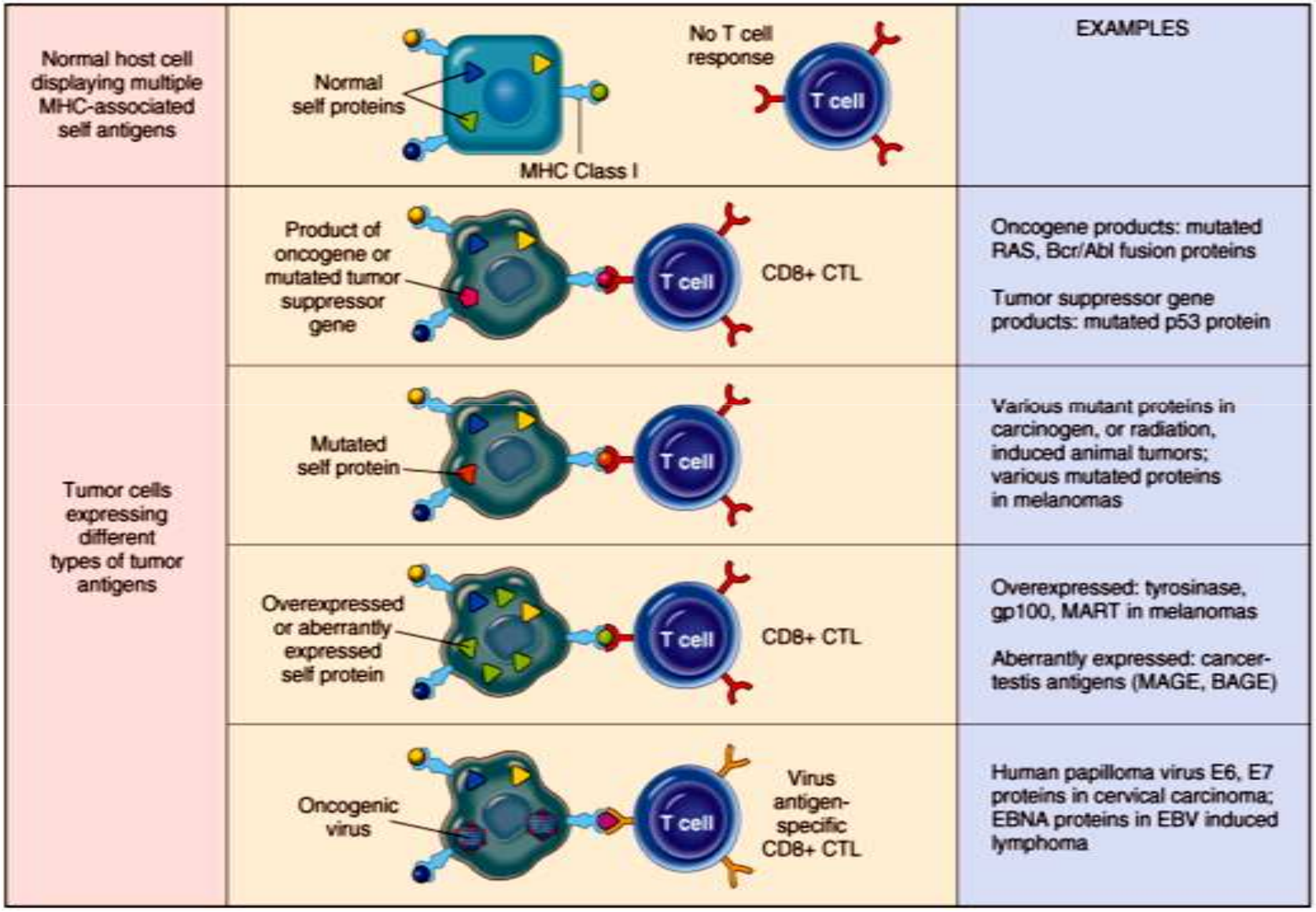
Immunologic control over tumors

Tumor evasion of the immune system.



Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. WB Saunders, 2003

Tumor antigens recognized by CD8+ T cells



Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. WB Saunders, 2003