Concept of „Read and Write“ genome and consequences of its application in oncology prof. A.Vašků

TBCM, September 22, 2017

Theoretical Bases of Clinical Medicine

VLTU0451c – Theoretical Bases of Clinical Medicine – interactional seminar
5th Semester
1-2 hrs a week, A11/ 114 Campus

3/5 Cancerogenesis

Guarantor of topic: prof. MUDr. Anna Vašků, CSc.

22. 9.2017	A11/114 Campus 8:30-10:10	Concept of „Read and Write“ genome and consequences of its application in oncology (prof. A.Vašků, 2 hrs)
29. 9.2017	A11/114 Campus 8:30-9:45	„Next generation sequencing“ in oncology (doc. Mráz, 1.5 hrs)
6. 10. 2017	A11/114 Campus 8:30-10:10	Importance of cytogenetics in hematological malignancies (doc. Ševčíková, 1.5 hrs)
13.10. 2017	A11/114 Campus 8:30-10:10	Importance of epigenetics in oncology (doc. Slabý; 1.5 hr)
20. 10. 2017	A11/114 Campus 8:30-9:45	Pathogenesis of leukemia´s (prof. Mayer, 0,5 hod) Pathogenesis of multiple myeloma (doc. Ševčíková, 1 hr)
27.10. 2017	A11/114 Campus 8:30-9:20	Pathogenesis of solid tumors (doc. Křen, 1 hr, přednáška)

Theoretical Bases of Clinical Medicine

VLTU0451c – Theoretical Bases of Clinical Medicine – interactional seminar
5th Semester
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4/5 Hypertension

Guarantor of topic: prof. MUDr. Miroslav Souček, CSc.

3. 11.2017	A11/114 Campus 8:30-10:10	Dysregulation of blood pressure in hypertension, hypertension in childhood, methodology of blood pressure measurement –misinterpretation of values (2 hrs- dr. Z. Nováková)
10.11.2017	A11/114 Campus 8:30-9:20	Dysregulation of blood pressure in hypertension, hypertension in childhood, methodology of blood pressure measurement –misinterpretation of values (1 hr- dr. Z. Nováková)
17. 11. 2017	A11/114 Campus	Bank Holiday
24. 11.2017	A11/114 Campus 8:30-10:10	Clinical and pharmacological aspects of hypertension II (2 hrs, prof. Souček)
1.12.2017	A11/114 Campus 8:30-10:10	Strokes I (2 hrs, prof. Smrčka)
8.12.2017	A11/114 Campus 8:30-9:20	Strokes II (1 hr, prof. Smrčka)

„Read and Write genome“ concept

- Genomes are DNA databases containing coding and formatting sequences that permit heritable transmission of the capacity to synthesize biologically adaptive RNA and protein molecules. In the course of evolution, the coding sequences and formatting signals change to encode novel adaptations. Traditionally, these genome changes have been attributed to accidental causes. But research on the mechanisms of DNA mutability has led to a different picture of active biologically mediated genome restructuring (Shapiro, 2011 and Shapiro, 2013).
- The fluid read–write (RW) genome replaces the constant read-only memory (ROM) genome.

„Read and Write (RW) genome“ concept

- The basic idea of the RW genome is that cells use DNA as a modifiable data storage medium to encode the RNA and protein molecules they need to deal with changing circumstances.
- Change is continual for living organisms. Alterations occur as cells progress through the cell cycle, as environmental conditions vary, as cells experience damage, as multicellular morphogenesis proceeds, and as they interact with other cells and organisms.

„Read and Write (RW) genome“ concept

- To manage recurring short-term variations, cells primarily deploy transient nucleoprotein complexes to regulate expression of genome data and carry out the necessary tasks of cell growth and replication.
- For longer-term changes, such as cellular differentiation and multicellular morphogenesis, heritable epigenetic modifications of the genome come into play over a number of cell generations.
- For the longest-term changes that create new biological functions in evolution, cells engage their natural genetic engineering (NGE) capabilities to acquire and alter DNA sequences and reconfigure genome organization. Sometimes, NGE functions also alter DNA structure to meet shorter-term needs, such as rapid generation of diversity among adaptive immune system receptors and countervailing variation of surface antigens on microbial invaders.

„Natural genetic engineering systems“

- The phrase “natural genetic engineering” (NGE) reflects the reality that living cells possess all the biochemical tools necessary for cutting, splicing, polymerizing and modifying DNA (*i.e.*, writing on their genomes.
- NGE functions are analogous to the tools we use in artificial genetic engineering. In addition, there are certain complexes of biochemical activities and DNA sequences that constitute specialized genome change cassettes, summarized under the title “mobile DNA”(Craig et al., 2015).

NGE

Endo- and exo-nucleases

Ligases

Error-free and mutator DNA polymerases

Site-specific recombinases

Homologous recombination complexes

Non-homologous end-joining (NHEJ) complexes

Reverse transcriptases (RTs)

Transposons and transposases

Retroviruses / retrotransposons and cDNA integrases

RNA chaperones / endonucleases and RTs for target-primed reverse transcription (TPRT) and genome insertion of cDNA copies of retroelement or cellular RNAs

DNA secretion and uptake systems

RNA secretion and uptake systems

Viruses

RW concept and cancer

- Cancer occurs when cells decouple from an organism's normal regulatory apparatus and proliferate uncontrollably to form a neoplasm, which may spread around the body to colonize remote organs (metastasis).
- Cancer remains deeply mysterious. Shapiro's attempt to redefine the field of genomics has the potential to transform the conceptual basis for understanding cancer and thereby suggesting more effective therapies.

RW concept and cancer

- Cancer comes in many varieties (it is, for example, specific to the organ of origin), but its progression generally follows a pattern. Clinicians have identified a dozen or so “hallmarks” of cancer, such as genome instability and heterogeneity, a shift in metabolism from oxidation–phosphorylation to fermentation (glycolysis), disabling of apoptotic pathways, transition to a motile state, evasion of the immune system, and a degree of cell–cell cooperation including the recruitment of normal cells in the vicinity of the primary tumor and in metastatic niches.

RW concept and cancer

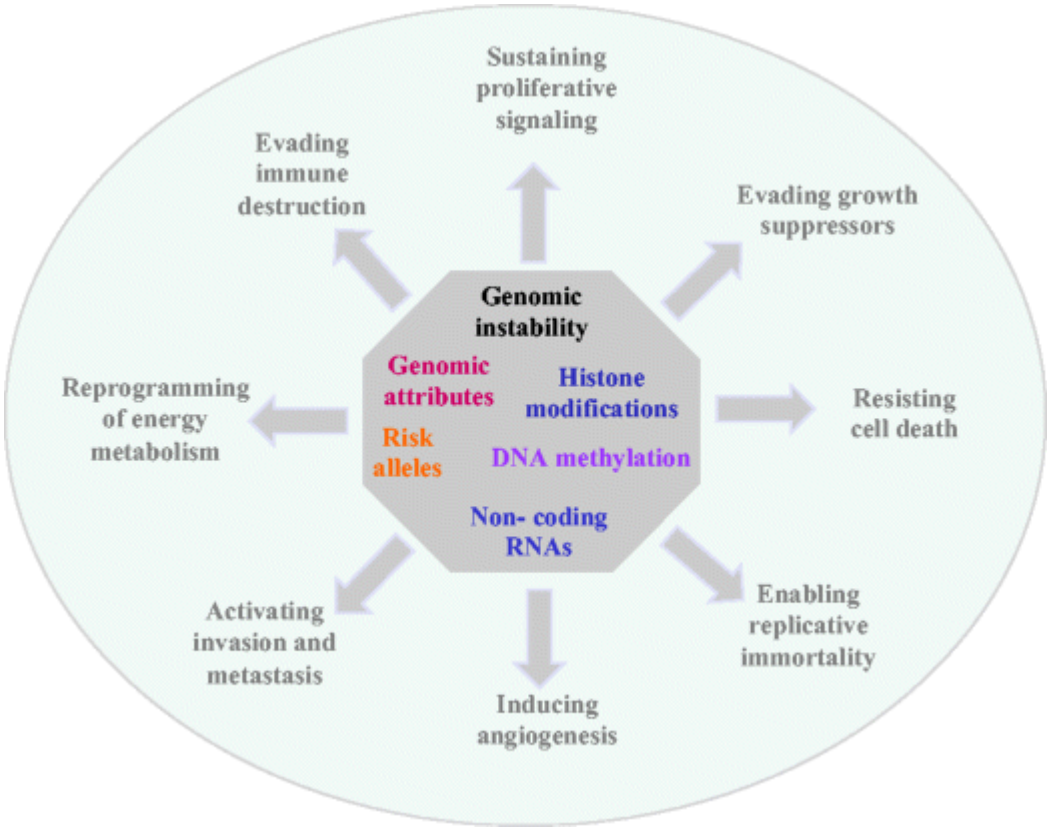
- In spite of the focus on cancer as a human disorder, it is found to be widespread in biology, occurring among mammals, fish, reptiles and even plants. This suggests ancient evolutionary roots.
- Multicellular organisms outsource their heritability to specialized germ cells, and in return somatic cells are subject to strict controls over their proliferation, such as apoptosis and tumor suppressor mechanisms.
- Cancer represents a breakdown in these controls, and thus an abrogation of an ancient covenant that dates back to the dawn of multicellularity over 1 billion years ago. Even “primitive” organisms such as sponges, with lineages hundreds of millions of years old, possess tumor suppressor genes.

Cell division dynamics during lifetime

- Cell division is carefully controled to serve to actual needs of the organism.
- Early in life, cell division capacity outweighs their destruction, in adulthood, it is in dynamic equilibrium and in senescence, involution dominates.
- Some cells are able to obviate the replication control. They change their phenotype to tumor ones.
- Benign tumor cells
- Malignant tumor cells (invasivity, metastatic potential).

Cancer

- Cancer does not seem to be a series of genetic accidents, but a deeply pre-programmed, ancient, systematic response to an insult or stress!
- The “cancer subroutine” is unlikely to be as simple as a cassette of genes activated in an orderly sequence but a gene regulatory network with a pre-defined dynamical trajectory.



Tumorigenesis is a multistep evolutionary process involving myriad, genetic, and epigenetics alterations. Epigenetic alterations during cancer initiation and progression provide an abundant source of variability that not only promotes rapid tumor adaptation but also provides a rich source of labile biomarkers to complement genetic analysis

Tumour Biol. 2016 Jul 28. **Biomarkers of genome instability and cancer epigenetics.** Reis AH, Vargas FR, Lemos B.

Genome stability

- Since our genomes are constantly exposed to exogenously-derived (e.g. UV radiation) and endogenously-derived (e.g. metabolically generated reactive oxygen species) DNA damaging agents, an impaired ability to detect and/or respond appropriately to these efects can impact on the maintenance of genetic stability.

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

Genome stability

- There are many examples of human Mendelian disorders defective in the repair of or response to DNA damage.
- The importance of these pathways is demonstrated by the increase in cancer predisposition and developmental abnormalities associated with these conditions.

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

Copy number variants

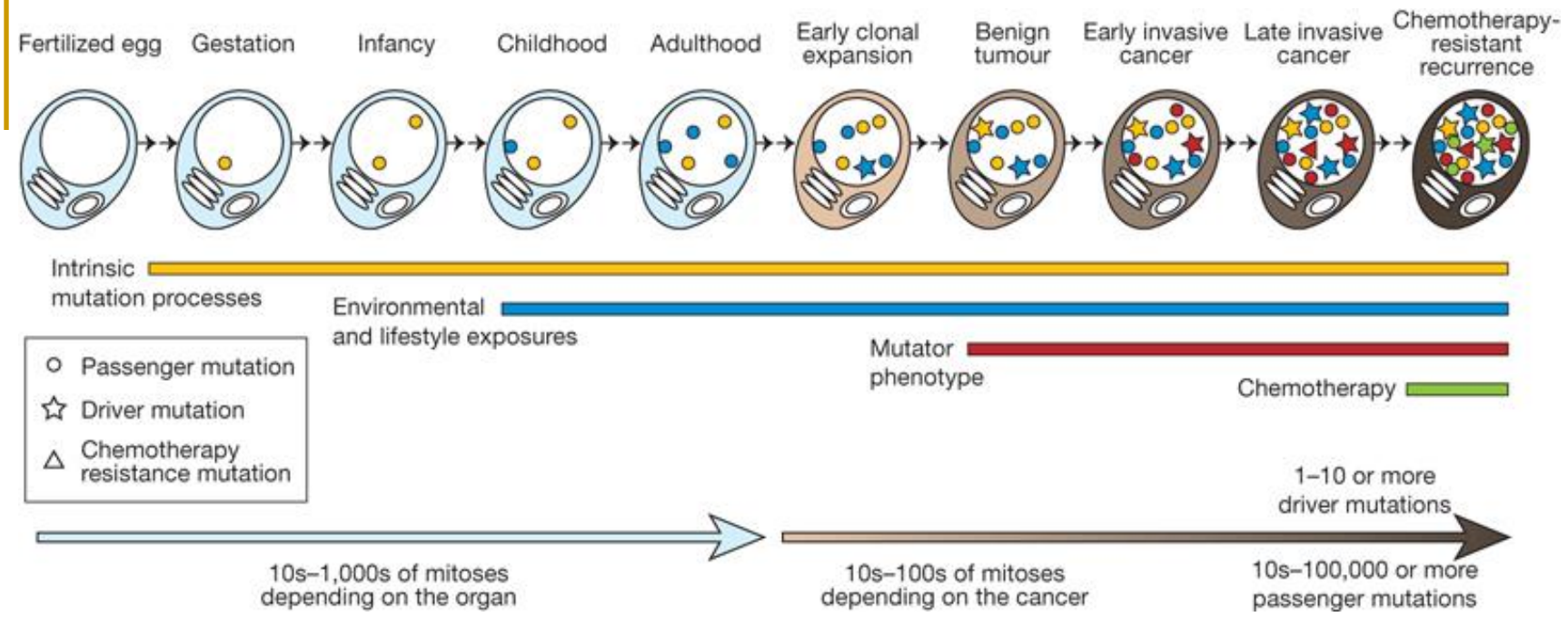
- The changes in gene copy number are associated with different phenotypes in humans. Perhaps, the most well known example of this is the *trisomy 21 causative of Down syndrome.*
- An increased expression of the genes on chromosome 21 results directly or indirectly in a clinically heterogeneous disorder incorporating *cognitive impairment, facial dismorphology, growth retardation, cancer predisposition, microcephaly, heart and skeletal abnormalities*

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

Gene mutations as a cause of variability in genes

- Rare alleles (prevalence less than 1% in population as a result of selection pressure and/or „recent“ mutation). These mutations represent „great genetic factors“ causing *monogenic diseases*
- Polymorphisms (prevalence more than 1% in population, smaller genetic factors in interactions with environmental factors conditioning *complex diseases* (including most of sporadic cancers).

Somatic cell mutations: an example
Sporadic colorectal cancer

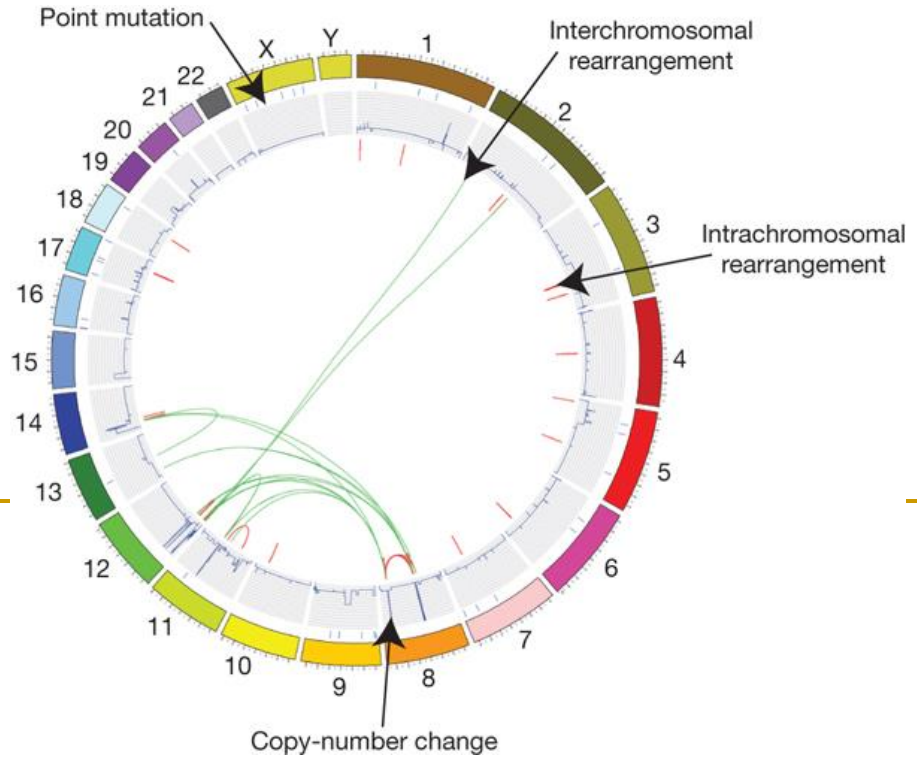


MR Stratton *et al. Nature* **458**, 719-724 (2009)

The lineage of mitotic cell divisions from the fertilized egg to a single cell within a cancer showing the timing of the somatic mutations acquired by the cancer cell and the processes that contribute to them.

nature

Figurative depiction of the landscape of somatic mutations present in a single cancer genome.



MR Stratton *et al. Nature* **458**, 719-724 (2009)

Somatic cell mutations: an example
Sporadic colorectal cancer

nature

Tumor phenotype

- Is characterised by the process of malignant transformation of the cell with
  - *loss of control of cell division* (alterations in cell cycle, antiapoptotic state, „immortality „ of tumor cell, alteration in signal transduction),
  - *loss of cell-cell contant inhibition, invasivity,*
  - *changes in metabolism*
  - *proangiogenetic activation*
- Malignant transformed cells seem to be resistent to external stress factors as hypoxia, low pH, hypoglycemia, malnutrition.

Malignant transformed cells

- Continue their division.
- Needs for presence of hormones and growth factors decreased.
- Production of own growth factors *(autocrinnal stimulation*).
- Decrement of ability to stop the growth.
- Decreased ability to stop the growth in worse nutrition conditions-

Malignant transformed cells

- Occur most frequently in the tissues with quick proliferation
- Environmental factors have a great impact on gene expression of the target cells
- A plenty of signals accepted by the cell leads to activation of specific transcription factors which decide about either

- ✓ *division or*
- ✓ *differenciation or*
- ✓ *apoptosis*

Tumors and immortality of cells

- Increased activity of telomerase.
- Telomers are key stabilisation factors of terminal part of the chromosome.
- Telomeres have repetitive sequence TAGGG.
- The length of telomers is decreased after multiple divisions of the cell (1 cell cycle is shortening of 1 telomere).
- Their renewal is catalysed by telomerase.

Two mechanisms during tumorigenesis:
mechanisms TERT („telomerase reverse transcriptase“)
„alternative lengthening of telomere (ALT) pathway“),

Metastatic phenotype

- Increased *motility, invasion* and ability to form *metastases*.
- Increased production of receptors (adhesive molecules)
- Increased production of hydrolytic enzymes
- Function of chemokines

Metastatic phenotype

- *Loss of dependence* to adhere to substrates (tumor cells are able to divide even in tissue culture)
- *Loss of gap junctions*.
- *Changes in cell membrane* (glycolipids and glycoproteins modifications).

Phenomenon of tumor suport

- Chemical cancerogenes:
- *Initiators:* compounds with cancerogenic potential after their metabolisation (P450).
- *Promoters:* effects possible after initiators *(phorbolester)*.

Tumor genetics

- The development of cancer is associated with a fundamental genetic change within the cell. Evidence for the genetic origin of cancer is based on the following:
- Some cancers show a familial predisposition.
- Most known carcinogenes act through induced mutations.
- Susceptibility to some carcinogens depends on the ability of cellular enzymes to convert them to a mutagenic form.
- Genetically determined traits associated with a deficiency in the enzymes required for DNA repair are associated with an increased risk of cancer.
- Some cancers are associated with chromosome 'instability' because of deficiencies in mismatch repair genes.
- Many malignant tumours represent clonal proliferations of neoplastic cells.
- Many tumours contain well-described cytogenetic abnormalities, which involve mutated or abnormally regulated oncogenes and tumour suppressor genes with transforming activity in cell lines.
- Mutations may occur in the germline and therefore be present in every cell in the body, or they may occur by somatic mutation in response, for example, to carcinogens, and therefore be present only in the cells of the tumour.

Tumor genotype

Mutations in

- ✓ Proto-oncogens
- ✓ Tumor-supressor genes
- ✓ Genes for genome stability
- ✓ Genes - modifiers.

Tumor genetics

- There is a small group of autosomal dominant inherited mutations such as *RB* (in retinoblastoma) and a small group of recessive mutations.
- Carriers of the recessive mutations are at risk of developing cancer if the second allele becomes mutated, leading to 'loss of heterozygosity' within the tumour although this is seldom sufficient as carcinogenesis is a multistep process.

Table 9.3
Familial cancer syndromes

	Tumour suppressor gene	Neoplasms
Autosomal dominant		
Retinoblastoma	*RB1*	Eye
Wilms' tumour	*WT1*	Kidney
Li–Fraumeni	*p53*	Sarcoma/brain/leukaemia
Neurofibromatosis type 1	*NF1*	Neurofibromas
Familial adenomatous polyposis (FAP)	*APC*	Colon
Hereditary non-polyposis colon cancer (HNPCC)	*MLH1* and *MSH2*	Colon, endometrium
Breast ovary families	*BRCA1* and *BRCA2*	Breast/ovary
Melanoma	*p16*	Skin
Von Hippel–Lindau	*VHL*	Renal cell carcinoma and haemangioblastoma
Autosomal recessive		
Xeroderma pigmentosa	*XP*	Skin
Ataxia telangiectasia	*AT*	Leukaemia, lymphoma
Fanconi's anaemia	*FA*	Leukaemia, lymphoma
Bloom's syndrome	*BS*	Leukaemia, lymphoma

Table 9.4
Acquired/somatic mutations and proto-oncogenes

Point mutation	
ras	Pancreatic cancer
DNA amplification	
myc	Neuroblastoma
HER2	Breast cancer
Chromosome translocation	
BCR-ABL	CML, AML, ALL
PML-RAR	APML
EWS	Ewing's sarcoma
IGH-bcl2	Follicular lymphoma

Abbreviations: CML, chronic myeloid leukaemia; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; APML, acute promyelocytic leukaemia

Tumor genetics

- Malignant transformation may result from a ***gain in function*** as cellular proto-oncogenes become mutated, (e.g. *ras*), amplified (e.g. *HER2*), or translocated (e.g. *BCR-ABL*).
- Alternatively, there may be ***a loss of function*** of tumour suppressor genes that normally suppress growth and differentiation.
- However, these mutations are insufficient to cause malignant transformation by themselves.

Gene mutations as a cause of variability in genes

- *Mutations in somatic cells*
- ✓ are generating in somatic cells during the lifetime
- ✓ are cell and/or tissue specific, without transfer to offspring

- *Mutations in germ cells*
- ✓ they become components of genetic predisposition
- ✓ they are present in all cells of the individual
- ✓ they are transfered to offspring

Proto-oncogenes and oncogenes

- Up to 100 of various human protooncogenes in each somatic cell.
- Targets for transduction signals (mitogenic), by which their expression is regulated

Proto-oncogenes and oncogenes

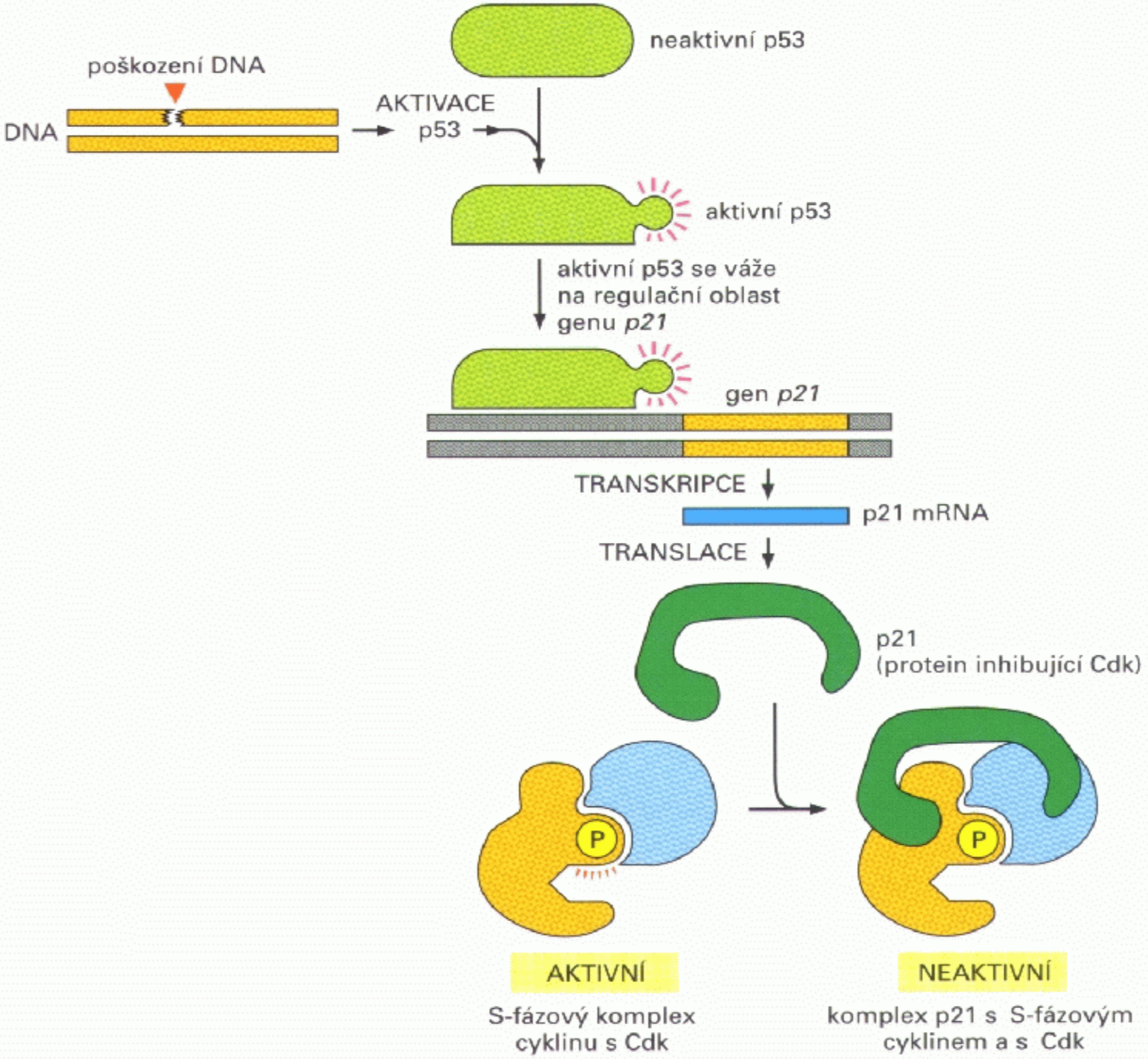
- Mutations of proto-oncogenes change them to oncogenes.
- Dominant mutation.
- Different pathways of proto-oncogenes activation:
- (a) viral transduction (e.g. onkogene *src*)
- (b) gene amplification (*myc, abl)*,
- (c) viral insertion (*myc*)
- (d) chromosomal alteration (*myc, abl*)
- (e) mutation (*ras*)

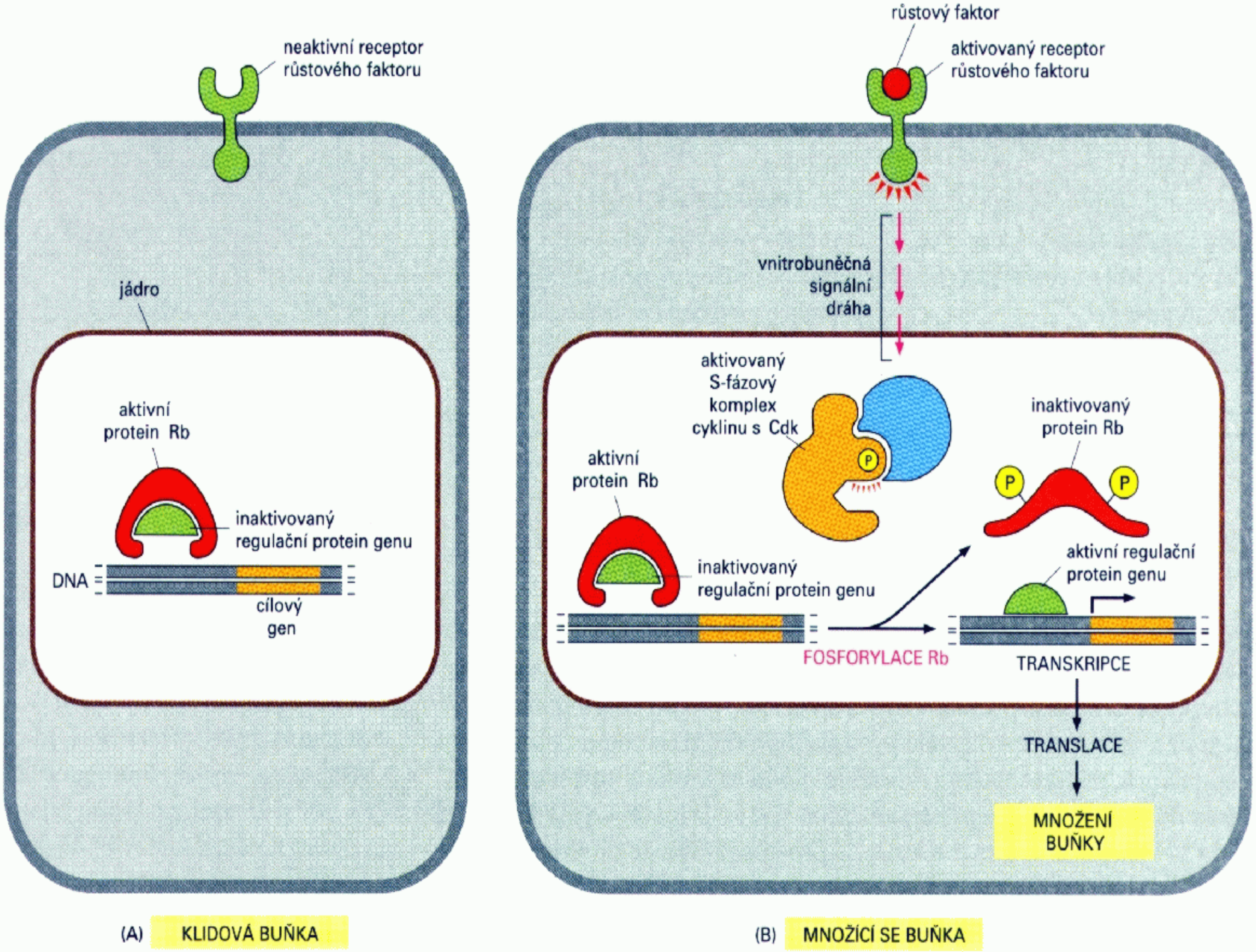
Types of oncogenes

- They are classified to 5 classes:
- (1) growth factors
- (2) growths factors receptors
- (3) intracellular transducers of the signals
- (4) *nuclear transcription factors*
- (5) *proteins controlling cell cycle*

Tumor-supresor protein p53 (TP53)

- Is a nuclear protein with key role between G0 and G1 phase of the cell cycle.
- Mutant variants of the p53 gene can be found in many cancer types.
- Somatic mutations-usually
- Germ mutations (*syndrome Li-Fraumeni).*


neaktivní p53
poškození DNA
DNA
AKTIVACE
p53
aktivní p53
aktivní p53 se váže
na regulační oblast
genu p21
gen p21
TRANSKRIPCE
p21 mRNA
TRANSLACE
p21
(protein inhibující Cdk)
P
P
AKTIVNÍ
S-fázový komplex
cyklinu s Cdk
NEAKTIVNÍ
komplex p21 s S-fázovým
cyklinem a s Cdk


neaktivní receptor
růstového faktoru
jádro
aktivní
protein Rb
inaktivovaný
regulační protein genu
DNA
cílový
gen
(A) KLIDOVÁ BUŇKA
růstový faktor
aktivovaný receptor
růstového faktoru
vnitrobuněčná
signální
dráha
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S-fázový
komplex
cyklinu s Cdk
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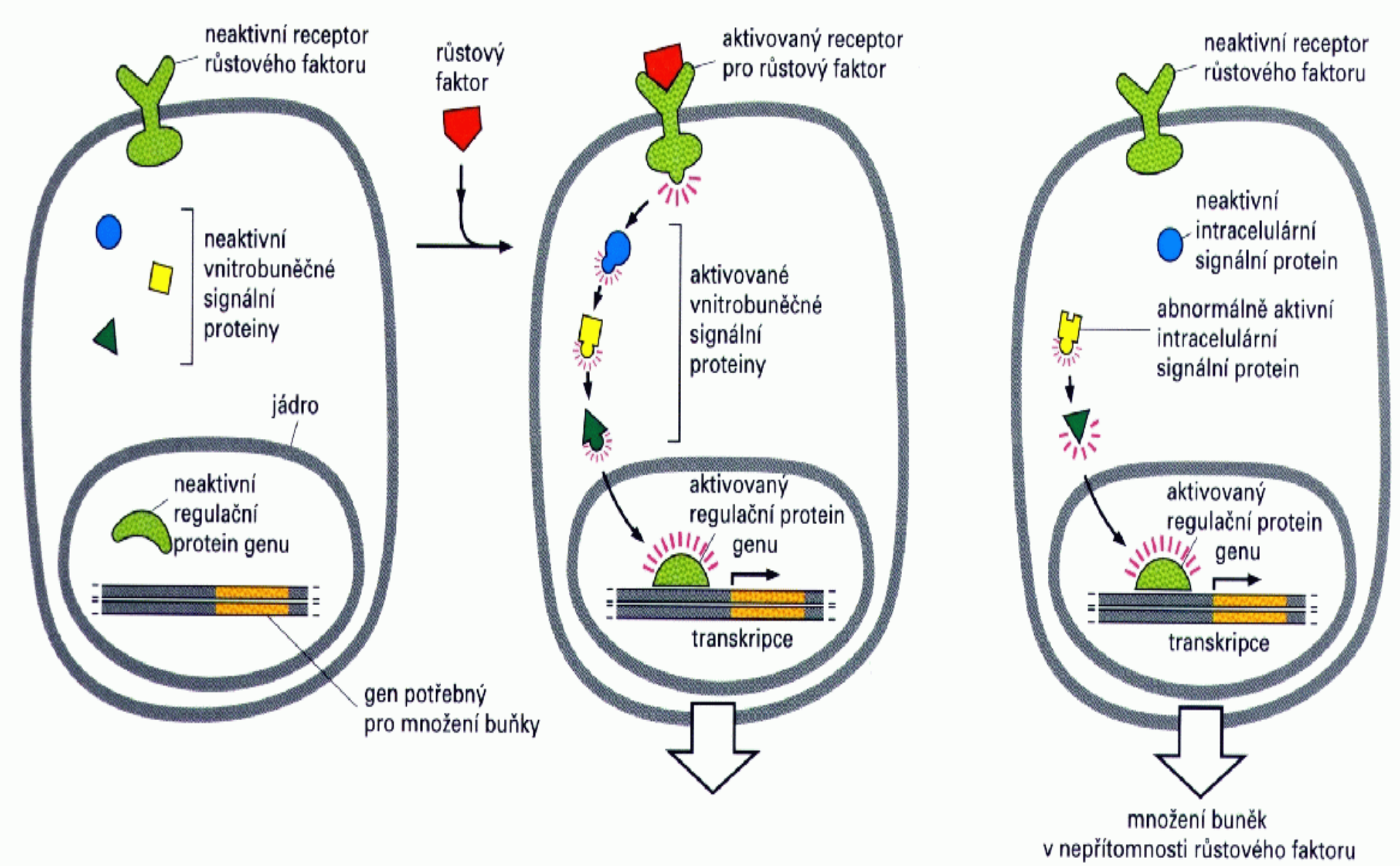
P
inaktivovaný
regulační protein genu
aktivní regulační
protein genu
FOSFORYLACE Rb
TRANSKRIPCE
TRANSLACE
MNOŽENÍ
BUŇKY
(B) MNOŽÍCÍ SE BUŇKA

p53-germ cell mutation

- Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome that is commonly associated with a germline mutation in the tumor suppressor gene p53.
- Loss of p53 results in increased expression of CD44, a cancer stem cell (CSC) marker, which is involved in the scavenging of reactive oxygen species (ROS).



(A) NORMÁLNÍ KLIDOVÁ BUŇKA

(B) NORMÁLNÍ MNOŽÍCÍ SE BUŇKA

(C) MNOŽÍCÍ SE RAKOVINNÁ BUŇKA

Tumor epigenetics

- It is now generally accepted that human cancer cells harbor global epigenetic abnormalities and that epigenetic alterations may be the key to initiating tumorigenesis(Baylin and Jones, 2011; Sandoval and Esteller, 2012; Sharma et al., 2010).
- The cancer epigenome is characterized by substantial changes in various epigenetic regulatory layers.
- Differences in DNA and histon methylation and acetylation state of histones in cancer DNA have been found

Inflammation and tumorigenesis

- Epigenetic switches play an important role in cancer development.
- Most of the currently discovered switches are induced by inflammation or involve inflammatory signaling.
- Chronic infections and inflammation contribute to about 25 % of all cancers.
- In many examples, the same inflammatory trigger (e.g., IL-6) that induced the switch is constitutively activated in cancer cells to maintain the new (tumor) phenotype.

Inflamation and tumorigenesis

- This suggests that inflammatory signaling, which initially originates from immune cells is adopted by cancer cells through epigenetic switches. Since epigenetic switches are reversible, they might represent excellent targets for anticancer therapy.

Apoptosis and tumorigenesis

- *Overexpression of Bcl2* (B-cell lymphoma 2) gene was found to be related to inhibition of apoptosis during tumorigenesis.

Tumor neoangiogenesis

- Dysequilibrium between antiangiogenetic and proangiogenetic factors („angiogenetic switch“).
- Without neoangiogenetic switch, the tumor growth is possible only to 1 – 2 mm^3, when O2 and nutrients support is possible by difussion from surrounding tissue.
- Hypoxia of tumor cells
- HIF- alpha induced transcription angiogenetic factors

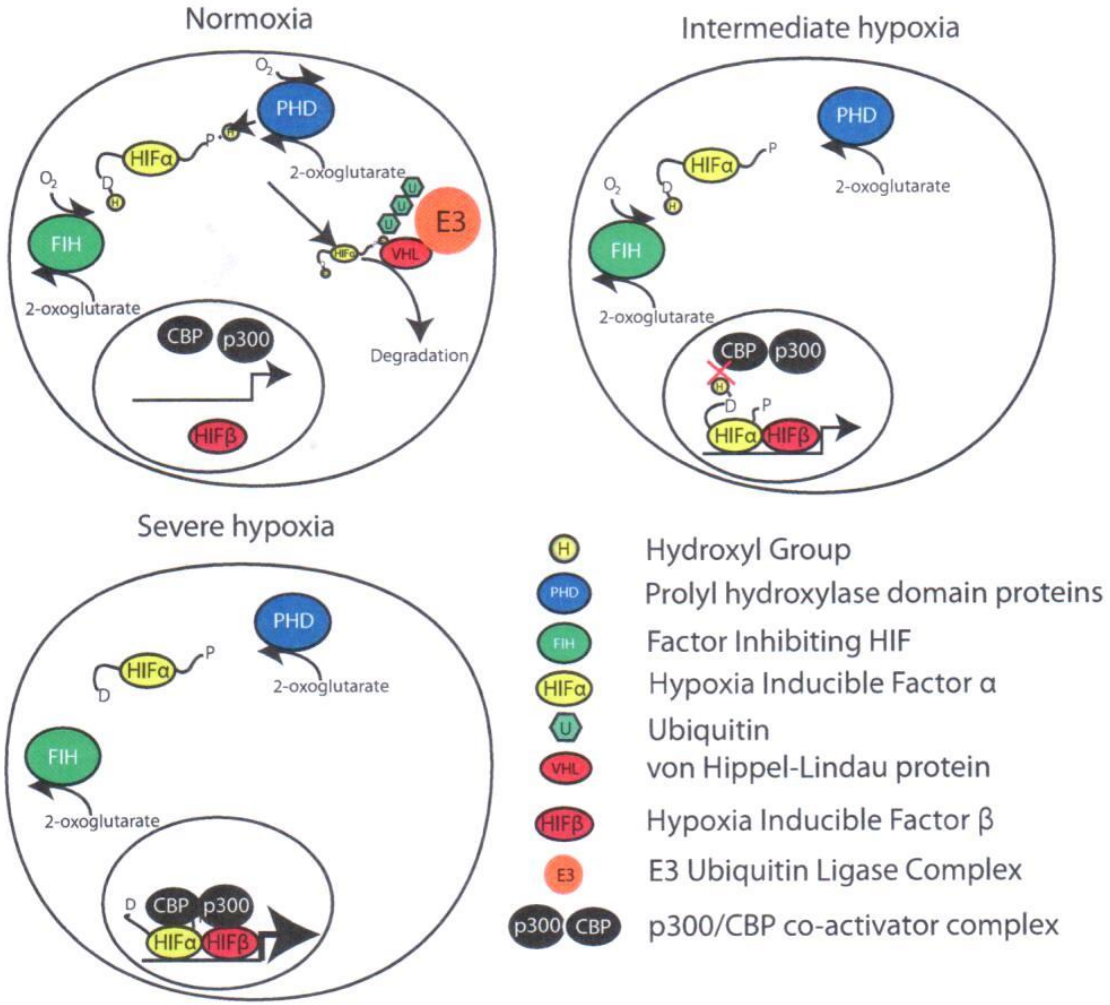


Figure 1. Mechanism of HIF activity. Under normoxic conditions, HIFα subunits are hydroxylated on proline residues. Hydroxylated prolines are recognised by the von Hippel-Lindau protein, ubiquinated by the E3 ubiquitin ligase, and targeted for proteosomal degradation. As oxygen levels fall, HIFα is stabilised and enters the nucleus to form a transcriptional complex with HIFβ subunits. FIH activity is maintained at lower oxygen levels than PHDs and remains active, hydroxylating asparagines. Hydroxylation of asparagines by FIH prevents association of the CBP/p300 coactivator complex with the HIFα/HIFβ transcriptional dimer. Under very low oxygen conditions, FIH becomes inactive and maximal HIF transcriptional activity is promoted.

doi:10.1371/journal.pbio.1001116.g001

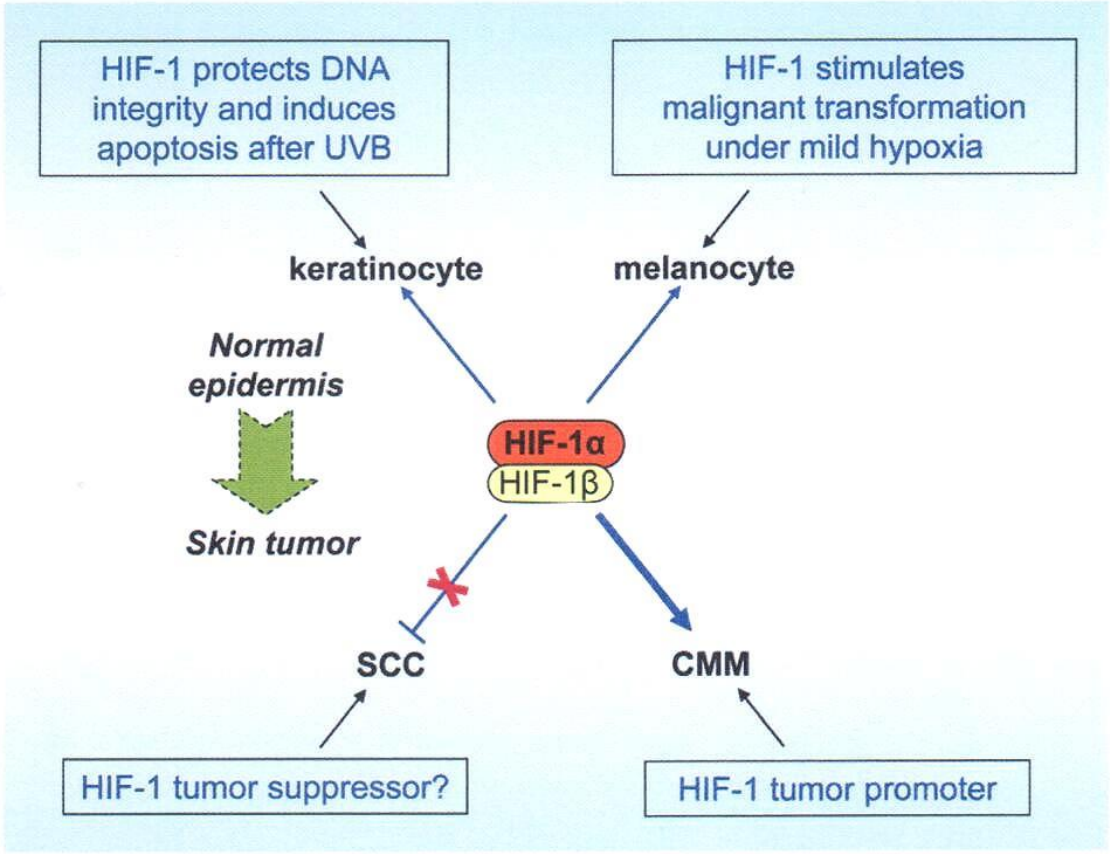
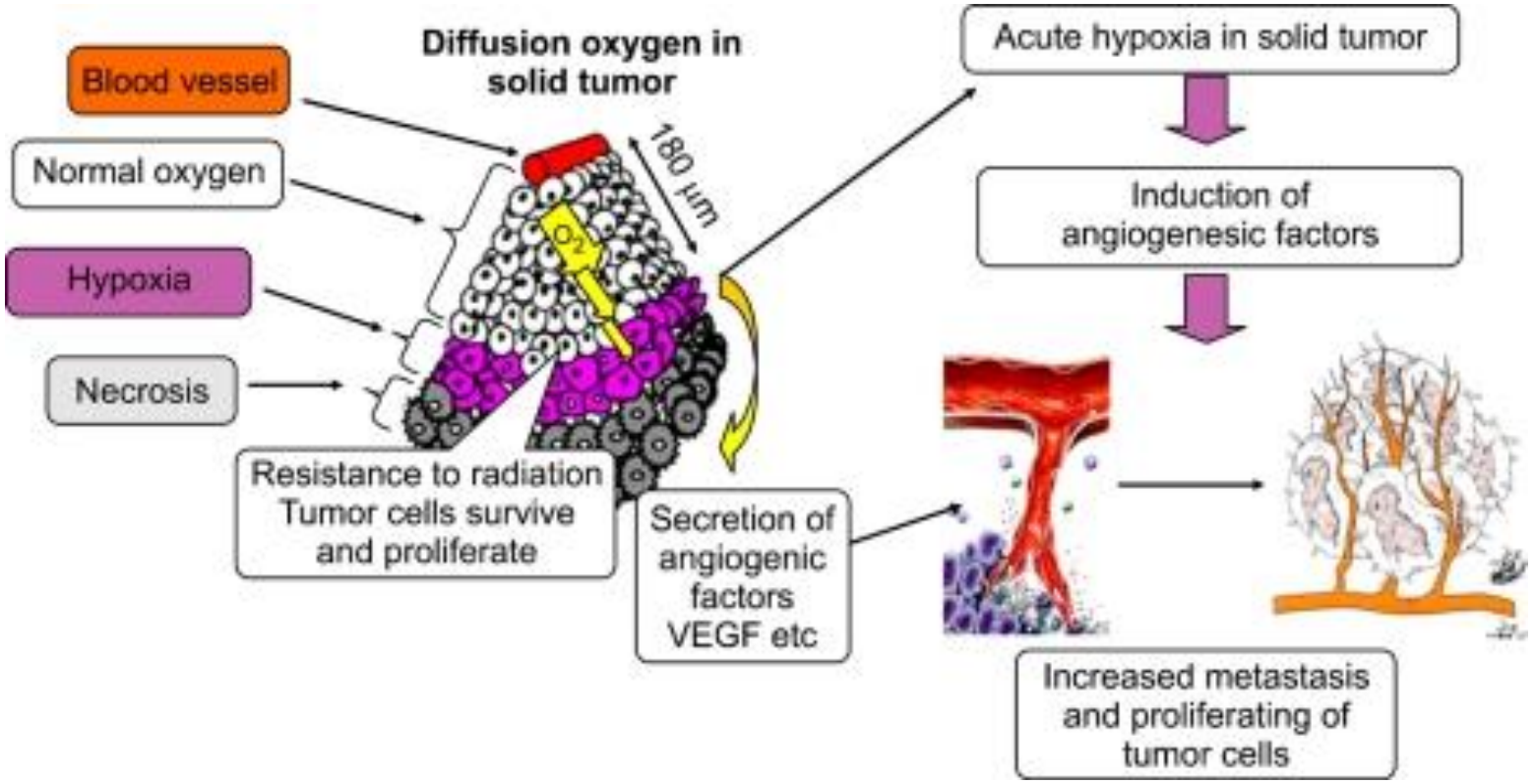


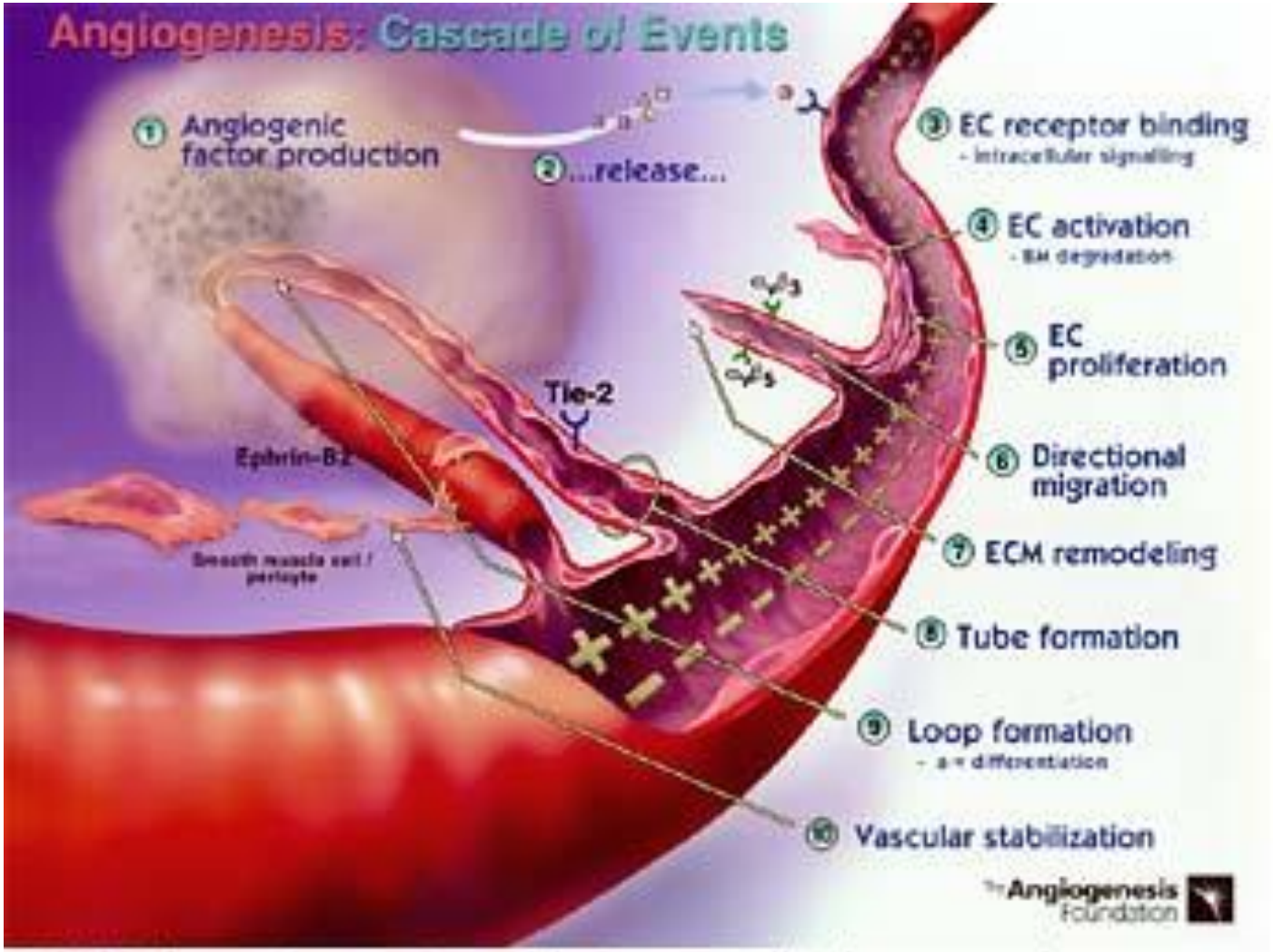
Fig. 4. Possible differential role of HIF-1 in skin homeostasis and carcinogenesis. In the epidermis, HIF-1 could play a dual role dictated in a cell specific manner. From one hand, HIF-1, by facilitating DNA integrity, cell cycle arrest or elimination of severely UVB damaged keratinocytes by the induction of apoptosis, could act as a tumor suppressor preventing the formation of non-melanoma skin cancer (SCC). On the other hand, HIF-1 along with skin mild hypoxia and oncogene expression could contribute to the transformation of melanocytes in to melanoma (CMM).



Chen L. et aL. Exp Mol Med. 2009; 41(12): 849–857.

Hypoxia and tumor angiogenesis

- Hypoxia occurs in chronic and acute vascular diseases and tumor formation.
- It is toxic to normal cells, but cancer cells can survive and continue to proliferate in hypoxia.
- Human tumors grew like cords around blood vessels, and tumor cells located > 180 μm from the blood vessels were observed to become necrotic.
- Hypoxia induces angiogenesis during tumor growth; after tumor growth progresses, an oxygen gradient develops from the oxygen source to the periphery of the tumor. Cells lacking oxygen and nutrition because of their distance from the blood supply become necrotic, whereas those closer to the blood supply begin sensing hypoxia and secrete angiogenic factors. As a result, angiogenesis occurs and the tumor develops its own vasculature, independent of the original tissue.



Thank you for your attention