

Mechanisms of carcinogenesis

Basic characteristics of malignant transformation

Malignant tumour is a genetic disease. It occurs as a result of accumulation of somatic alterations of specific genes induced by genotoxic effects: physical (ionising radiation), chemical (genotoxic substances) or biological (failure of endogenous replication processes, viruses). Creation of malignant tumour can be induced or slowed by existing hereditary mutations (alterations of highly penetrating predisposition genes vs. alterations of genes with limited penetration). Epigenetic factors influencing expression of genetic information (mostly histone modification) have also major influence on the creation of tumours.

The most typical characteristic of cancer cells is their uncontrollable growth throughout all physiological structures of organism. Physiological structures begin on cellular levels from which specialised tissues and organs are form. Physiological structures have multiple layers of regulation. On cellular level in the form of a local signalisation (growth factors, cytokines, immunomodulatory molecules) while neurohumoral systems are effecting organs and organ systems from distance (mainly hormones). Correct function of cellular components is under the surveillance of immune system. The formation of malignant tumour is caused by a defect in homeostasis beginning on a genome level of a malignantly transformed tissue, which combined with the failure of controlling mechanisms of immune system and as the result of spreading metastases, can cause symptoms of systemic collapse leading to death. Even though the progression of cancer transformation is different among malignant tumours, neoplastic tissue always exhibits similar characteristics resulting from defects of transforming cells on the level of:

- **Interpretation of local and systemic signalisation.**
- **Internal homeostasis** - mostly affected genome.
- **Local intracellular** communication and communication of a cell with its surroundings.

These processes have regulatory function for basic behaviour of each cells within tissues:

- **Regulation of mitosis** (replication, apoptosis).
- Regulation of functional capacity within tissue (differentiation and migration of cells).

All those processes are contributing to **tissue homeostasis** - dynamic balance between a creation and death of cells with respect to actual needs of specific tissue within organism (unscathed hierarchy of cell structures which is a condition of physiological integrity of organism). Failure of such processes leads to characteristic symptoms of transformed cancer cells populations:

- Autonomy in production of growth factors (GF).
- Reduced sensitivity to inhibition GF .
- Faulty apoptosis.
- Unlimited replication potential (immortalisation).
- Defective repair of DNA (genome instability).
- Pronounced angiogenesis.
- The ability of metastasis and tissue invasion.

Even though there is a number of deregulated intracellular signals that are causing malignant transformation uncontrollable growth is mainly the result of a failure of two critical systems: cell cycle and apoptosis. These two systems have complementary effect in influencing tissue homeostasis (balance between creation of cells in the process of cell cycle and death of used/damaged cells in the processes of apoptosis defines normal physiological state in tissue of adult organism). Cancer cells are always characterised by defection of such balance caused by increased speed of cell cycle and resistance against the induction of apoptosis. Defects of both systems are caused by mutations along with epigenetic alteration of genes which regulate both processes (oncogenes and protooncogenes that are participating in stimulation of cell cycle and inhibition of apoptosis and tumour suppressor genes which are in opposition to oncogenes). Genetic alterations are tolerated in cancer cells because the third critical condition of malignant transformation is deregulation of DNA reparative mechanisms. Failure of reparative mechanisms causes genome instability resulting in cumulation of faulty genetic alterations which can damage other regulatory mechanisms responsible for the creation of heterogeneous cancer populations with high malignant potential.

The origin of cancer cells

What is the corner stone from which is created initial cluster of carcinogenic transformed immortalised cells? In each tissue there are three basic cell populations that are needed for physiological regeneration during human lifetime because regeneration time of most cells (except pyramid nerve cells) resembles a fraction of organisms lifespan:

- **Tissue specific stem cells** - with unlimited replication potential in tissues: they restore reserve of progenitor cells .

Characteristics: replication potential is similar to the whole lifespan of an organism. Concentration in tissues is very low. Unlimited however low mitotic potential. The absence of most phenotype signs of matured cell populations.

- **Progenitor cells** - supplement the continuous reduction of damaged mature cells in tissues.

Characteristics: limited regeneration potential; fast proliferation; the ability to migrate and the ability to differentiate to specialised cells of tissue at need. Depending on the degree of proliferation there can be phenotype signs of mature cells.

- **Specialised tissue cells** are created by differentiation from its progenitor and make up >99,9% of cell population in tissues.

Characteristics: exert tissue specific functions; have low replication potential (they age considerably in the process of a few replications and have to be replaced by a new population from progenitor cells); terminal and specialised phenotype; limited or no ability to migrate (applies to solid tissues).

With regard to known characteristics of cancer cells (unlimited replication potential, loss of contact inhibition, migration, incomplete phenotype expression signs of fully differentiated cells etc.) it is likely the first transformed cancer cell (malignant stem cell) clone forms by gradual accumulation of genetic and epigenetic alterations. Origin of such cell comes most likely from a line of stem cells or progenitor cells rather than fully matured tissue cells.

Intracellular signalisation defects leading to cancerous transformation

If the defect in regulation of cell cycle, apoptosis and simultaneous DNA reparative mechanism inactivation is the cause of cancerous transformation which specific genes and signal processes are the root cause? Even though there have been recognised a thousands of somatic DNA alterations and epigenetic insults, there are specific signal transduction pathways whose defects are critical for cancerous transformation.

Mitosis signalisation defects and the failure of the cell cycle

The failure of the cell cycle is, with regard to its regeneration mechanism, effecting mainly initial phase - cell cycle activation and regulation of cell cycle control steps. Pro-mitotic signalisation includes detection, transmission, spread and amplification of mitotic signal which eventually initiates such changes in gene expression of stimulated cell that induce synthesis of protein regulators controlling progression through cell cycle (for example cytokines). Those pathways are carried out by signal cascades on biochemical level including intrinsic signal molecules (ligands), protein receptors and transducers transporting mitotic signal on kinases. Those kinases modulate activity of specific transcription factors responsible for gene expression of genes regulating progression through cell cycle. Such actions are represented by signalisation of:

- **Growth factors** and its receptors - receptors with intrinsic tyrosine kinase activity including RF>RRFs>adaptors (Grb/SOS)>transducers (Ras)>kinase system (MAPKKK>MAPKK>MAPK) and target TFs (Elk). All components mentioned stimulate cell cycle.
- **Cytokines** and its receptors - as an example of direct signalisation typically based on cytokine receptor activation (without intrinsic TK activity) which (as a result of ligand induced conformation change) associates with membrane binding molecule Jak kinase phosphorylating its own receptor and with consequently phosphorylated receptor associating molecule STAT protein (signal transducers and activators of transcription). Activating STAT protein phosphorylation enables its dimerization and translocation into the nucleus in which they function as specific TFs.
- **Signalisation incorporating protein kinase B (AKT)** - integrating signalisation from membrane receptors (RTKs, cytokine receptors) and influencing not only cell cycle but even apoptosis.
- **Beta-catenin signalisation** - regulated by contact inhibition provided by adhesion molecules (cadherines and beta-catenin) or stimulated by signal molecules of secretion (often autocrine) signal molecules Wnt proteins interacting with its receptor complex (Frizzled/LRPs) and influencing processes of differentiation in cell.
- Others - Notch, Hedgehog, NFkB (nuclear factors kappa B), nuclear and intracytoplasmatic receptors of lipophilic signal molecules (for example: steroids)

Oncogenes mutations coding individual signal pathways protein components which result in activation of proteins without regulation of superior signal molecule are typical signs of somatic alterations in cancer cells (for example: mutations in genes **EGFR**, **k-Ras**, **PI3K**) and cause pro-mitotic hyper stimulation independent on the presence of superior regulation signal. Similar effects have gene amplifications (amplification of alleles) of genes coding individual transduction cascade proteins (for example: ErbB-2 coding her2/neu receptor, heterodimerising with other members of EGFR family).

Mutation in tumour suppressor genes can have similar effect. Physiological function of such genes is signal cascade inhibition. For example:

- Mutation in **neurofibroma** (NF-1) causing the loss of GAP activity (GTPase activating proteins), enabling prolonged existence of activated Ras-GTP and therefore pro-mitotic hyperstimulation. tím promitotickou hyperstimulaci.
- Inactivating mutation in **APC** whose gene product APC (adenomatous polyposis coil) protein in normal circumstances inactivates beta-catenin signalisation by retention of beta-catenin in cytoplasm. Mutation consequently enables release and translocation of beta-catenin into nucleus and the start of cell cycle

regulatory protein transcription.

- Mutation of tumour suppressor gene **PTEN** causing inactivation of PTEN (phosphatase and tensing homolog) phosphatase which is dephosphorylating 3,4,5-phosphatidylinositol 3-phosphate (PIP3), essential in the PKB/AKT kinase cascade activation, to signal inactive 4,5-phosphatidylinositoldiphosphate (PIP2). Prolonged PIP3 halftime activates AKT kinase cascade without the need of superior regulatory signals.

Research findings from the last couple of years indicate that the important role in the gene expression regulation of signal regulators influencing mitotic signalisation have microRNA (**miRNA**) molecules functioning as oncogenes and tumour suppressor signals. miRNA gene expression mutations or alterations are probably contributing to malignant transformation.

Apart from mutations, negative regulators of mitotic stimulation can be influenced by epigenetic changes on the level of hypermethylation (for example: hypermethylation of SOCS promoters physiologically inhibiting cytokine signalisation).

Cell cycle defects are (except for mitotic signalisation defects mentioned above) effecting regulation of its own cell cycle. On the level of cell cycle action it is often deregulation of the main control point in G1 phase. Molecular basis for this control point is overcoming of the **Rb protein** inhibiting influence which is physiologically achieved by sufficient stimulation leading to the synthesis of genes of early (E2Fs) and consequently late (cytokines from D and E family) response. Cyclins creating active complexes with CDKs are hyperphosphorylating Rb protein, hence disabling its interaction (and inhibition) with the number of regulatory proteins (for example: TFs from the E2Fs family). Rb protein can be pathologically inhibited on the level of gene mutations or pathological cyclin hyperexpression (by gene amplifications, rarely by mutations) hyperphosphorylating (inactivating) Rb protein.

Another important cell cycle deregulation mechanism in cancer cells is factor inhibitors inactivation, which are physiologically blocking cell cycle progression during mitotic hyperstimulation, defects in S-phase DNA replication or faulty chromosome segregation in M-phase. Those factors include two main products of tumour suppressor genes from the CIP/KIP (cyclin-dependent inhibitory proteins/kinase inhibitory proteins; with p21, p27, p57 proteins) and Ink4 (inhibitors of kinases; with p15, p16, p18 and p19 proteins) family. Except for the direct inactivation with mutations there is often defect in the form of functional inactivation of those cell cycle negative regulators on the level of signal pathway and TFs alterations which regulate gene expression (for example: **TGF-beta/SMAD** and **p53**).

Defects in apoptosis

Malignant cell growth during initial clonal expansion of transformed cells in primary deposit is also enabled by defect in the cells ability to initiate apoptotic mechanisms. **Defects in apoptosis** include extrinsic pathway initiation (including DISC complex creation) - eliminating regulatory effect of immunocompetent cells, intrinsic parts of apoptosis activation (characterised by the creation of apoptosome) - enabling the existence and proliferation of cancer cell despite damage to its genome DNA and even defective caspase cascade mediated apoptotic signals.

DISC (death-induced signalling complex) **complex** creation is enabled by death receptors (DRs) trimmerisation. DRs present on the surface of all cells (with the exception of immunologically privileged tissues) are stimulated by death ligands (DL) - signal ligands on membranes of immunocompetent cells. Interaction between DRs and DLs is inhibited in cancer cells by decreasing expression of DRs on the level of gene expression by inactivation of TFs (inhibitions/ mutations/ubiquitinations p53, inhibitory phosphorylation TFs of the FOXO trans activating promotor family for CD95/Apo1/FasR) or inhibition of DL on the basis of decoy receptors (DR lacking intracytoplasmatic DD, which would enable association of adaptor molecules and active DISC complex creation).

Since most active DRs complexes lead to the creation of changeable signal complexes in relation to actual presence of adaptor molecules in cell the **change in adaptor protein gene expression** can critically influence the ability to induce apoptosis. For example while binding FADD protein (Fas-associated protein with death domain) containing DD and death factor domain (DED) enables the completion of DISC complex by connecting procaspase-8 and converting it to caspase 8, association of activated DRs trimmer with adaptors TRAFs (TNF receptor-associated factors) "signal shift" leading to, for example, activation of transformation factors NF kappa-B with significant antiapoptotic effects.

The creation of **apoptosome** (activation of APAF1 and bonding procaspase-9) in intrinsic part of apoptosis is influenced by cytochrome C translocation from inter membrane space of mitochondria to cytoplasm. The main protein family regulating such process is **Bcl-2** protein family including proapoptotic (Bax) and antiapoptotic (Bcl-2) proteins associated with the outer mitochondrial membrane. Ratio of pro- and antiapoptotic proteins regulates the ability to induce intrinsic part of apoptosis. Hyperexpression of antiapoptotic proteins (for example: caused by its gene amplification) and decreased expression of proapoptotic Bcl-2 protein family members (for example: induced by defected TFs, which regulate their gene expression - typically mutation in p53 gene coding p53 protein which transactivates expression of genes such as Bax, PUMA (p53-upregulated modulator of apoptosis; BBC3) and NOXA coding proapoptotic proteins) disabling initiation of **intrinsic apoptotic** pathway.

Caspase activation defect is another mechanism of apoptosis inhibition in cancer cells. Inhibition of proximal caspase-8 activation in extrinsic apoptosis pathway can be caused by over expression of CFLAR (Caspase-8 and FADD-like apoptosis regulator) which is lacking proteolytic activity and competing with procaspase-8 molecule over binding receptor on DISC complex and therefore disabling its activation. The activity of executive caspases (caspase-3) is inhibited in a number of cancer cells by protein family IAP (inhibitors of apoptosis; for example: BIRC5-bacuviral IAP repeat-containing protein 5-survivin) which is highly translated in such cells.

DNA reparative mechanisms defects

Alterations of genes regulating cell cycle and apoptosis is enabled in cancer cells thanks to DNA reparative mechanisms defects whose full activity would under normal circumstances prevent the accumulation of gene insults and its tolerance. Physiologically DNA reparative mechanisms create effective protective barrier preventing creation of malignant transformed cells. Reparative processes are closely linked to regulation of cell cycle and apoptosis because: (1) under physiological circumstances can enter the cell cycle only the cell with intact genome DNA, (2) progression through cell cycle and into its final phase (M-phase) can achieve only the cell with correctly replicated genome DNA and (3) mitosis and final cytokinesis can happen only when segregating genetic material is symmetrically duplicated. If such conditions are not fulfilled the cell cycle process is ceased and reparative mechanisms come into action. Should the reparative mechanisms be unable to repair the damage the cell undergoes apoptosis. On molecular level the relation between cell cycle regulation, DNA reparation and apoptosis represented by regulatory proteins which influence signalisation in all processes mentioned above (for example: ATM (ataxia-telangiectasia mutated), activated double strand breaks in DNA initiate not only activation of DNA reparation (phosphorylation of protein BRCA1 for example) but thanks to the phosphorylation of p53 activate this transcription factor which is responsible for quick increase of expression not only cell cycle inhibitors p21 but also apoptosis stimulating Bcl-2 family members (Bax, PUMA, NOXA)).

DNA reparative mechanisms defects in cancer cells can influence all reparative mechanisms:

- Defects of correction mechanisms on the level of bases (**BER/NER** system) resulting in the creation of micromutations (missense/nonsense).
- Very serious are alterations of reparations in double strand DNA breaks repaired by either less precise mechanism **NHEJ** (non-homologous end joining) or more precise **HR** (homologous recombination) whose inactivation leads to chromosomal translocations/ losses/ amplifications of genetic material in cancer cells which leads to physiological degradation of alleles of tumorsuppressor genes and amplification of chromosomal segments containing oncogenes.

DNA reparative mechanisms defects are one of the most common symptoms of initial cancerous stages. In the case of sporadic (non hereditary) cancerous diseases are number of genes coding proteins engaged in reparative mechanisms inactivated by somatic mutations (point mutations even large deletions effecting whole genes) and/or by hypermethylation of promoters. Relatively rarely are hereditary mutations in such genes part of severe hereditary cancer syndromes (creating usually <5-6% tumours; for example MSH/MLH genes, ATM, BRCA1/2, MRE11, and others) where preexisting alterations of usually one allele in gene significantly increase the risk of malignant transformation in tissue.

Purpose

There is steady increase of malignant diseases in our population. Understanding causes for creation and progression of malignant diseases is serious question not only for research in molecular oncogenetics but also an important presumption for rational use of targeted biological treatment which is as opposed to classical chemotherapy focused against specific molecular signs (for example: over translated receptor her2/neu; mutated k-Ras or B-Raf oncoproteins) to each patient with specific malignant disease. Despite its good results high price of such treatment requires application only at indicated patients with good chance of positive treatment response. This decision is up to oncologist who has to be able to understand strict biological criteria originating from molecular principles of treated malignant disease and phenotype/genotype of cancerous cells with specific patient in order to make valid decision.

Another area that is improving thanks to progress in molecular biology of tumours is **oncological diagnostics**. Uncovering molecular principles of malignant transformations enables usage of molecular taxonomy for classification of malignant diseases which enables better classification of each malignant form. Better responding their biological principles than it was classified up to now with only pathological and imunohistochemical analysis. Important diagnostic area is detection of pathogenic mutations in tumorous predisposition genes.

For example answering the origin of cell populations in malignant tumour is not only theoretical problem but can bring significant progress in ontological therapy. Most current treatment strategies enable destruction of most cancer cells however it is not adequately efficient against tumour's stem cells. Their persistence in tissues is probably partial cause for relapse of the disease.

Finally restoration of negative regulation of cell cycle and increase of sensitivity to proapoptotic mechanisms in cancer cells is basic concept of conventional chemotherapy and radiotherapy.

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