Causes of tumors, carcinogenesis, carcinogens

History:

Since early 1900 – two models of cancer disease development: 1. prof. Yamagiwa was the first who proved that cancer disease can be induced by chemical carcinogens (tar initiates cancer in rabbits). 2. Francis Peyton Rous was the Nobel Prize-winning virologist who discovered the role of viruses in the transmission of certain types of cancer. He described that sarcoma can be induced on a domestic chicken by a transfer of a cell-free filtrate (obtained from sarcoma) to another healthy bird. Both hypothesis are seemingly mutually exclusive but subsequently it was realized that cancer is a genetic disease and that both, oncogene viruses and carcinogenes, can initiate changes in genetic information which ultimately leads to cancer disease development. Approximately 25% of cancers are consequence of virus infection (polyomavirus, Papillomavirus, SV40 virus, HTLV-1) Numerous mechanisms leading to cancer have stochastic character – the longer we live, the more likely these events occurs. It is estimated that prevalence of cancer diseases will double in next decades. However, the absolute number of cases of cancer in persons younger than 50 years is not predicted to increase significantly over the next 30 years. -- Cancer is a group of heterogenous genetic diseases arising as a consequence of aberrant gene expression or alterations in DNA sequence of a specific genes. Each cancer is different. Even cancer of the same organ in two patients vary. However, a common feature of all cancer diseases is a disruption of genomic integrity, which leads to disbalance between cell division and apoptosis. This changes in cell homeostasis could be given by the effect of physical (ionizing radiation), chemical (genotoxic substances) or biological (e.g. DNA replication, epigenetic modifications, shortening of telomeres, miRNA regulation, viruses etc) influences. Moreover, malignant transformation could be driven by hereditary mutations in tumor-supressor or proto-oncogenes. Tumor-supressors: P53, Bcl-2, Chk1, Chk2, INK4 inhibitors, p21, p27, BRCA1, Rb etc. Proto-oncogenes: Ras, Raf, Myc, Cyclin D,E,A,B, AKT, Src, miR-21, HOTAIR etc.

Cell cycle deregulation:

Cell cycle is a highly coordinated protein orchestra which leads into divission of one cell into two daughter cells. Its essential process for the preservation of genome integrity. Cell cycle involves distinct parts, so called G1, S, G2 and M phase. Enterance into the cell cycle and passage through the each mentioned phase is strictly regulated with expression of cyclins, interacting protein partners of cyclin dependent kinases. - Cyclin D + Cdk 6,4 (G0 -- G1 phase) - Cyclin E + Cdk 2 (G1 – S phase) - Cyclin A + Cdk 2 (S and G2 phase) - Cyclin B + Cdk 1 (M phase)

Cyclin D

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Expression of cyclins (cyclin D) is driven by signalisation cascades including for example:
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MAPK pathway

(also known as the Ras-Raf-MEK-ERK pathway). MAPK pathway is important protein cascade which leads (while activated by binding of eg. epidermal grow factor on its receptor) to the expression of genes important for cell cycle progression. MAPK pathway is named after its effector kinase MAPK which phosphorylates and activates Myc. Activated transcription factor Myc in turn regulates expression of Cyclin D, key protein for enterance into the cell cycle. Inhibition of complex Cyclin D-Cdk4 using INK4 (eg. palbociclib, ribociclib and abemaciclib - triple-negative breast cancer) inhibitors seems to be a potential drug for cancer treatment. Uncontrolled cell growth is a necessary step for malignant transformation. Mutations in upstream activator of MAPK, proto-oncogene from Ras family (most notably H-Ras, K-Ras, B-Raf), play crucial role in cancer development (particularly in melanoma and colorectal cancer). Some compounds are nowadays used for inhibiting MAPK pathway as a potential drugs for cancer treatment (e.g. sorafenib, cetuximab, panitumumab).

Wnt Pathway

Myc and consequently cyclin D expression is regulated also by canonical Wnt pathway. Wnt pathway is continually activated in stem cells for preservation of “stemness” by binding of ligand Wnt on receptor Frizzled which in turn activates protein Dishevelled. This cascade leads to inhibition of “destruction complex” (formed by protein Apc, Axin and GSK3-beta), which decreases level of beta catenin. Beta-catenin forms a complex with transcription factors and regulates expression of genes important for cell cycle. Wnt pathway (Apc or beta-catenin) is mutated in 90% of colorectal cancers.

PKB/AKT pathway

Activated by insulin-like grow factor binding on its receptor -- activation of PI3K which in turn phosphorylates PIP2 on PIP3 (Phosphatidylinositol 3-phosphate). Phosphatase PTEN remove phosphate from PIP3 and inhibits AKT pathway. PTEN is frequently mutated in cancer disease. PIP3 activates PDK1 and AKT respectively. Activated AKT: - Inhibits apoptosis (stabilization of Bcl-2, inhibition of Bax) - Stabilization of NF-kappa B and Cyclin D - Activation of proliferation (PKB phosphorylates and inhibits GSK3-beta) - Activation of HIF-1 which phosphorylates pyruvate kinase dehydrogenase (production of lactate in cancer cells).

NF-kappaB pathway

NF-kappaB is activated by TNF- alpha – activation of IKKalpha, beta and NEMO – inhibiton of IKB and activation of NF-kappaB. Activated NF-kappaB leads to regulation of about 150 target genes (eg. cytokines, chemokines, COX-2). COX-2 for example activates production of pro-inflammatory prostaglandins from arachidonic acid. NF-kappaB plays a crucial role in cancer disease and cell cycle regulation.

• Retinoblastoma protein

Target substrate of Cyclin D/ Cdk complex is protein Rb (retinoblastoma protein). Rb is a tumor suppressor protein and its function is to prevent entrance into the cell cycle and excessive cell growth respectively. Phosphorylation (inhibition) of Rb by Cyclin D/ Cdk complex release E2F transcription factor which in turn regulates expression of S phase genes. Some viruses can also inhibit Rb protein (for example SV-40 large T antigen produced by Simian virus).

• Transcription factor E2F

Transcription factor E2F creates heterodimer with protein DP1. Activated heterodimer E2F/ DP1 regulates expression of cyclin E which interacts with Cdk2. Cyclin E/ Cdk2 complex increases phosphorylation of Rb protein. Activated Cdk can be inhibited by inhibitors of Cdk (p15, p16, p18, p19, p21, p27, p57). Inhibitors of Cdk are activated either by DNA damage response effector proteins (p53) or by TGF-beta pathway.

TGF-beta pathway

TGF-beta pathway is activated by TGF-beta ligand - phosphorylation and activation of SMAD 2, 3 and 4. SMAD4 in turn regulates transcription of INK4 inhibitors (p15, p16, p18, p19). SMAD4 is frequently mutated in colorectal cancer.

• P53

Expression of p21 is regulated by p53 mainly during DNA damage response. P21 slows down cell cycle progression and allows DNA repair. However, if DNA damage remain unrepaired, p53 stabilizes proapoptotic proteins. P53 pathway is mutated in almost 100% cancers (p53 itself is mutated in approximately 50% of cancer cases). Importance of p53 can be illustrated by Li-Fraumeni syndrome, which is characterized by p53 mutations (in one allele- haploinsufficiency) and predisposition to various cancer diseases.

Apoptosis deregulation

Tumor growth is driven not only by uncontrolled cell division but also by deregulation of apoptosis. All cancer cells have acquired resistance to the mechanisms leading to their programmed cell death. Mutations in pro-apoptotic and anti-apoptotic genes and in their upstream signaling pathways (AKT, p53 etc.) are the main reason for apoptosis deregulation. Several distinct apoptotic pathways - Intrinsic - Extrinsic - Perforin/ Granzyme pathway The target protein of all apoptotic pathways is caspase 3. Caspases are effector proteins of apoptosis. Function of caspases is for example fragmentation of DNA (can serve as a marker for liquid biopsy). **Extrincis and intrinsic pathways:** TNF or Fas receptor (CD95), situated in cytoplasmatic membrane, can be stimulated by TNF-alfa and Fas ligand respectively. Its activation creates binding site for FADD and TRADD adaptor proteins. FADD associates with caspase 8 (frequently mutated in colorectal cancer) and creates DISC complex. Caspase 8 in turn activates caspase 3 (effector caspase) and protein Bid. Protein Bid is a key “bridge protein” connecting extrinsic and intrinsic pathways. Bid inhibits Bcl-2 proto-oncogene and activates Bax. Bax creates a pore in mitochondrial membrane and enable cytochrome c to create Apoptosome complex (with 7 Apaf-1 and procaspaze 9 proteins). Activated caspase 9 activates effector caspase 3 **Perforin/ granzyme pathway:** is a key pathway for cytotoxic T- lymphocytes and NK cells during immune response (for example against cancer cells). Perforine/ granzyme pathway is named after Granzyme A and B (serine proteases released during immune response). Granzyme A activates caspase independent pathway. Granzyme B activates protein Bid.

Mutations in apoptotic pathway: - p53 is a key regulator of apoptosis (while mutated, Bcl-2 can´t be inhibited by downstream proteins of p53 pathway, PUMA and NOXA, and a cell with damaged DNA can survive). - Mutations in AKT and Bcl-2 respectively are also involved in cancer progression.

Cell immortalisation

Telomeres are nucleoprotein structures at the end of each eukaryotic chromosome (TTAGGG repetitions). Telomeres shorten during each round of the cell cycle due to “end replication problem”. Telomere length is preserved by telomerase (TERT/ TERC nucleoprotein structure), which lengthen 3´-end of telomeres (enabling RNA polymerase during DNA replication to create primer for DNA polymerase). When telomeres reach their critical length, affected cells undergo senescent state or apoptosis. However, if the cell continues in cell division (due to mutations in p53 or other genes regulating apoptosis), genomic instability occurs. Genomic instability is a driving force in tumor progression. Cancer cells need to reactivate telomerase during a malignant transformation. Recent studies indicate that TERT gene in malignant transformation is reactivated by mutations in TERT promoter together with mutations in Ras protooncogene.

Mutations

Carcinogens:

Chemical: o Arsenic, benzene, beryllium, cadmium, ethylene oxide, vinyl chloride, alcohol. o promoters and initiators. Promoters: fenobarbital, sacharin, croton oil, pyrocatechin, D-limonene, 12-miristate 13-acetate. Initiators: 7,12-Dimethylbenz[a]anthracene, 3-Methylcholanthrene, Benzo[a]pyrene diol epoxide Physical: UV radiadion, Gamma- radiation, asbestos, erionite, glass wool Biological: Aflatoxin, HBV, HPV, helminths, Helicobacter pylori

DNA damage induced by carcinogens: - Depurination - Depyrimidination - Deamination - Oxidation - Alkylation **Carcinogenesis is divided into three stages:** Initiation: Cells with acquired mutations in key target genes can gain a growth advantage over normal surrounding cells. However, one genetic change is not sufficient enough for induction of a malignant cell transformation (it usually occurs after several gene mutations over a number of years). **Promotion:** A promotion phase lasts for years to decades. Cells with acquired driver mutations is stimulated even more and creates excessively extended cell mass. **Progression:** Includes metastasis and blood vessel supply.