Carcinogenesis

Carcinogenesis is a multi-phase process during which a normal cell changes to a neoplastic one.

Malignant Transformation and Cell Division

In malignancy, the regulation of the cell cycle transition **from G1-phase to S-phase** is most commonly disrupted. The mechanisms of regulation are several and very complex. **Three cyclin-dependent kinase (CDK) complexes** with different types of cyclins:

- cyclin D + CDK4
- cyclin D + CDK6
- cyclin E + CDK2

Influence of the Rb gene

The resulting complexes phosphorylate **the retinoblastoma gene product (Rb gene)** at ten different sites. This alters the ability of Rb to associate with other proteins. Rb is a hit site for transforming viruses such as SV40 large T-antigen, adenovirus E1A and human papillomavirus E7 antigen.

The E2F protein is a transcription factor that forms a heterodimer with the DP1 transcription factor, thereby activating several genes required for S-phase development. This involves activation of dihydrofolate reductase, thymidine kinase, DNA polymerase A, and the genes c-myc, c-myb and cdc2. In addition to this growth factor support, phosphorylated Rb protein also promotes differentiation through association with transcription factors such as MyoD and activated transcription factor. This results in complexes of ATF and cAMP-response element binding proteins (CREB).

CDK activity is also regulated by CDK inhibitors, which are low molecular weight proteins with a general inhibitory effect on a number of cyclin-dependent kinases such as p21Cip1/Waf1, p27Kip1, p57Kip2 or with a specific inhibitory effect on cyclin D/cdk4 and cyclin D/cdk6 complex such as p16INK4a, p15INK4b and p18. The first member of this family has been identified as p21, which inhibits both cdk and proliferating cell nuclear antigen (PCNA), a subunit of DNA polymerase d. Induction of p21 production, resulting from transcriptional activation by p53 upon DNA damage, halts the development of the cell division cycle at several sites, including G1 and S-phase, allowing the onset of the DNA repair machinery. If the DNA damage is too extensive and therefore irreparable, the cell becomes "abnormal" and is eliminated by apoptosis. However, if apoptosis does not occur, the resulting mutation persists and can be passed on to daughter cells during division of the mutated cell. This creates a phenomenon of gene instability that promotes malignant transformation. This happens more frequently in HNPCC (hereditary non-polyposis colorectal cancer) gene carriers. There is a defect in the 'repair read' so that the DNA replication error is not detected and the repair mechanism is not initiated.

Malignantly transformed cells are characterized primarily by continued division. At the same time, they have a reduced requirement for the hormones and growth factors needed by a normal cell, which come from the outside. Some transformed cells produce their own specific growth factors (autocrine stimulation). **The ability to stop growth is lost.** In normal cells, a decrease in the levels of isoleucine, phosphate, epidermal growth factor and other growth regulating substances below a certain threshold concentration induces a shift to a quiescent state (G0 phase). Normal cells begin to grow (divide) only when their nutritional requirements are properly provided for. Tumor cells lack this ability to stop growing in response to a lack of nutrients and growth factors; they even continue to proliferate, although they may die in the process.

It is believed that most tumors arise from a single cell and that tumor progression is the result of acquired genetic variation in the original clone, allowing sequential selection of aggressive subclones.

Aberrant regulation of the cell division cycle is one of the key points of tumorigenesis. Tumor cells tend to escape the physiological mechanism of cell division control. Factors involved in this include Efp (estrogen-responsive RING-finger protein), which controls the breakdown of cell cycle inhibitors through ubiquitination and directs the shift of breast tumor growth from a hormone-dependent to a hormone-independent mechanism. This mechanism is important in the treatment of mammary adenocarcinoma with tamoxifen (an estrogen antagonist) or other estrogen antagonists (SERMs) when tumor cells shift to a hormone-independent type. A critical component of ubiquitination is E3-ubiquitin-ligase, which has two roles:

1. it acts as a substrate for ubiquitination
2. it stimulates the formation of E2-ubiquitin-conjugating enzyme.

The protein that is tagged by this conjugation undergoes rapid destruction by the proteasome.

Tumor-suppressor protein p53 (TP53) is one of the nuclear proteins that plays a key role in the regulation of the cell division cycle during the transition from G0 to G1 phase. It contains domains for binding to a specific stretch of DNA, as well as oligomerizing and transcription-activating domains. It binds as a tetramer to a binding site on a specific stretch of DNA and thereby activates the expression of genes encoding factors that inhibit proliferation and promote cell invasiveness. Mutants of the p53 gene are found in a number of malignant tumors. The altered TP53 loses the ability to bind to the corresponding DNA locus, leading to insufficient production of

tumor growth suppressor factors. Alteration of the p53 gene occurs not only as a somatic mutation but also as a germ cell mutation in some cancers with familial occurrence (Li-Fraumeni syndrome). Retinoblastoma protein 1 (RB1) is a nuclear phosphoprotein with DNA-binding activity; it coactivates with histone deacetylase to inhibit transcription.

Tab.
Cell cycle regulators of malignant tumors.

Gene	Alteration	Tumor
Retinoblastoma gene (Rb)	Deletion, point mutation	Retinoblastomas, osteosarcomas, soft tissue sarcomas, small cell lung carcinomas, bladder and breast carcinomas
Cyclin D	Chromosome translocation, gene amplification	Parathyroid adenoma, some lymphomas, carcinomas of mammary gland, head and neck, liver (primary), esophagus
cdk4	Amplification, point mutation	Glioblastoma, sarcoma, melanoblastoma
p16INK4a (p15INK4b)	Deletion, point mutation, methylation	Pancreatic, esophageal, lung (small cell) carcinomas, glioblastomas, sarcomas, familial melanomas and pancreatic carcinomas

Defects in Apoptosis

The growth of cancer cells is enabled not only by uncontrolled cell division but also by the ability not to respond to **apoptotic signals**. Most, if not all, cancer cells have acquired resistance to mechanisms leading to their programmed death. There is experimental evidence that disruption of apoptosis signaling is a general prerequisite for the existence and development of cancer cells. The oncogenic potential of the Bcl2 family factor, physiologically responsible for inhibiting apoptosis, is likely to play a very important role.

The Bcl2 gene

The Bcl2 gene (B-cell lymphoma 2 gene) was originally discovered as a gene linked to the immunoglobulin locus during a chromosomal translocation in follicular lymphoma. Its overexpression has also been found to affect apoptosis rather than proliferation. This means that nascent neoplastic cells gain a selective advantage by this inhibition of programmed death. They can remain nested as foci in the host tissue, especially in places where cytokines and oxygen cannot reach. This escape from apoptosis is then promoted by other oncogenic self-preservation mechanisms, leading to the emergence of more aggressive clones. Defective apoptosis also facilitates metastasis, as cells can ignore restriction signals coming from the environment and survive separate from the extracellular matrix. Mutations that favor tumor development reverse the response to cytotoxic therapy, resulting in refractory clones. The role of Bcl2 (and its homologues, Bcl-xL and Bcl-w) in the mechanism of inhibition of apoptosis is likely to be a protective effect on mitochondrial integrity by **preventing cytochrome c exit into the cytoplasm**, which prevents Apaf-1 activation and subsequent activation of the caspase cascade.

Mitotic "immortality" of Cells

The tumor cell becomes mitotically immortal. This is caused by increased activity of the enzyme telomerase. Repeated cell division is physiologically limited by telomere shortening. Telomere length decreases after multiple passages (1 cell cycle = 1 telomere shortening) and further in cell cultures from advanced age patients. Their renewal is catalyzed by telomerase. Telomere length correlates with telomerase expression and activity. Therefore, it is hypothesized that loss of DNA from the end of chromosomes by telomere shortening leads to deletion of essential genes, resulting in cell damage and subsequent apoptosis.

The number of telomeres serves as a generation clock that counts down the individual cycles of cell division to determine cell life and replication potential. Cancer cells have a high replicative potential. This is made possible by at least two mechanisms by which they maintain a sufficient number of telomeres, or telomere renewal. The most common (activity has been demonstrated in 85-90% of tumor cells) is the **TERT mechanism**, which is a protein component of telomerase. Only a small fraction of cancer cells use another mechanism called **alternative lengthening of telomeres (ALT)**, which allows maintenance of telomere number without the action of telomerase.

Malignant Transformation of the Cell

Cell proliferation is very carefully controlled to match the needs of the whole organism. In the early stages of an individual's life, the capacity for cell proliferation outweighs cell death; in adulthood, it is in dynamic balance; in old age, involution begins to predominate. However, this varies for different cell types. The cells of the mucosa of the small intestine disappear and are renewed in a few days, like some forms of leukocytes. In contrast, the lifespan of red blood cells is 120 days on average. Hepatocytes in healthy adults are rarely lost. The same applies to nerve cells, but they are not able to regenerate.

However, some cells escape replication control (they do not need external signals to control their division, they are autonomous) and thus turn into tumor cells. Those that retain at least approximately the appearance and function of normal cells and, above all, still remain in the place where they originated are benign cells and their proliferation gives rise to benign tumors. Cells that have lost most of the properties of the cells from which they are derived and tend to penetrate into their surroundings (invasiveness) and to distant sites (metastasis) are malignant cells and form malignant tumors.

A malignant tumor is a genetic disease (DNA disease), but its expression begins in an individual cell (monoclonal theory). It is a multi-step process where mutations (alterations) in genes controlling cell proliferation (division), differentiation and death are gradually accumulated at the chromosome level.

Stages of carcinogenesis

The first mutation marks the 'kicking off process'. Cells with mutations 1 and 2 gradually outgrow or replace cells with mutation 1 in the tumor tissue. Further mutations (genetic changes) encourage the cell population to become more aggressive. The subclonal genetic heterogeneity of the tumor reflects the progressive development of the tumor tissue.

Although some forms of tumors are hereditary, the majority arise from mutations in somatic cells and are caused by endogenous errors in DNA replication or changes leading to malignant transformation are induced by the action of carcinogens. For example, UV radiation of the appropriate wavelength can be absorbed by the DNA bases, which are thus damaged to form dimers of two adjacent pyrimidines. This alteration interferes with normal transcription and DNA replication. The effect of ionizing X-rays is different: the DNA strand is cleaved (broken), resulting in loose DNA strands that must be reattached without residue by a repair mechanism. If this does not happen (the cell does not tolerate free DNA ends), certain chromosome segments can be translocated, which is usually the cause of proto-oncogene activation. However, one genetic change is not sufficient to induce a malignant transformation of the cell. This usually occurs after several (five to ten) gene mutations over a number of years.

Genetic alteration induces a tumor phenotype

- Epithelial cell proliferation
- Hyperplasia
- Adenoma
- Dysplasia
- „in situ" carcinoma

The transformation of normal body tissue into an invasive cancer takes an average of 5-10 years. This is influenced by hereditary genetic factors and somatic epigenetic factors.

The course of carcinogenesis is divided into three stages:

Initiation

The initiation stage, which represents the initial genetic event, i.e. the mutation of a critical gene. This is a short but irreversible period of time; it confers a growth selection advantage on the initiated cells. The cell thus acquires the potential for malignant transformation; at this stage, the process may stop.

Promotion

The graduation stage, which lasts for years or even decades; the affected cells (clone) are stimulated to proliferate even more intensively. However, promotional factors alone are not able to induce malignant tumor transformation, only to promote it. The intensity of the promoter mechanisms must reach a certain level to stimulate the initiated clone, and conversely, the removal of the promoters can slow or even stop the process of carcinogenesis.

Progression

The progression stage is characterized by further gradual accumulation of genetic changes such as:

- Uncontrolled growth for sustained activation of growth stimulus signal transduction,
- alteration of critical points in the cell cycle,
- deregulation of DNA transcription factors.

The tumor initially remains at its site of origin, but through activation of other factors it begins to spread to the immediate vicinity (invasion) and through the bloodstream to distant sites (metastasis). A very important condition for tumor growth is a sufficient supply of nutrients and oxygen, which must be ensured by the establishment of a vascular supply (tumor neoangiogenesis).

Carcinogens

Carcinogens

Mutations in genes with **oncogenic potential** can be spontaneous, but are more often induced by **environmental factors (mutagens)**, divided into

- chemical agents
- physical agents
- biological agents

Chemical agents

- polycyclic and aromatic hydrocarbons, chlorinated hydrocarbons, aromatic amines, nitrosamines, asbestos,

heavy metals, mycotoxins and others
- **most carcinogenic chemicals** are active in the body only after they have been converted into **their own carcinogens**
- **the metabolic activation** of chemicals involves an individual's genetic disposition, which in turn **influences the frequency of cancer**
- inappropriate dietary habits play a major role in the development of **chemically induced gastrointestinal cancers** - the effect of inappropriate food composition and preparation is cumulative with the effect of carcinogens
- the development of colon cancer is **directly related to dietary habits**
- among **the most risky foods** are animal fats and products containing them
- **inappropriate preparation of foods** - frying, baking, smoking - increases their content of carcinogens (nitrosamines, polycyclic aromatic hydrocarbons)
- fruits, vegetables, legumes (containing vitamins, chemoprotective substances detoxifying carcinogens) and foods with a high content of fiber (soluble - pectin and mucilage, insoluble - cellulose, lignin), which help digestion and emptying of the digestive tract, are considered to be **suitable or even protective.**

Physical agents

- **UV radiation, ionizing radiation** (e.g. gamma rays, X-rays) are carcinogenic
- radiation increases the risk of certain types of cancer (ionizing radiation e.g. leukemia, UV radiation for skin tumors)
- **ionizing radiation** causes in particular chromosome breaks and chromatid rearrangements; the formation of **thymine dimers** is typical for UV radiation
- similarly to chemical carcinogenesis, the frequency of radiation-induced tumors is influenced mainly by the **activity of genes for the repair of DNA errors**
- statistical studies on families have shown that the offspring of parents exposed to carcinogens or mutagens can acquire a predisposition to cancer by mutations in **the prezygotic stage of germ cells** exposed to these factors (gametic mutations)
- in the case of occupational exposure to radiation, even higher numbers of affected children per family have been reported

Environment and lifestyle are important factors influencing carcinogenesis - by influencing these factors, the possibility of cancer can be prevented.

Biological agents

- Cancers caused by biological influences include those of **viral etiology**
- **viruses** identified in this context in humans to date:
  - **DNA viruses** from 4 distinct families - herpesviruses, hepadnaviruses, papovaviruses and adenoviruses
  - **RNA viruses** - retroviruses
- **Human papillomaviruses** have been shown to be related to malignant transformation - the cause of cervical cancer, laryngeal papillomatosis and oral squamous cell carcinoma
- **EB virus** (herpesvirus family) is associated with B cell lymphomas
- **Hepatitis B virus** (HBV) has been shown to be associated with an increased incidence of hepatocellular carcinoma
- the high endemicity of these cancers is in South Africa, and the carcinogenic effect of aflatoxin B (from mold) is compounded by poor dietary habits - the accumulation of these two effects results in an increased frequency of hepatocarcinomas
- the target gene for mutagenesis is **TP53** - point mutations underlie malignant transformation
- **HIV** (human immunodeficiency virus) is associated with **Kaposi's sarcoma** and **B lymphoma**
- another human retrovirus, **HTVL-1**, is reported to cause adult-onset T-cell leukemia and **HTVL-2** has been isolated from T-cell hairy cell leukemia
- **oncogenic viruses** do not act as infectious agents during malignant transformation - malignant cell reversal occurs when the virus affects the host cell DNA and acts as an **oncogenic factor**
- **integration of the papillomavirus** into the genome of a eukaryotic cell is an example of the influence of the host cell genes on the regulatory region of the virus genome
- in **Burkitt's lymphoma**, it is EB virus associated with a structural aberration of the translocation type
- TP53 in hepatomas, where hepatitis B virus is considered to be inducible, is a point mutation of the substitution type
- sometimes viral DNA oncoproteins act in inducing malignant transformation, viral antigens (oncoproteins) bind with **p53 protein** and inactivate it

Burkitt's lymphoma

- the best known and most studied **human tumor of viral etiology** is Burkitt's lymphoma and nasopharyngeal carcinoma - conditioned by the mutagenic effect of **EB virus**
- has a significant territorial incidence in children in equatorial Africa

In cancers of viral etiology, environmental influences and the dietary and hygienic habits of the family usually cumulate with the viral influence.

- **immunology**

  - the development of tumors also depends on **the immunological status** of the individual or the population as a whole - in Burkitt's lymphoma and in children in equatorial Africa are said to be associated with a high prevalence of **the malaria agent** (plasmodium)
  - **plasmodium** induces immunodeficiency in its host and thus prevents the elimination of emerging malignant cells by the immune system
  - the existence of other **tumors with EBV inducing agents** - **Hodgkin's lymphoma** and **certain types of T cell lymphomas** - has been described
  - the disease of this type is not bound to a specific territory, but is related to the immunological state of the individual
  - **immunosuppression**, e.g. drug-induced or acquired (e.g. AIDS), increases the frequency of tumors associated with viral infection

Literature

Related articles

- Protein degradation
- The degradation system of the cell
- Ubiquitination
- Deubiquitination
- History of the ubiquitin-proteasome system
- Proteasome inhibitors
- Carcinogens

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