Chapter 10

Pathophysiology of malignant disease

10.1 Carcinogenesis

In the last years cancer research has provided an enormous amount of information. But a unitary theory of carcinogenesis has not been created still today.

The study of carcinogenesis has historically been devided into two major areas:

1. carcinogenesis induced by chemical and physical agents
2. viral carcinogenesis

Within the last decade has been found that these two areas of carcinogenesis are in fact closely related. Chemical and physical agents as well as viruses act via the alteration of a limited number of target genes – activation of protooncogenes and inactivation of tumor supressor genes.

In **cellular transformation process**, by which normal cell becomes a cancer (malignant) cell, two clearly separated stages were defined: **initiation** and **promotion**. (Fig. 10.1)

Initiation is induced by carcinogens. **Carcinogens** (initiators) include chemical agents, irradiation, viruses and hormones. Carcinogen causes **an irreversibile DNA damage, which predisposes to cancer**. DNA changes induced by carcinogens include interruptions of the DNA chain, errors in DNA repair, elimination of a base pair etc. **Promotion** is mediated by **cocarcinogens** (promoters). These agents are not themselves mutagenic or carcinogenic but accelerate or promote the transformation when applied repeatedly after a carcinogen (initiator). Promoters include chemicals, hormones, drugs etc. They may induce some alterations in initiated cells, such as alteration of cell surface sensitivity to various growth factors, alteration of cell surface glycoproteins and glycolipids, alteration of cell morphology, increase phospholipid and glucose metabolism, stimulation of DNA synthesis and cell proliferation, increase production of free oxygen radicals, activation of a latent provirus DNA, induction of disproportionate DNA replication within one cell cycle with gene amplification, etc.

A classic example of initiation and promotion is such model, in which the carcinogen (polycyclic aromatic hydrocarbon, benzo(a)pyren) is applied to mouse skin. Following this croton oil or acetone is repeatedly applied. Croton oil produced a more significant increase in malignant tumor formation compared to acetone. Initiator is represented by benzo(a)pyren and promoters include croton oil and acetone.

Perhaps the best example of the **multistage, multifactorial nature of cancer** in humans is the interaction of asbestos and cigarette smoking in the etiology of lung cancer, with the combined factors increasing the risk almost 900-fold compared to that in nonsmoking nonasbestos workers.

It is accepted that human cancer does not arise all of a single mutation, but there is still controversy concerning the actual relevant number of exposures to initiators and promoters that must occur in incubation period of cancer.

For many human cancers the probability that all target genes will be altered by environmental factors is closely associated with **age**. IndividualÝs age determines how long the cell has been subjected to environmental carcinogenic factors and therefore cancer incidence increases with age.

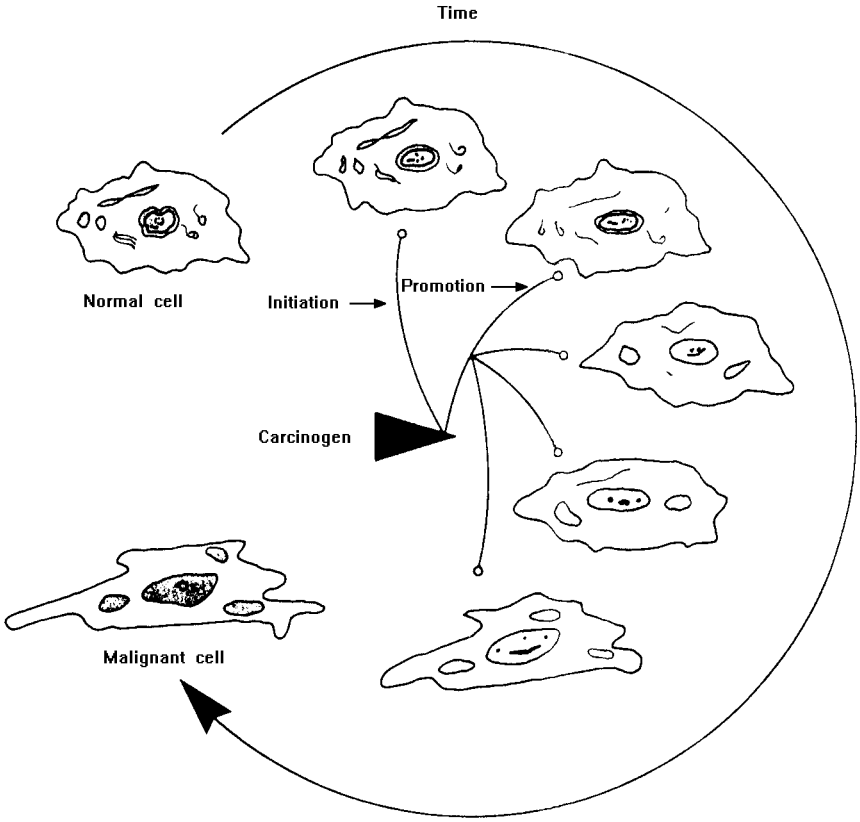

Figure 10.1: Two-stage carcinogenesis: initiation and promotion

There are many experts who claim that as much as 90 % of all human cancers are of environmental origin (caused by chemicals, radiation, hormones, viruses).

10.1.1 Chemical carcinogenesis

The number of known chemical carcinogens grows. The problem of their identification in humans is due to by **long latent periods** (even 20 and more years) between exposure to these agents and the first appearance of cancer. It may be the time required for a sequence of promoting events. Chemical carcinogens include naturally ocurring and synthetic compounds.

The higher incidence of human cancer is associated with approximately 30 chemicals. Some substantial groups of carcinogens are as following:

1. Polycyclic aromatic hydrocarbons (i.e. benzoapyrene)
2. Nitrosamines (i.e. dimethylnitrosamine)
3. Aromatic amines (i.e. beta-naphtylamine)
4. Heavy metals (i.e. arsenic)
5. Alkylating agents (i.e. melphalan)
6. Toxins (i.e. aflatoxin)
7. Others (i.e. vinyl chloride)

A wide range of different chemicals associated with cancer induction requires metabolic activation for reactivity with DNA. The process of metabolic

activation requires cellular enzymes, which are induced by the carcinogen. Individuals vary in their abilities to activate chemical carcinogens. This genetically determined variability in the degree of inducibility of the activating enzymes may be correlated with cancer susceptibility.

One system of such activating enzymes is aryl hydrocarbon hydroxylase system, which converts proximate carcinogen benzo(a)pyrene, a component of cigarette smoke, into its ultimate carcinogenic form. Individuals with lung cancer appear to have a genetically higher level of inducibility of this enzyme system than those smokers without lung cancer.

Chemical carcinogens are **dose-dependent**. Many of them have been shown to induce cancer when used in high concentrations. But little is known about the effects of these carcinogenes when humans are exposed to minimal amounts of these agents in the environment over a period of many years.

Many chemical carcinogens are present in the food, air and water.

10.1.1.1 Nutritional factors and carcinogenesis

Some carcinogenic agents are formed in the food through **cooking** and **charcoal broiling**. Any **burnt food** may have carcinogenic potential. Carcinogenic potential of food may depend upon, how it is **store**d and its age when consumed. Extremely dangerous carcinogens, such as aflatoxin, may be produced by **food molds**. Some **food preservatives** and **additives used at high concentrations** are associated with carcinogenic activity.

Other dietary elements, such as high fiber diet, vitamins A, E, C and selenium, have been shown to inhibit carcinogenic potential of food.

10.1.1.2 Cigarette smoking and carcinogenesis

Cigarette smoke contains about 7000 chemical substances including many different types of carcinogens, such as benzo(a)pyren, nitrosamines, aromatic amines etc. The incidence of lung cancer appears to be 10 fold higher in smokers than in non-smokers. After smoking of approximately 200 000 cigarettes there is 30 fold increased risk of lung cancer. Cancer in smokers may be associated with cigarette smoke chemicals and irritating of mucose in respiratory system. The risk of developing lung cancer is increasing also in passive smokers.

Smoking contributes also to esophagus, bladder, pancreas, larynx and other cancers.

High incidence of cancers falls in those individuals who stopped smoking.

10.1.2 Radiation carcinogenesis

Ultraviolet light and ionizing irradiation are the most universal carcinogenic factors.

Radiation damages DNA directly, causing **mutations**. In support of this theory is the frequent occurence of multiple skin tumours after exposure to ultraviolet light in individuals with xeroderma pigmentosum. In this rare hereditary defect the cellular enzymes reparing ultraviolet-induced mutations within cellular DNA are missing. Deffective DNA repair mechanism is associated with the early development of skin cancer on skin exposed to sunlight. Radiation can also act through the formation of highly reactive **free-oxygen radicals** that have capability of peroxidating cellular molecules. Radiation may also **activate cancer-causing viral DNA sequences** present within the genetic code of individuals. Another observation in the exposed population is the higher than expected presence of **chromosomal abnormalities**.

Skin cancer results from sunlight exposure. Negative effects of ultraviolet light and increased risk of skin cancer may be associated also with damage of the ozone layer of the earth.

Radiation damage is a linear response to dose received.

Human radiation carcinogenesis caused by ionising irradiation has been studied in the survivors of the atomic bombings of Hiroshima and Nagasaki. The incidence of leukaemia was greater than in nonexposed Japanese populations and were characterized by a latency period even of 20 to 30 years after exposure.

Another groups of patients developing malignancies with greater than average frequency are those who received radiation therapy. The incidence peaks 4 to 8 years following radiation. Also therapeutic injection of bone-seeking radioactive isotopes such as ^{224}Ra also leads to high incidence osteogenic tumors.

The most common sites for radation induced cancer are the lymphoid system, the thyroid, the female breast and the lung.

10.1.3 Viral carcinogenesis

In 1911 Peyton Rous described Rous sarcoma virus, an agent that induced malignant tumors – sarcomas in inoculated chickens. The gene responsible for the tumor inducing capabilities of Rous sarcoma virus was isolated in the 70's. This gene was named according virus from which this oncogene was isolated – v-src. It has been identified **more than 20 oncogenes, most of which are carried by RNA viruses**.

RNA viruses are replicative and very minimally transforming or nonreplicative and highly transforming. Highly oncogenic retroviruses have evolved from their non-oncogenic retrovirus counterparts. This evolution include several steps:

RNA viruses contain a gene (pol gene) for **enzyme reverse transcriptase**. Using this enzyme RNA virus makes a complementary DNA copy of its own RNA. (For this reason these viruses containing reverse transcriptase are called retroviruses.) Thus **virus can incorporate its own viral DNA into the genome of the host cell in a form of provirus**. Only in an integrated form oncogenic virus may cause cell transformation. Provirus contains also genes for structural proteins (gag, env gene), for transcription (long term repeat – LTR) etc.

During process known as transduction **the virus incorporates part of the cellular DNA material into its own viral genome. Accidental viral and proto-oncogenes combination may occur. Somewhere along this path proto-oncogene mutates and viral oncogene may arise de novo**. Also such changes as removal of signals for transcription termination and other rearrangements of cellular DNA may occur. The virus now becomes oncogenic. **Viral oncogenes are mutants of normal cellular genes – proto-oncogenes. Proto-oncogenes are cellular prototypes – progenitors of viral oncogenes**. Viral oncogenes are not homologues with proto-oncogenes as was initially suspected. For example c-myb is a cellular progenitor of viral oncogene – v -myb, however structure and expression of v-myb is not identical with the structure and expression of c-myb (Fig. 10.2).

Oncogenicity of viral-oncogenes is due to their products – the quantitative or qualitative altered transforming proteins. They are protein-kinases responsible for alterations in cellular morphology and growth.

Another family of viruses – replication competent viruses (slowly transforming viruses) do not carry oncogenes of their own. If the proviral DNA is integrated in the proximity of a target gene - protooncogen, also these viruses without v-onc could begin to regulate the expression of proto-oncogenes.

Viral infection may also contribute to carcinogenesis **undirectly**, not only directly – by insertion. Viruses may stimulate cell proliferation associated with normal repair or healing process. Actively dividing cells are at high risk of mutations induced by carcinogenes. During some viral infection, the host's immune system is compromised and thus cancer likelihood increases.

Oncogenic viruses (mostly from RNA family) are responsible for many animal malignancies (in chickens, mice, monkeys, cats etc.)

Only one human cancer has been unequivocally shown to be result of a retroviral infection – adult T cell leukaemia (ATL) caused by C type RNA viruses, human T cell leukaemia-lymphoma viruses – **HTLV I and II**. Epidemiological data indicate that there is a consistent association between HTLV I and II infection and adult T-cell leukaemia. The HTLV I provirus sequences integrated in genome of tumor cells have been detected in patients with ATL from different parts of the world. These include the southwestern islands of Japan and the Caribbean. Approximately 5 % of the normal population and 100 % of patients with ATL have a high titer of HTLV antibodies. Patients who develop ATL have a higher titer of antibodies to the HTLV viruses. Transmission of HTLV I by close contact is suggested by the greater antibody prevalence in family members of HTLV–positive patients compared to the prevalence in the general population. The infection with members of the HTLV family may also produce immunosupression. HTLV I and II are members of the same family as the virus that causes AIDS (HTLV III and HIV.)

The close relationship is supposed between Burkitt's lymphoma (malignancies of jaw region found predominantly in Central Africa) and the **Epstein-Barr virus**, (EBV is a DNA, herpes like virus infected B lymphocytes – causative agent of infectious mononucleosis). High titer of antibodies to the EBV and EBV provirus sequences have been detected in DNA of malignant cells. The antibody titer against the EBV rises and falls with tumor progression and regression. Despite this evidence, however,

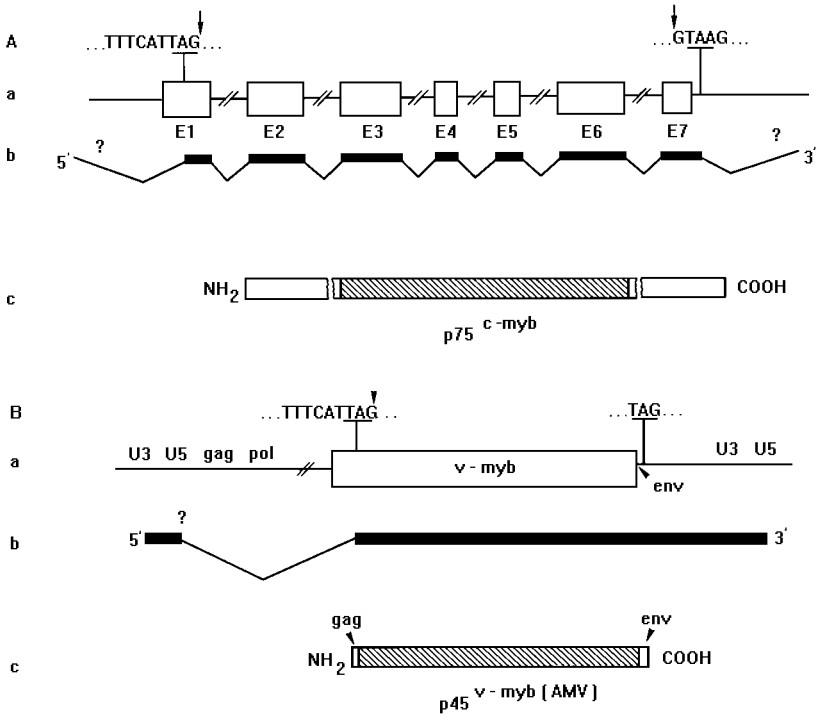

Figure 10.2: A comparison of structure and expression of c-myb and v-myb (a: DNA, b: RNA, c: protein encoded by RNA)

it is not clear if the virus is a cause of the lymphoma or whether other oncogenic stimuli produce the cancer in infected cells.

Infection of the liver with **hepatitis B virus** (HBV) is associated with an increased incidence of primary liver cancer (hepatoma). In areas of the world where there is a high prevalence of HBV carriage, there is a corresponding high prevalence of hepatoma (in Southeast Asia, Central and South Africa). The frequency of finding persistent HBV infection in hepatoma patients is 10 to 40 times higher than in controls living in the same area. Chronic infection with HBV often leads to cirrhosis. Prospective studies have shown that that HBsAg-positive patients with cirrhosis will develop liver cancer with more frequency in contrast to HBsAg-negative cirrhosis patients (e.g. those with alcoholic cirrhosis). In other prospective studies the risk of developing hepatoma was about 250 times higher among the virus carriers than in matched controls. It is suggested that these tumors appear after about 35 years of persistent HBV infection.

The applications of the new hepatitis B vaccines are promising in prevention not only viral infection but also the hepatoma.

It seems also likely that viral infection may depress the detoxification of chemical carcinogens in liver and by this way it may contribute to carcinogenesis.

Herpes simplex type II virus and papillomavirus can cause genital infection, which has been shown in females to be associated with the later development of cervical cancer. Patients who develop malignancy have a higher titer of antibodies to the virus and the malignant cells contain viral DNA, RNA and proteins.

Human breast cancer is associated with B type RNA virus infection – **Bittner factor**.

Viruses are often suspected also in acute leukaemia

causation because a nonspecific viral like illness characterized by fever, malaise, leukocytosis, respiratory symptomes etc. Viral infection may also decrease the immunologic capability of the individual to protect against cancer.

10.1.4 Hormones and carcinogenesis

Hormones are supposed to be carcinogenic agents, especially when elevated.

Estrogens may be responsible for development of breast cancer and endometrial cancer. Some malignant cells of breast cancer appear to have estrogen hormone receptors that allow binding to hormone molecules to take place, which then causes cellular division and growth. Also endometrial cancer may be associated with estrogen replacement therapy.

Diethylstilbestrol administered to prevent abortion has been linked to an increased incidence of vaginal and testicular cancer in children of women with such treatment in pregnancy.

Elevated levels of hormones may act as promoters by increasing of normal cellular proliferation or they may promote also tumor growth and dissemination.

10.1.5 Irritation and carcinogenesis

Irritation may also play a role in carcinogenesis. Continous irritation of skin or mucose may promote malignancy. It is well known that irritation support development of malignant melanoma from a previously benign pigmented mole. Also using intrauterine devices may subject uterus to prolonged irritation and may increase risk of development of cancer. Some components of smoke and alcohol may promote carcinogenesis acting as irritating factors.

10.2 Cellular oncogenes

Mammalian cells possess a set of normal cellular genes – proto-oncogenes (c-onc) and tumor supressor genes.

Proto-oncogenes are termed also as celullar oncogenes (c-onc) with acronyme from corresponding viral oncogene (e.g. c-src, c-myc, c-myb etc). They are not cancer genes. The term "proto-oncogenes" is used to denote their ability to require an oncogenic potential.

In normal cells they have functions in cell growth and differentiation: They

- code for phosphorylate proteins
- influence DNA replication
- control mRNA production
- bind GTP

Since the retroviral oncogenes are mutants of normal proto-oncogens, they have provided a key to open a door to understand proto-oncogene – oncogene conversion. It was accepted that following crucial events may be responsible for conversion of proto-oncogenes to "cancer" genes:

- **mutations** (point mutations of proto-oncogenes or their regulatory genes caused by chemicals, radiation, viruses)
- **amplifications**
- **translocations** (proto-oncogene is placed in proximity to a strong promoter)

An example is found in Burkitt's lymphoma, where the translocation of c-myc proto-oncogene from chromosome 8 to chromosome 14 is often observed. In this instance, the oncogene c-myc of chromosome 8 becomes activated when is transcripted in tandem with either heavy chain genes (IgH genes) of chromosome 14 or with immunoglobulin light chain genes of chromosomes 2 and 22.

Altered c-myc play an important role in B cell tumorigenesis. Normal proto-oncogene c-myc is expressed in normal proliferating lymphoid cells and presumably this expression is a signal for continued growth. In terminally differentiating B cells, c-myc transcription is arrested, resulting in a nonproliferating plasma cell. However, when chromosomal rearrangements bring c-myc into the proximity of an immunoglobulin gene locus, c-myc transcription can be positively driven by that locus, thus providing continued proliferation to plasma cell.

Another example is the translocation 9, 22 in 90 % patients with chronic myelogenous leukemia, where the proto-oncogene c-abl from chromosome 9