

Underwood: Chapter 9: Carcinogenesis

Carcinogenesis is the process which results in the formation of tumours (neoplasms). Cells converted from normal to neoplastic, and thus capable of growing autonomously and forming tumours, are said to have been *transformed* and the conversion from normal to neoplastic is referred to as *transformation*. The abnormal behaviour of transformed cells result from genetic alterations or mutations.

Tumours arise from single cells that have become transformed by cumulative mutational events. Tumours are therefore said to be *monoclonal*. Spontaneous mutations during DNA replication are probably common, but many are rectified by repair mechanisms. The probability of neoplastic transformation increases with the number of cell divisions experienced by a cell; this may explain why the incidence of cancer increases with age. Exposure to carcinogens increases the probability of specific mutational events.

Tumours are classified according to their behaviour into *benign* and *malignant* categories: benign tumours remain localised to their site of origin; malignant tumours invade adjacent tissues and spread to other organs (metastasis). Carcinogenesis embraces the causation of both categories of tumours, but, because malignant tumours are much more serious, most epidemiological, clinical and experimental observations have been concentrated on them. Indeed, *carcinogenesis strictly applies to the causation only of malignant tumours (cancers), whereas oncogenesis includes the causation of all tumours, benign and malignant.*

A *carcinogen* is an agent known or alleged to participate in the causation of tumours. Such agents are said to be *carcinogenic* (cancer causing) or *oncogenic* (tumour causing). The ultimate site of action of all carcinogens is the DNA in which genes are encoded. Carcinogens are therefore also *mutagenic*. Very often more than one carcinogen is necessary to produce tumours from normal tissues and cells, and there is good evidence that the process may occur in possibly several discrete steps; this is the *multistep hypothesis*.

Once started, the process does not require the continued presence of the carcinogen. It is rather a 'hit-and-run' situation and evidence of the specific causative agent(s) is not usually found in the eventual tumours. Exceptions include some suspected carcinogenic viruses, genetic material which persists in the resulting tumours, and some insoluble substances, such as thorium dioxide and asbestos, which cannot be eliminated from the tissues.

Genetic alterations are absolutely fundamental to the carcinogenic process. They result in the transformation of normal cells into cells capable of proliferating autonomously to form a tumour; further genetic abnormalities determine the ability of the tumour cells to invade and spread to other sites (i.e., a *malignant tumour*).

Identification of Carcinogens

- Most cancers are attributed to environmental causes
- Laboratory testing can identify some carcinogens

- Some carcinogens can be suspected from epidemiological studies
- Many carcinogens require co-factors
- Long latent interval between exposure and detection of the consequent tumour hampers identification.

Carcinogens may be identified from:

- epidemiological studies
- assessment of occupational risks
- direct accidental exposure
- carcinogenic effects in laboratory animals
- transforming effects on cell cultures
- mutagenicity testing in bacteria.

Epidemiological Evidence

Liver-Cell Carcinoma

In the UK and USA, liver-cell carcinoma is a relatively uncommon tumour and, when it does occur, it is usually associated with cirrhosis. Epidemiology reveals two factors which may be involved in the high prevalence in endemic areas; mycotoxins and hepatitis B virus.

Mycotoxins

There is a positive correlation between the incidence of liver-cell carcinoma in different regions of Uganda and the frequency with which food samples in those regions are found to be contaminated with aflatoxins. Aflatoxins are mycotoxins produced by the fungus *Aspergillus flavus*, and are a highly carcinogenic group of compounds.

Hepatitis B Virus

There is a strong correlation between the incidence of HBV infection and liver-cell carcinoma in many countries. Suspicion that the HBV may be oncogenic is reinforced by the discovery of a copy of the viral genome incorporated within the genome of the liver cancer cells.

Oesophageal Cancer

The very high incidence of oesophageal cancer in China and in the Caspian littoral region of Iran has been intensively studied.

Lung Cancer

It is a major public health problem in many countries. In the UK, approximately 35000 deaths are attributed to this cause annually; the actual incidence is only marginally higher because this form of cancer has an extremely poor prognosis.

Occupational Risks

Scrotal Cancer

Percival Pott is credited with the first observation, in 1777, linking a particular tumour with a specific occupation. He noticed a high incidence of cancer of the scrotal skin in men who were or had been chimney sweeps, and postulated that the soot was responsible. It was not until 150 years later that the specific carcinogen, a polycyclic aromatic hydrocarbon, was identified.

Cancer of the Cervix

The risk of cancer of the cervix is strongly associated with sexual intercourse, in particular with the number of partners and thus the risk of exposure to a possible carcinogenic agent conveyed by the male. Evidence favouring a human papillomavirus (HPV) is more compelling because the viral DNA can be found incorporated into DNA extracted from many cervical carcinomas.

Bladder Cancer

In the 1980s, epidemiologists noted a higher than expected incidence of bladder cancer among men employed in the aniline dye and rubber industries. Further analysis led to the identification of beta-naphthylamine as the causative agent.

Direct Exposure

Thorotrast

Thorotrast was a colloidal suspension of thorium dioxide widely used in many countries during 1930-1950 as a contrast medium in diagnostic radiology. It is naturally radioactive, emitting alpha radiation and has an extremely long half-life of 1.39×10^{10} years. In 1947 the first report was published of a patient who developed angiosarcoma of the liver after Thorotrast administration.

Thyroid Cancer and Thyroid Irradiation

Several decades ago it was fashionable to treat many children with a variety of non-specific ailments by thyroid irradiation to promote involution of the organ. The thyroid gland is particularly sensitive to the carcinogenic effects of ionising radiation.

Experimental Observations

Three types of test systems are employed:

- laboratory animals in which the incidence of tumours is monitored
- cell and tissue cultures in which growth-transforming effects are sought
- bacterial cultures for mutagenicity testing (Ames test).

Known or Suspected Carcinogens

The main classes of carcinogenic agents are:

- chemicals
- viruses
- ionising and non-ionising radiation
- hormones, mycotoxins and parasites
- miscellaneous agents.

Chemical Carcinogens

- No common structural features
- Most require metabolic conversion into active carcinogens
- Major classes include polycyclic aromatic hydrocarbons, aromatic amines, nitrosamines, azo dyes, alkylating agents.

Some agents act directly, requiring no metabolic conversion. Others (*procarcinogens*) require metabolic conversion into active carcinogens (*ultimate carcinogens*). If the enzyme required for conversion is ubiquitous within tissues, tumours will occur at the site of contact or entry; for example, polycyclic aromatic hydrocarbons induce skin tumours if painted on to the skin, or lung cancer if inhaled in tobacco smoke. Other agents require metabolic conversion by enzymes confined to certain organs, and thus often induce tumours remote from the site of entry; for example, aromatic amines require hydroxylation in the liver before expressing their carcinogenic effect. In a few instances the carcinogen is synthesised in the body from ingredient in the diet; thus, carcinogenic nitrosamines are synthesised by gut bacteria utilising dietary nitrates and nitrites.

Polycyclic Aromatic Hydrocarbons

They were the first chemical carcinogens to be intensively studied. In 1917, Yamagiwa and Itchikawa in Japan reported that skin tumours could be induced in rabbits by painting

their skin with tar. Tar was a suspected carcinogen because of the high incidence of skin cancer among tar-workers, particularly on the hands, which were frequently in contact with it. In the 1930s in London, Cook and Kennaway fractionated tar and showed that the carcinogenic effect was attributable to the polycyclic aromatic hydrocarbons. These are procarcinogens, requiring metabolic conversion to form ultimate carcinogens. In this case, the carcinogenic effect is invariably at the site of contact because the hydroxylating enzymes (i.e., aryl carboxylase) are ubiquitous in human tissues and readily induced in susceptible individuals. However, if the substance is absorbed into the body, this may lead to a risk of cancer at sites remote from the point of initial contact; there is, for example, an increased incidence of bladder cancer in tobacco smokers.

Tobacco

There is overwhelming epidemiological evidence incriminating tobacco and its combustion products in carcinogenesis. The tumour most commonly associated with smoking is carcinoma of the lung. This tumour is much more common in smokers than in non-smokers and the risk to an individual or group parallels the quantity of tobacco consumed. Tobacco smoke contains many candidates for carcinogenic activity, the most important is probably 3,4-benzpyrene. Tobacco is also chewed in some countries, and there it is associated with a risk of carcinoma of the mouth.

Aromatic Amines

The high incidence of bladder cancer in workers in the dye and rubber industries has now been attributed to beta-naphthylamine. Unlike the polycyclic aromatic hydrocarbons, this substance has no local carcinogenic effect. It requires conversion by hydroxylation in the liver into the active carcinogenic metabolite, 1-hydroxy-2-naphthylamine. However, the carcinogenic effect is masked immediately by conjugation with glucuronic acid in the liver. Bladder cancer results because the conjugated metabolite is excreted in the urine and deconjugated in the urinary tract by the enzyme glucuronidase, thus exposing the urothelium to the active carcinogen.

Nitrosamines

There is epidemiological evidence linking carcinomas of the GIT to dietary nitrates and nitrites. Nitrates are used widely as fertilisers, and are eventually washed by the rain into rivers and underground water tables where they can contaminate drinking water. In addition, both nitrates and nitrites are used as food additives. Although these radicals are not in themselves carcinogenic, they are readily metabolised by commensal bacteria within the gut and converted to carcinogenic nitrosamines by combination with secondary amines and amides.

Azo Dyes

Azo dyes are derivatives of aromatic amines. In laboratory animals, dimethylaminoazobenzene - otherwise known as 'butter yellow' because it was once used to impart a tasteful yellow colour to margarine - causes liver cancer.

Alkylating Agents

Many categories of chemical carcinogen, including cyclic hydrocarbons, have alkylation as the ultimate common pathway, so it is not surprising that alkylating agents themselves can be carcinogenic. Alkylating agents bind directly to DNA, the ultimate site of action of all carcinogens. Nitrogen mustard is a well-known example, but these agents are not otherwise widely implicated as a major cause of human cancer.

Oncogenic Viruses

- Clusters of cancer cases in space and time suggest a viral aetiology
- Tumours associated with viruses tend to be more common in youth
- Immunosuppression favours viral oncogenesis
- Viruses implicated in human carcinogenesis include Epstein-Barr virus (Burkitt's lymphoma) and human papillomaviruses (cancer of the cervix)
- Oncogenic DNA viral genome is directly incorporated into host cell DNA
- Oncogenic RNA viral genome is transcribed into DNA by reverse transcriptases prior to incorporation (oncogenic retrovirus).

Viruses were first implicated as carcinogenic agents through the experiments of Rous (in 1911) and Shope (in 1932) who studied fowl sarcomas and rabbit skin tumours respectively. 'Bittner milk factor' is associated with the high incidence of mammary cancer in certain strains of mice, which has been discovered to be an RNA virus which is oncogenic in the oestrogenic milieu of female mice of strains with the appropriate genetic constitution.

Human tumours for which a viral aetiology has been proposed or proven include:

- carcinoma of the cervix (human papillomaviruses)
- Burkitt's lymphoma (Epstein-Barr virus)
- nasopharyngeal carcinoma (Epstein-Barr virus)
- liver cell carcinoma (HBV)
- T-cell leukaemia/lymphoma in Japan and the Caribbean (RNA retrovirus).

Human Papillomaviruses (HPV)

HPV, with many subtypes, are known to cause the common wart (squamous cell papilloma).

Epstein-Barr Virus (EBV)

EBV was first discovered in cell cultures from Burkitt's lymphoma, a B-cell lymphoma endemic in certain regions of Africa and occurring only sporadically elsewhere. Infection by the virus causes infectious mononucleosis. A cofactor is involved and epidemiological evidence suggests that this is malaria.

Radiation

- UV light is a major cause of skin cancer
- Exposure to ionising radiation is associated with an increased risk of cancer of many site, including leukaemia.

UV Light (UVL)

Most types of skin cancer are associated with UVL exposure, but the risk is particularly high for malignant melanoma and basal cell carcinoma ('rodent ulcer'). The risk is greatly exaggerated in patients with *xeroderma pigmentosum*, a rare congenital deficiency of DNA repair enzymes, in whom numerous skin cancers occur due to unrepaired damage to the DNA of the skin cells induced by UVL.

Ionising Radiation

Industrial exposure includes the risk of carcinoma of the lung associated with the mining of radioactive uranium.

Particularly sensitive tissues include thyroid, breast, bone and haemopoietic tissue.

Biological Agents

Oestrogens, parasites, mycotoxins.

Hormones

Oestrogens can be shown experimentally to promote the formation of mammary and endometrial cancers. Androgenic and anabolic steroids are known to induce liver-cell tumours. Oestrogenic steroids may make pre-existing liver-cell lesions (i.e., adenomas and focal nodular hyperplasia) abnormally vascular, thus causing otherwise asymptomatic lesions to present clinically.

Mycotoxins

Aflatoxins produced by *Aspergillus flavus*. Particularly, aflatoxin B₁ is among the most potent carcinogens. High incidence of liver-cell carcinoma in some parts of Africa is linked to them.

Parasites

Schistosoma is strongly implicated in the high incidence of bladder cancer, usually of squamous cell type.

Clonorchis sinensis, the Chinese liver fluke, dwells in the bile ducts where it induces an inflammatory reaction, epithelial hyperplasia and sometimes eventually adenocarcinoma of the bile ducts (cholangiocarcinoma).

Miscellaneous Carcinogens

Asbestos

Inhalation of asbestos fibres results in various lesions: asbestosis, pleural plaques, mesothelioma and carcinoma of the lung. Mesothelioma is exceptionally rare in the absence of asbestos exposure.

Metals

Exposure to compounds containing nickel leads to a risk of carcinoma of the mucous lining the nasal cavities and of the lung.

Host Factors in Carcinogenesis

In addition to the extrinsic or environmental factors in carcinogenesis, there are also several important host factors which influence the cancer risk:

- race
- diet
- constitutional factors (gender, inherited risk, etc.)
- premalignant lesions and conditions
- transplacental exposure.

Race

Diet

There is a possible positive correlation between dietary fat and the risk of breast and colorectal cancer. Alcohol appears to be a risk factor for breast cancer. There is experimental evidence to suggest that a low protein diet has a protective effect against certain chemical carcinogens by reducing the levels of mixed function oxygenases in the liver. Dietary fibre appears to be protective for colorectal cancer by promoting more rapid intestinal transit.

Constitutional Factors

There is an *inherited predisposition* to breast cancer. A woman whose mother and on sister have developed breast cancer has a 50% probability of developing one herself. Xeroderma pigmentosum. Polyposis coli is an autosomal dominant inherited predisposition to develop multiple adenomatous polyps of the large bowel. Retinoblastoma is familial and often bilateral in approximately one-third of cases. In these patients there is usually an abnormality of chromosome 13.

The incidence of cancer increases with *age*.

Hormonal status may explain the constitutional risk for breast cancer. It is more common in women who are nulliparous or who have not breast fed their children, and those who have experienced an early menarche and/or late menopause.

Premalignant Lesions and Conditions

A *pre-malignant lesion* is an identifiable local abnormality associated with an increased risk of a malignant tumour developing at that site. Examples include adenomatous polyps of the colon and rectum, and epithelial dysplasia in various sites, notably the cervix.

A *pre-malignant condition* is one which is associated with an increased risk of malignant tumours. In chronic ulcerative colitis, for example, there is an increased risk of colorectal cancer and this can be predicted by seeking the pre-malignant lesion (in this case dysplasia) in rectal biopsies. Sometimes congenital abnormalities predispose to cancer; the undescended testis is more prone to neoplasms.

Transplacental Carcinogenesis

In the 1940s some pregnant women with threatened miscarriages were treated with diethylstilbestrol, a synthetic oestrogenic compound, in an attempt to avert the fetus being aborted. The female progeny of those pregnancies which went successfully to full term were later discovered to have a high incidence of vaginal adenocarcinoma, an otherwise rare tumour, in early adult life. This is an example of transplacental carcinogenesis; the carcinogen, presumably diethylstilboestrol, was administered to the mother, but the carcinogenic effect was exhibited only in the child resulting from the pregnancy, when she reached young adulthood.

The Carcinogenic Process

- Multistep process
- May require initiating and promoting agents
- Growth persists in the absence of causative agents.

Latency

Part of the reason for the long latent interval between exposure to a carcinogen and clinical recognition of the tumour is the fact that tumours result from the clonal proliferation of single cells; it takes an appreciable time for this transformed single cells to grow into a nodule of cells large enough to cause signs and symptoms. With the possible exceptions of ionising radiation and of some fast-transforming oncogenic retroviruses, the change from a normal cell into a growing and potentially lethal neoplasm is thought to entail more than one event. Evidence for this *multistep theory* of carcinogenesis is derived mostly from observations on the effects of chemical agents on laboratory animals.

Initiation and Promotion

These are two major steps in the transformation from normal to neoplastic. *Initiation* is the event that actually induces the lesion in the cell's genome that bestows neoplastic potential. *Promotion* is the event stimulating clonal proliferation of the initiated transformed cell. For example, successive applications of methycolanthrene and croton oil on mouse skin.

Persistence

The stage of persistence is reached when the clonal proliferation of the tumour cells no longer requires the presence of initiators or promoters.

Genomic Abnormalities in Tumours

- Current evidence favours genetic rather than epigenetic explanations of carcinogenesis
- Chromosomal abnormalities, sometimes consistent (i.e., Philadelphia chromosome), are common
- Oncogenes, genes directing cell growth and differentiation, are abnormally expressed in many tumours
- Oncogene expression results in autocrine growth stimulation.

Chromosomal Abnormalities in Tumours

Until quite recently the only technique for examining the genome of cells was chromosomal (karyotypic) analysis. This involves culturing the cells in the presence of colchicine, which blocks formation of the mitotic spindle and arrests mitosis in metaphase. On exposure to a hypotonic medium, the osmotic shock causes the cells to explode and spill their chromosomes on to the surface of a glass slide where they can be stained, counted and examined in detail. Individual genes cannot be localised precisely without resorting to in situ hybridisation. Abnormalities such as additional chromosomes and translocation of part of one chromosome to another are very common, but few are constant even among a single tumour type.

One of the few exceptions is the Philadelphia chromosome which is commonly found in chronic myeloid (granulocytic) leukaemia.

In Burkitt's lymphoma there is a translocation of c-myc oncogene from chromosome 8 to an immunoglobulin gene locus on chromosome 14 and results in expression of c-myc gene.

In chronic myeloid leukaemia there is a translocation involving chromosomes 9 and 22 (Philadelphia) which results in fusion of c-abl and bcr genes. bcr-abl protein has tyrosine kinase activity.

In follicle centre cell lymphoma there is a translocation involving chromosomes 14 and 18 which results in expression of bcl-2 gene.

In Ewing's tumor (peripheral neuroectodermal tumour) there is a translocation involving chromosomes 11 and 22 which distinguishes these tumours from neuroblastoma which they may resemble histologically.

Genetic Mechanisms in Carcinogenesis

Two genetic mechanisms have been proposed:

- Tumours result from the enhanced expression of stimulatory genes. These genes (*oncogenes*) act dominantly.

- Tumours result from the loss or inactivation of inhibitory genes. These genes (*anti-oncogenes*) act recessively.

The first inhibitory gene to have been well characterised is the Rb1 gene associated with retinoblastoma. Individuals with hereditary retinoblastomas show a germ-line deletion on chromosome 13, corresponding to the known site of the Rb1 gene. Therefore, only one further mutational loss of the paired gene in the target retinal cell is required for the tumour to develop. Sporadic retinoblastoma cases have a normal chromosome 13 and therefore require two mutational losses before the tumour can develop. A similar mechanism is proposed for colorectal carcinoma arising in patients with familial adenomatous polyposis (FAP), which is associated with an allelic loss on chromosome 5.

Oncogenes

Oncogenes are genes governing the neoplastic behaviour of cells. Originally proposed as a hypothesis, oncogenes were 'discovered' as a result of studies of oncogenic RNA retroviruses. These are RNA viruses which have the ability to transfer their genome, or parts of it, to the genome of the cells they infect. Normally the transfer of genomic information is in the opposite direction: DNA sequences are transcribed into RNA, which then determines the amino acid sequence of a peptide or protein. However, retroviruses contain an enzyme, *reverse transcriptase*, which enables the viral RNA to be transcribed into complementary DNA which is then incorporated into the infected cell's genome.

The next major discovery was of the presence of DNA sequences identical to viral oncogenes (v-oncogenes) in the genome of normal cells (cellular or proto-oncogenes). However, in normal cells these oncogenes are present at the frequency of only one copy per haploid genome, and their transcription is tightly controlled as required for cell growth and differentiation.

Normal or partially transformed cell cultures can be transformed by the addition of DNA bearing oncogenes, a process known as *transfection*. Alternatively, oncogenic (or carcinogenic) retroviruses can transform cells by transferring oncogenes from another cell, a process known as *transduction*.

Some oncogenes, *myc* and *ras*, have well characterised effects corresponding to the early stages of tumourigenesis.

Oncogenes can be classified into five groups according to the function of the gene product (oncoprotein):

- nuclear-binding oncoproteins involved in the regulation of cellular proliferation (i.e., *myc*)
- tyrosine kinase activity (i.e., *src*)
- growth factors (i.e., *sis* coding for platelet derived growth factor)
- receptors for growth factors (i.e., *erbB* coding for epidermal growth factor receptor)
- cyclic nucleotide binding activity (i.e., *ras* and GTP) disrupting intracellular signalling.

Oncogene Expression in Tumours

It can become altered to result in either:

- normal quantities of the oncoprotein molecule altered by mutation in such a way that it is abnormally active
- normal oncoprotein produced in excessive quantities because of gene amplification or enhanced transcription.

Mutant oncoproteins may have less or greater biological activity than the normal molecule. This can have profound effects on receptor function and intracellular signalling. For example, the mutant protein product of the *ras* oncogene family is a hyperactive protein acting on cyclic nucleotides (GTP); the oncoprotein binds GTP and has GTPase activity.

Increased expression of oncogenes may be detected by:

- the presence of more of the oncogene product (*oncoprotein*) within or on the cells

- increased production of mRNA transcripts of the oncogene
- increased numbers of copies of the oncogene in the genome.

Increased numbers of copies result from infection by a retrovirus, which causes reverse transcription of its RNA and insertion of *multiple copies* of the resulting DNA into the DNA of the host cell genome.

A more common example in humans is DNA amplification resulting in multiple gene copies, such as in the *myc* family of oncogenes in neuroblastoma; this can be recognised in chromosome preparations from tumour cells by the presence of *homogenously staining regions* and *double minute chromosomes*. *Increased transcription* can occur if the oncogene, not normally transcribed in the genome, is moved to another part of the genome where active transcription occurs.

Role of Oncogene Products in Tumour Growth

Cell cultures transformed by carcinogens, and showing increased or mutant oncogene expression, exhibit a variety of changes corresponding to the abnormal behaviour and appearance of tumour cells *in vivo*:

- independence of the requirement for extrinsic growth factors
- production of tumour when injected into immunotolerant animals
- production of plasminogen activator and proteases to assist invasion
- reduced cell cohesiveness, thus assisting metastasis
- immortalisation
- an increase in plasma membrane and cellular motility
- growth to higher cell densities
- abnormal cellular orientation.

The Genetic Theory

In some instances, the neoplastic behaviour of tumour cells can be influenced by epigenetic factors.