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The pathogenesis of neoplasia

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ABSTRACT

Many different factors are involved in the development of tumors. A cancer-producing agent or carcinogen, and presumably promoting agents, must be present. Carcinogens may be chemical or physical, e.g. ionizing radiation or ultraviolet light. Identified chemical carcinogens include hydrocarbon carcinogens present in coal tar and a series of chemicals used in the rubber industry. Animal experiments suggest that viruses may be associated with the initiation of some cancers, mainly the leukemic group; viruses also seem to be associated with some types of human cancer. The role of oncogenes has already been mentioned. It is also becoming obvious that there is often an interaction between chemical carcinogens and viruses in tumour induction.

THE PATHOGENESIS OF NEOPLASIA

Tumour growth or neoplasia

It is not possible to define a tumour cell in absolute terms. Tumors are usually recognized by the fact that the cells have shown abnormal growth, so that a reasonably acceptable definition is that tumour cells differ from normal cells in that they are no longer responsive to normal growth-controlling mechanisms (1). Since there are almost certainly many different factors involved, the altered cells may still respond to some but not to others. A further complication is that some tumour cells, especially soon after the cells have been transformed from the normal, may not be growing at all. In the present state of knowledge any definition must be 'operational'. Malignant tumors are diseases with unnormal genetic expression. Their expression depends of age, degree of cell differentiation, growing, invasion and metastatic potential as well as therapy responses (2). Given these qualifications we can classify tumors into three main groups:

1. Benign tumors may arise in any tissue, grow locally, and cause damage by local pressure or obstruction. However, the common feature is that they do not spread to distant sites.

2. In situ tumors usually develop in epithelium and are usually, but not invariably, small. The cells have the morphological appearance of cancer cells but remain in the epithelial layer. They do not invade the basement membrane and supporting mesenchyme. Some authorities recognize a stage of dysplasia (epithelial irregularity) which is not absolutely identifiable as cancer in situ but which may sometimes precede cancer in situ. Theoretically, cancers in situ may arise also in mesenchymal, reticuloendothelial, or nervous tissue, but they have not been recognized.

3. Cancers are fully developed (malignant) tumors with a specific capacity to invade and destroy the underlying mesenchyme local invasion. The tumour cells need nutrients that are provided through the blood stream in normal tissues. Some tumour cells produce a range of proteins that stimulate the growth of blood vessels into the tumour, thus allowing continuous growth to occur. The new vessels are not very well formed and are easily damaged so that the invading tumour cells may penetrate these, and lymphatic vessels. Tumour fragments may be carried in these vessels to local lymph nodes or to distant organs, where they may, produce secondary tumors (metastases). Cancers may arise in any tissue. Although there may be a progression from benign to malignant, this is far from invariable. Many benign tumors never become malignant. Some of these problems of definition may be more easily understood if we consider the whole process of tumour induction and development (carcinogenesis).

The process of carcinogenesis

Carcinogenesis is a multistage process (Figure 1). The application of a cancer-producing agent (carcinogen) does not lead to the immediate production of a tumour. There are a series of changes after the initiation step induced by the carcinogen. The subsequent stages tumour promotion may be produced by the carcinogen or by other substances (promoting agents), which do not themselves "produce" tumors. Initiation, which is the primary and essential step in the process, is very rapid, but once the initial change has taken place the initiated cells may persist for a considerable time, perhaps the life span of the individual. The most likely site for the primary event is in the genetic material (DNA), although there are other possibilities. The carcinogen is thought to damage or destroy specific genes probably in the stem cell population of the tissue involved (3,4).
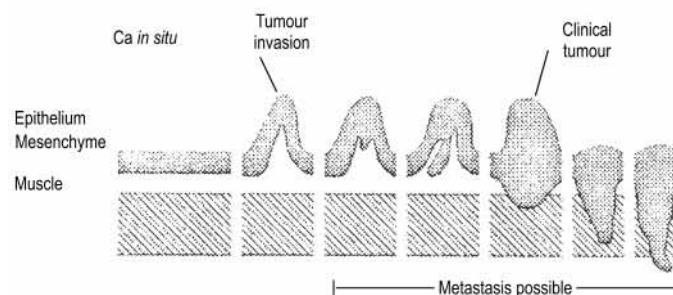

Figure 1. Factors influencing tumour development showing the progression from normal to invasive tumor

The earliest events, initiation and promotion, require exogenous exposures to carcinogenic chemicals. Initiation is mutagenic in nature and generally results from DNA damage produced by a metabolically activated genotoxic carcinogen. In this regard, it is an irreversible phenomenon. In contrast, tumor promotion is epigenetic in nature and is often reversible. Promoters induce changes in epidermal homeostasis that provide a tissue environment conducive for the clonal expansion of initiated cells. The consequence of initiation and promotion is the formation single clone of initiated cells (5).

Initiated cells remain latent until acted upon by promoting agents. Many of these 'transformed' cells may not grow at all or grow very slowly. It is at this stage that the influence of growth appears. Promoting agents are not carcino-

genic by themselves but they induce initiated cells to divide. Many agents will induce cell division, but only promoters will induce tumour development, so that although cell growth is necessary for tumour development there must also be other factors involved. The suggestion is that promoting agents may interfere with the process of differentiation that normally takes place when cells move from the dividing stem cell population into functioning, and usually non-dividing, cells. Even though these growth-promoting stimuli are acting on the cells, they may still be sensitive to the normal growth-inhibiting factors in the body so that the final outcome depends on the balance between the factors and the extent of the changes in the initiated cells. This explains why pre-neoplastic, or even apparently fully transformed tumors, can be found but do not appear to be growing, and sometimes even regress (3,5).

The whole sequence of events in the process of tumour formation is almost certainly a consequence of gene changes, although the host may influence gene expression. We are now beginning to understand some of these changes, although there are still many problems unsolved. The discovery that oncogenes of tumour-producing viruses are related to genes (protooncogenes) in normal, as well as some tumour, cells has led to intensive research into the relationship of these genes to normal development and tumour growth and development. These genes have been localized to specific chromosomes and some to sites of chromosome abnormalities in tumors. Much speculation now centers on the question of whether the initiation, progression, and maintenance of some tumors depends on overexpression through gene amplification (an increase in the number of copies of a particular gene), or inappropriate expression (i.e. at the wrong time) of normal genes, or whether mutations in a critical region of a gene are necessary. A possible hypothesis is that a mutation may be necessary for the initiation event but that some or all of the later stages may depend on over or inappropriate expression. These possibilities are discussed in the sections on carcinogenesis (6).

One theory, for which there is now increasing evidence, was proposed by Knudson (7). He suggested that at least two independent mutations are needed before tumors can develop. In cases of inherited (familial) tumour predisposition, the first mutation is present in the germ cells (sperm or ovum) and is therefore inherited by every cell. Only one further mutation is required in these cases. In the more common, non-familial cases two mutations (which may include gene deletions) in the same cell are required and the chances of this happening are consequently much smaller. It now seems certain that the changes must occur at the same site in each of the pair of homologous chromosomes, and in some cases the exact chromosome has been identified. An attractive hypothesis for some tumors is that the deleted or altered genes normally produce a product that suppresses the expression of transforming growth factors by another pair of genes. The term tumour suppressor gene has been used to describe DNA sequences (genes) that act as dominant suppressors of malignancy and the identification of such genes, e.g. p53, and their relationship to the genes identified by Knudson and others in familial tumors is a field in which there is now much activity (8).

Another major and unexplained area is concerned with the time-scale of carcinogenesis. The latent period between initiation and the appearance of tumors is one of the least understood aspects of tumour development. In humans, after exposure to industrial carcinogens, it may take over 20 years before tumors develop. Even in animals given massive doses of carcinogens, it may take up to a quarter or more of the total life span before tumors appear. The time for the reduction in risk after removing the carcinogen is equally long. Alcohol induces cancer of the esophagus but stopping drinking reduces the risk. For former moderate drinkers the risk returns to normal after 10-14 years, but for heavy drinkers high risk remains for 15 years or more. Yet another unexplained fact is that only a very small number of cells 'initiated' by a carcinogen will eventually produce tumors; perhaps only one or two from many millions of treated cells.

Factors influencing the development of cancers

Many different factors are involved in the development of tumors. A cancer-producing agent or carcinogen, and presumably promoting agents, must be present. Carcinogens may be chemical or physical, e.g. radiation or ultravio-

let light which causes skin cancer in Caucasians exposed to tropical sunlight but rarely in dark-skinned races. Genetic and enzymatic disorders can be induced even by low intensity microwave radiation (9). Identified chemical carcinogens include hydrocarbon carcinogens present in coal tar and a series of chemicals used in the rubber industry. Several specific promoting agents have now been identified and work on the mechanism of action of the different types is in progress. Animal experiments suggest that viruses may be associated with the initiation of some cancers, mainly the leukemic group; viruses also seem to be associated with some types of human cancer. The role of oncogenes has already been mentioned. It is also becoming obvious that there is often an interaction between chemical carcinogens and viruses in tumour induction. A good example of this is seen in the association between hepatitis B virus and environmental chemicals in the development of liver cancer (10) and there is suggestive evidence in other tumors, particularly in cancer of the cervix (11). In other cases, such as with cigarette smoking, no single agent has been isolated but cigarette smoke is a very complex mixture of chemicals many of which may contribute to the carcinogenic effect of smoking. We know that cigarette smoking leads to the development of lung cancer and that the more an individual smokes the greater his (or her) chance of developing lung cancer; but all cigarette smokers do not develop lung cancer. There is considerable individual variation in response. We know from animal experiments and epidemiological studies that there is a genetic (DNA-associated) basis for this. Analyses of these changes are now being done at cellular and molecular level. Some genetically homogeneous, inbred strains of mice are particularly susceptible to tumour induction by particular viruses or chemicals; some species are more susceptible than others to particular chemicals. In human populations some families and some races are more prone to develop certain cancers. This may be owing to genetic or environmental factors. The chance of a particular individual developing cancer depends on the balance between the various factors concerned. For example, exposure to a massive dose of a carcinogen may override an inherent genetic resistance, or genetic susceptibility may be so high that, the development of specific tumors is invariable. With some tumors, particularly lung cancer and some industrial cancers, exposure to the carcinogen alone is sufficient to almost override other factors, but for the so-called spontaneous tumors, i.e. those that develop without a so-far, recognizable cause, we have little idea of the relative importance of the various factors.

Another factor that influences the type of cancer that develops is age. One of the few definite facts we have about cancer is that there is an age-associated, organ-specific tumour incidence. Most cancers in humans and experimental animals can be divided into three main groups depending on their age incidence. (i) Embryonic, e.g. neuroblastoma (tumors of embryonic nerve ceils), embryonic tumors of kidney (Wilma' tumors), retinoblastomas, etc. (ii) Those predominantly in the young, e.g. some leukemias, tumors of the bone, testis, etc. (iii) Those with an increasing incidence with age, e.g. tumors of prostate, colon, bladder, skin, breast, etc (12).

The incidence of human tumors is highly related to age. There are at least three possible explanations for this last group of age-associated tumors which includes the most frequently occurring human tumors, (i) There is a continuous exposure throughout life to low levels of a cumulative carcinogen, (ii) With age, humoral changes are induced, i.e. in the cellular environment, by alterations in the immune or hormonal systems, which allows or encourages neoplastic change to take place, (iii) There are age-associated changes in some cells, which increase their susceptibility to neo-plastic transformation. There is some evidence for each of these possibilities. The first is the most likely for many tumors. The relationship between tumour development in endocrine-sensitive organs, such as the breast or prostate, to age associated hormonal changes in the patient is still to be defined, but seems likely to be involved in the rate of growth of these, tumors. There are also relationship between the immune system and cancer (13), because it was noticed that immunodeficiency states are connected with increasing prevalence of some tumour types (e.g. AIDS and Kaposi sarcoma). Finally, there is some experimental evidence for an increased sensitivity to neoplastic change in tissue

culture and in transplants after the application of carcinogens to cells from some organs, such as bladder from old animals (12). We still do not know which of these explanations is correct or whether more than one process is involved.

The natural history of cancer or tumour progression

A series of changes takes place after a tissue cell is 'initiated' but the rate at which this occurs depends on changes in the cell and on changes in the host. Most chemical and physical cancer-inducing agents are very highly reactive and when they react with DNA in the affected cell they usually damage many other sites as well as the relatively few that are thought to control neoplastic transformation. Thus, the same agent may produce tumors in a given organ that differ greatly from each other, depending on the specific genes that have been altered or lost. At one extreme, if only the 'transforming' sites have been altered, the resulting tumour cells will still retain much of the normal differentiated structure and function of the cell from which they have arisen. In the skin, for example, the tumour cell will still resemble a skin cell (Fig. 2) and may still produce normal skin products and be responsive to normal growth-controlling factors. If the genes responsible for normal structure are more severely damaged, the resulting tumour cells have fewer normal properties.
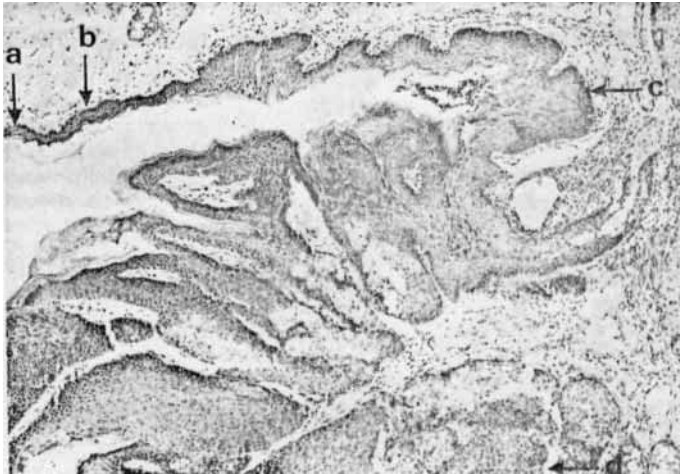

Figure 2. Section (stained with haematoxylin and eosin) if the edge of a squamous carcinoma of skin, with normal skin (a) on the left and increasing dysplasia (b) and (c) leading into the main mass of the tumour (d) below right. (x50)

At the other extreme, the cells may have lost almost all the normal properties of the cell from which they have arisen. The loss of normal characteristics is known as dedifferentiation or anaplasia. The pathologist can grade tumors by making an approximate assessment of the degree of structural dedifferentiation by examining sections of tumors under the microscope. As a rule, there is an approximate correlation between the tumour grade and the growth rate. The most differentiated tumors (low grade, i.e. Grade I) tend to be more slow in growing, and the most anaplastic (high Grade III or IV) more rapidly growing. Unfortunately, this relationship is not absolute but it does give a useful guide to tumour behavior. Human breast cancers have been graded in this way and it has been shown that about 80 per cent of patients with well-differentiated Grade I breast cancers will be alive and well at five years (and much longer), but only 20 per cent of patients with Grade IV tumors will survive for this time. It is of course equally obvious from these figures that although 80 per cent of patients with Grade I cancers survive, 20 per cent with the same structural type of tumour do not, hence tumour growth is influenced by factors other than tumour structure, particularly the reaction by the patients own defense mechanisms. Unfortunately, we have few ideas about the nature of these mechanisms. In hormone-responsive tissues such as the breast, the tumour cells may still retain some of the normal responsiveness to hormones. The pathologist's assessment of tumour grade is based only on alterations in structure and these are not invariably related to changes in function. Some cells may have lost their specific structural characters but still retain differen-

tiated biochemical characters, and others may still appear structurally differentiated but have lost many normal functional attributes.

Another practical problem in the assessment of tumors is that tumors are not homogeneous and some may contain areas with more than one tumour grade. Note that in the tumour shown in Figure 2, there is a progression from benign to malignant resembling that illustrated in Fig. 1, but the progression is in space rather than necessarily in time. It used to be thought that tumors arose from a single altered cell, i.e. were clonal in origin, but there is now some evidence to suggest that this may not be invariably true. Even if it were true, there is no doubt that by the time a tumour is detectable clinically, whether it, has arisen from one or many cells, it has been present for a long time and the cells have had to go through a large number of cell divisions so that variation and selection of different cell populations have occurred. A tumour about 0.5 cm in diameter, which is just detectable, may contain over 500 million cells. The developed tumour usually consists of a mixed population of cells, which may differ in structure, function, growth potential, resistance to drugs or X-rays, and ability to invade and metastasize. Many of these characteristics may not be stable and may be influenced by the host response or by treatment. An obvious example is the destruction of X-ray-sensitive cells by X-ray treatment. If the tumour also contains X ray-resistant cells, the cancer cells that are left after treatment will be X-ray resistant. Any individual character may vary independently. It is important to notice that tumour growing is closely related with vascular supply and neo-angiogenesis, which is under control of many growth factors and oncogenes (14).

Tumour progression is the development by a tumour of changes in one or more characters in its constituent cells. Although progression is usually towards greater malignancy, this is not invariably so. There are a number of cases (unfortunately small) in which rapidly growing tumors have ceased to grow, or have even disappeared completely. Although we do not yet have any explanation for this, it does show that there are natural mechanisms still to be discovered that will eventually allow us to control tumour growth.

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