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The mechanisms of neoplastic transformation

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Historically, the contemporary understanding of tumours developed hand in hand with Virchow's notion that the cell was the basic element of many diseases. Indeed, classical pathology began early on to describe a multitude of cancers, but, at the same time, it was also quick to recognise features that were common to them all, and this led to the concept of the cancer cell [1]. Current classifications comprise hundreds of types and sub-types of human tumours [2]; and today we can define cancer as a genetic disorder of somatic cells [3]. While this definition may sound reductionist, it offers an attractive framework of reconciling the heterogeneity of cancer with the underlying unique common misbehaviour of the cancer cell. In fact, somatic mutations are what all cancers have in common; however, the precise nature of these mutations and the cell types in which they take place must be the determinants of each individual type of cancer (see Fig. 1).

Within this framework, we have to consider three sorts of questions: (1) The number of mutations required to convert (or to pervert) a normal cell into a cancer cell. (2) The specific genes in which the mutations occur. (3) The nature of the mutations themselves.

1. *Two or more mutations are needed to produce a cancer cell*. As much as one would like to draw a sharp line between cancer and non-cancer when signing out a diagnostic report on a biopsy, a variety of lesions have intermediate features. Such lesions have been designated by a profusion of terminology (such as 'suspicious', benign tumour, indolent, dysplastic, non-invasive, *in situ*, pre-leukaemic, pre-malignant, etc.). Although these designations are qualitative rather than quantitative, it seems reasonable to presume that, at least in some cases, cells belonging to these lesions have undergone one or more somatic mutations, but short of the total number required to give cancer. The first estimate of this number (n) was derived from epidemiological data on the age dependence of the incidence of cancer in various populations: this resembles a simple power function with an exponent of approximately 5 [4]. However, Knudson's pioneering work on retinoblastoma came to be known as the two-hit model ($n=2$) [5]. To validate these estimates, more direct approaches are needed. In the case of human colon cancer, correlating histological stages with molecular analysis has again yielded a value of n around 5 [6]. In contrast, reconstructing malignancy in mouse animal models indicates that n may be as low as 2 [7]. There is no *a priori* reason why n should be the same for all tumours. At the moment, we should perhaps be content with accepting that n may range from 2 to 5.
2. *Many different genes may be mutated in cancer, but they belong to discrete functional sets*. Genes implicated in the pathogenesis of cancer have been identified by a number of different approaches, ranging from the study of oncogenic viruses, to the identification of specific karyotype abnormalities, to the isolation of genes by transformation assays (Table 1). Thus, cancer genes can be categorised in a variety of ways (Table 2). An all-inclusive classification is difficult; but popular dichotomies are oncogenes versus tumour suppressor genes; gatekeepers versus caretakers; proliferation-promoting genes or proliferation-permitting genes versus anti-apoptosis genes [8]. A concept of general importance is that, since mutation is a baseline liability of somatic cells, anything that increases the rate of mutation increases the risk of cancer; and anything that eliminates mutant cells decreases the prob-

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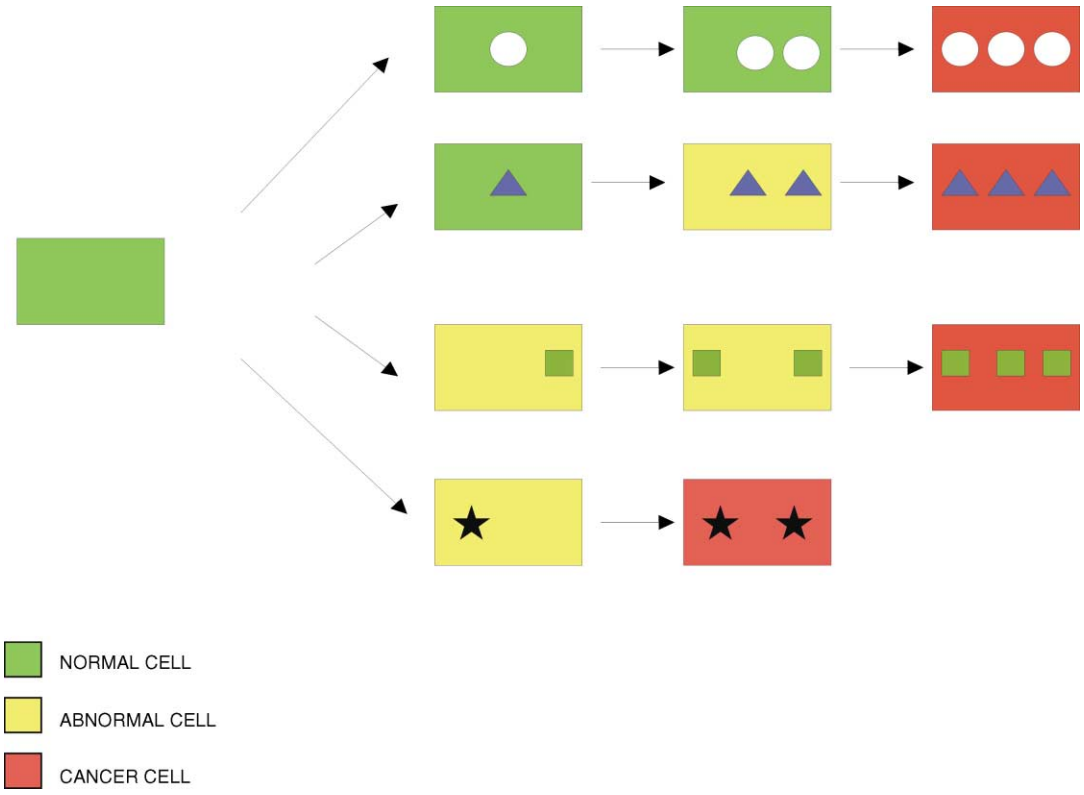

Fig. 1. *Multiple pathways from a normal to a malignant cell.* This cartoon illustrates several features of the way in which a sequence of discrete genetic events leads to malignant transformation. Each rectangle is a cell; each symbol within a rectangle is a somatic mutation. Green indicates a cell with a normal phenotype; yellow indicates a cell with an abnormal, but not malignant phenotype; red indicates a cell with a malignant phenotype. Note that: (i) the number *n* of somatic mutations required for malignant transformation may vary (in this cartoon it is either 2 or 3, but probably it can be up to 5); (ii) there are probably precise sequences required for malignant transfomation (i.e. a triangle after two circles may not produce cancer); (iii) in some cases the appearance of the cell may not change until the final mutation has taken place, whereas in others one or each mutation produces a phenotypic (pre-malignant) change; (iv) finally, this diagram accommodates the possibility that in any pathway the first mutation may be present in the germ-line, and therefore inherited: in this case the somatic mutation pathway would be reduced by one unit, and therefore the risk of reaching the malignant stage would be much higher.

ability of cancer. In practice, if we attempt to classify the kinds of genes which, when mutated, have been found to contribute to cause cancer, we can compile the following list: (a) genes encoding growth factors and growth factor receptors; (b) genes participating in signal transduction and otherwise in the cell cycle; (c) transcription-controlling genes and other genes encoding nuclear proteins; (d) genes involved in DNA repair and in chromosomal replication, mitotic segregation and telomere maintenance; (e) genes responsible for triggering apoptosis of abnormal cells; (f) genes involved in interactions of cells with the extracellular matrix and blood vessels (Table 2).

3. *Mutations may produce loss of function or gain of function.* At first sight the cancer cell may appear as a prime example of gain of function [9] since it proliferates too much. However, in many cases this is associated with a failure of differentiation, which can be regarded as a loss of function; and

Table 1
Genes responsible for oncogenesis can be identified in different ways

Approach	Likely type of gene	Example
Pedigree analysis	Tumour suppressor	Retinoblastoma (*Rb*)
Cell transformation	Oncogene	*RAS*
Specific chromosomal translocation	Oncogene	*MYC*
Retroviral homologue	Oncogene	*ABL*
Loss of heterozygosity	Tumour suppressor	*WT*

Table 2
Many different genes may be mutated in cancer: is any classification possible?

- Growth factor receptors
- G proteins
- Other signal transduction molecules
- Molecules controlling the cell cycle
- Transcription factors
- Other DNA binding proteins
- Cytoskeletal/adhesion molecules
- Signals and effectors of apoptosis
- Telomerase

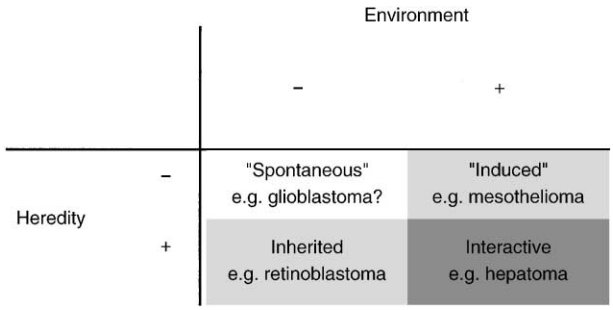

Fig. 2. *Causes and types of cancer*. Like for all diseases, also for cancer both genes and the environment must play a role, but their relative contributions may be quite different for different types of cancer. For instance, *retinoblastoma* is often due to inheritance of a mutant (inactive) *Rb* gene. Mesothelioma is almost invariably due to exposure to asbestos. Hepatoma, often arising in an already cirrhotic liver, may follow chronic hepatitis due to infection with the Hepatitis B virus, in which case we may regard it as caused by environmental factors; but it may also arise following a severe iron overload in a person homozygous for a mutant *haemochromatosis* gene, in which case we would have to regard it as of hereditary causation. For some types of cancer (e.g. glioblastoma), we know very little about the causes: except that somatic mutations must take place in those as well.

failure of controls on proliferation is also a loss of function [10] (to some extent, gain or loss is in the mind of the beholder). Mutations can be classified in terms of their consequences on the function of the gene product. In general, large deletions, frameshift mutations, nonsense mutations and many splice site mutations can all completely inactivate a gene. In many types of cancer, such mutations are probably the majority; they imply loss of function of a tumour suppressor gene. By contrast, a missense mutation can alter substantially the function of a gene only in a limited number of cases, e.g. when it modifies an active site or a binding domain. A different class of mutations, rather rarely transmitted through the germ line, but very frequent in certain types of tumours, are major gene rearrangements, such as those resulting from chromosomal translocations [11]. These comprise two distinct sub-types: those that produce fusion genes, and those where two genes become juxtaposed, whereby one influences

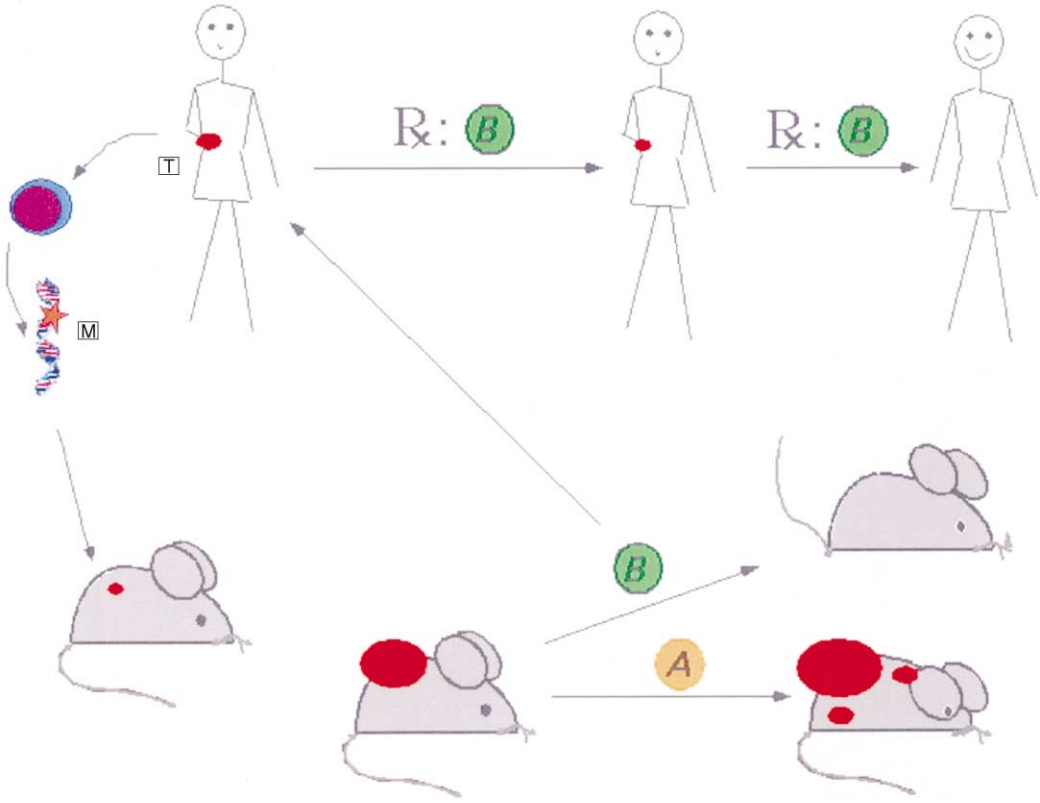

Fig. 3. Causes and types of cancer. Once the molecular lesions (M) in a human tumour (T) have been identified, it is possible to engineer them in a mouse by transgene technology and gene targeting technology. Thus, one can produce in the mouse a tumour which, unlike conventional rodent tumours, will be a close mimick of the original human tumour. By this technology, it is possible to obtain a large number of genetically identical tumour-bearing animals, in which specific therapeutic protocols can be tested. Once one treatment schedule (in this case B) is found to be effective, it can be promoted to a clinical trial in human patients. It is evident that this approach can decrease by orders of magnitude the time and cost of reaching a significant therapeutic result; in addition, much unnecessary suffering can potentially be avoided and ethical issues may be circumscribed. Rx, therapy.

abnormally the expression of the other. In either case, the consequences can be a major gain of function.

In this paper, these three cardinal aspects of the pathogenesis of tumours will be reviewed. It will also be emphasised that the concept of cancer resulting from a sequence of somatic mutations serves well to rationalise the interplay of inherited factors and acquired factors in carcinogenesis (Fig. 2). Thus, in a first approximation, the inheritance of a mutated tumour suppressor gene will decrease by 1 the number n of somatic mutations required to cause cancer. The homozygous state for a defective DNA repair gene [12,13] will increase the mutation rate and therefore potentially the rate of accumulation of n somatic mutations. Similarly, environmental factors that increase either the rate of cell proliferation (as in inflammatory processes), or the mutation rate (as with exposure to mutagenic agents) will also favour the accumulation of n somatic mutations.

The clinical implications of these concepts will not be explored in detail. However, it is relevant to mention, that, since specific molecular lesions (or sets of molecular lesions) are associated with specific types of cancer, the visible applied benefits from our improved understanding of cancer have been thus far in the area of diagnosis and prognosis. Of course, one would like to envisage that this progress will have an impact on cancer treatment as well. One development which holds promises relies on the power of animal models. In the past, a major limitation of experimental oncology has been that, precisely because there are so many different types of cancer, experimental tumours in animals have been very different from human tumours. By identifying molecular lesions in any particular type of human cancer, and by using transgenic technology and gene targeting technology, it is now possible to literally reconstruct that type of cancer in mice [14]. The mice bearing a human cancer can thus be used as an assay system for treatment protocols (Fig. 3).

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