Cancer

Step by step into carcinogenesis

from John Cairns and Jonothan Logan

MOST human cancers develop as the result of a succession of steps occurring over a period of many years. For example, the first visible step in the production of a cancer of the cervix is commonly the appearance of an expanding clone of abnormal cells (cervical dysplasia); the clone usually regresses and disappears, but sometimes it gives rise to a still more abnormal family of cells (carcinoma *in situ*), and it is among these that the fully invasive cancer arises. The sequence therefore involves the release of cells from the physical restraints that operate in an organized epithelium, a relaxation of the rules of differentiation and exemption from the rule of finite lifespan. Since we do not understand the molecular biology of the growth and form of even the simplest multicellular system, it is impossible to guess what changes in biochemistry would endow a family of cells with that rather abstract list of properties. In particular, we have no way of guessing whether cancers arise in many different ways, sometimes involving one set of gene products and sometimes another, or usually follow a well defined course involving the same set of functions.

It was therefore a great simplification to discover, during the last year, that some human cancers are abnormal in the same functions that are affected by the RNA tumour viruses of animals. In one kind of human lymphoma and in certain leukaemias, which were known to be associated with particular chromosomal rearrangements, the change was shown to involve the cellular homologues of certain known retrovirus oncogenes (proto-oncogenes). At about the same time, DNA from several human epithelial and lymphoid cancers was shown to be capable of transforming mouse cells in culture (though the majority of cancers which have been tested so far do not yield DNA active in this assay). In some instances this transforming DNA has also been identified as one of the known proto-oncogenes, behaving abnormally because it is overexpressed and/or bears some alteration in coding sequence. So there was the pleasing prospect that apparently diverse modes of carcinogenesis may actually proceed through a limited set of cellular pathways. Three papers in this issue of *Nature*, on pages 596, 602 and 648, extend the process of simplification at least one step further and also cast some light on the classes of function that have to be altered to make a cancer cell.

Originally, it was possible to identify oncogenes involved in certain cancers and to determine the localized sequence changes responsible for their activity, because the altered DNA, behaving like a dominant trait, could be incorporated into other cells by transfection and would make them tumorigenic. It was customary to use NIH 3T3 cells for the experiments because their properties had been well studied and they had proved to be very efficient recipients of transfecting DNA. This is, however, a line of mouse fibroblasts that have been 'immortalized' by passage through crisis in culture; furthermore, these cells occasionally undergo spontaneous transformation, suggesting that they may have already passed through all but the final step of carcinogenesis. So they may provide a very restricted assay for the genetic changes present in cancer cells. One of the built-in programmes protecting the whole organism against invasion by clones of uncontrolled variants is the programmed senescence that normally comes into play after a certain number of cell generations. This barrier has to be overcome whenever a cancer is formed or a cell line is established *in vitro*, and so it is not logical to expect that transfection experiments with any established cell line could show what changes are needed to circumvent programmed senescence. The key ingredient in the new experiments is the use of primary and secondary cultures of rat and hamster cells to observe what phenotypic changes are produced by tumorigenic oncogenes and to see whether it is possible to transform these more normal cells using some combination of oncogenes.

A year ago we learned that transfection of a gene isolated from the human bladder carcinoma cell line EJ will transform NIH 3T3 cells. This gene (Ha-*ras*), one of several related *ras* genes dispersed among the human chromosomes, is a homologue of the Harvey murine sarcoma virus oncogene and this version of the gene was tumorigenic for NIH 3T3 cells because it bore an alteration in one codon. The present set of experiments establishes that this mutant gene will not, by itself, transform normal rodent fibroblasts (though it can induce the ability to grow in soft agar). However, it will transform if the recipient cell is, at the same time, given a virally promoted *myc* gene (see Land, Parada and Weinberg, p.596) or certain genes from DNA tumour viruses (Land *et al.*; and Ruley, p.602), or if the cell has already been treated with carcinogens and has undergone immortalization (Newbold and Overell, p.648). For tumorigenicity, it is therefore necessary to alter at least two separate functions. There were, however, signs that sometimes a third step is necessary, because fibroblasts transformed with mutant *ras* together with virally promoted *myc* produced tumours (sarcomas) with a limited capacity for growth, whereas transfection of an established line of rat fibroblasts produced tumours that would grow without limit. In short, we seem to have here a sequence of two or three steps that would nicely fit the fact that the incidence of sarcomas in human populations increases roughly as the second power of age.

At this point, a sceptic could argue that even though the number of steps agrees with the epidemiological facts, the agreement might be misleading. The easiest way of making a sarcoma by transfection may well be to modify the functions encoded in *ras* and *myc*, but the usual way sarcomas arise 'spontaneously' could conceivably be by some other route. It is therefore very significant that the role of altered *ras* can be filled by the gene of polyoma virus that codes for middle-T antigen, and the role of altered *myc* can be filled by the polyoma gene for large-T antigen or by the *E1a* gene of adenovirus 5 (pages 596 and 602). As its name implies, polyoma can cause tumours in many different kinds of cell. So it is important news that a DNA virus, generically unrelated to the retroviruses in which the *ras* and *myc* oncogenes were first discovered, apparently produces cancers by achieving the same objectives, perhaps by modifying the very same two functions that had to be modified in the transfection experiments that use cellular oncogenes. It seems quite likely therefore that two critical steps in the formation of many human cancers — even those arising in completely

100 years ago

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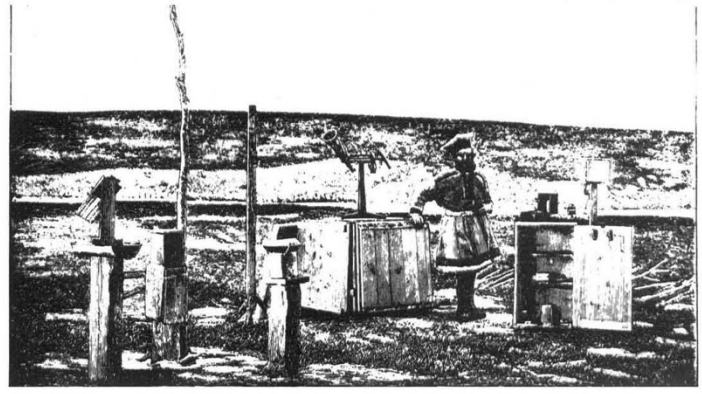

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