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Comments and Critique

Cancer Development: the Rise of Epigenetics

THINKING ABOUT the origin of cancer has tended over many years to oscillate between the genetic and epigenetic. Genetic thinking presumes that somatic mutation or some form of chromosome rearrangement is the dominant type of lesion in cancer. Epigenetic thinking is more complex, but basically assumes that defects in the type of control involved in normal differentiation, either at the level of the cell or the tissue, are the prime movers in cancer. Epigenesis may include genetic changes, just as the normal epigenetic development of immune lymphocytes or haploid germ cells does, but the genetic changes are not the primary aspect of the epigenetic process. Rather, it is the dynamic interaction between cells and tissues that underlies epigenesis.

Concomitant with the dominant position of molecular genetics in contemporary biology, the hypothetical genetic origin of cancer has become widely accepted. If a particular year were to be identified for the full ascendancy of this acceptance, 1981 might be chosen, since this is when there were three separate reports that DNA derived from long-term cultures of a human bladder cancer could transfect and then transform the NIH 3T3 line of mouse fibroblasts [1–3]. The responsible segment of human DNA was similar in base sequence to the RNA of the Harvey rat sarcoma (Ha-*ras*) virus, and was designated the cellular *ras* gene [4]. It differed from the normal cellular Ha-*ras* gene in one mutated codon. Since then, a great profusion of mutations have been found in the cells of human and animal cancers, and these have been dubbed cellular oncogenes, although few of them cause the transformation of NIH 3T3 or other cells. As it turns out, NIH 3T3 cells are uniquely sensitive to transformation by the *ras* family of genes, and readily undergo "spontaneous" transformation when subjected to moderate physiological constraints on growth or metabolism [5]. None of these findings proves that the alterations in tumour cell DNA actually caused the tumours; they simply show that genetic change is much more common in many, though not all, tumours than in normal tissue.

Perhaps the strongest arguments against the genetic origin of tumours have also come from studies of neoplastic transformation in cell culture in which the cellular change is not prefigured by a method like transfection of DNA. Studies on transformation by X-rays and chemical carcinogens indicate that most if not all the exposed cells are heritably altered so that their progeny have a higher probability of transformation than do the untreated cells [6, 7]. Such high frequency of heritable change is incompatible with mutation at one, or even at many genetic loci. The same has been found to be true for "spontaneous" transformation, which is not truly spontaneous, since it occurs only under conditions of moderate physiological constraint. The requirement for certain inductive conditions is itself inconsistent with a mutational origin. However, the most compelling evidence is that clones of cells transformed by X-rays or by moderate constraints revert to normal appearance and behaviour when placed under maximal growth conditions [8, 9]. The cells lose their tumour-producing capacity in the process [10]. These results are reminiscent of the finding that hepatocyte nodules, which are an early stage in the development of liver cancer in rats treated with chemical carcinogens, have a very high rate of redifferentiation when the carcinogen treatment is stopped [11]. Similarly, most of the nevi which precede the development of malignant melanoma in humans also redifferentiate [12]. And of course there is the well-known observation that mouse teratocarcinoma cells are capable of differentiating into a variety of normal tissues if transplanted into the early mouse embryo [13]. But these findings have been largely ignored or rationalised away in the rush to mutational judgement of the current era.

Now comes a report by Lavrovsky *et al.* (p. 17) on reversion of tumour cells, which is the most extensive and detailed of such studies to date. Soviet biologists have been studying "spontaneous" transformation of cells cultured from a number of inbred mouse and rat lines for several years. These cells produce sarcomas in syngeneic mice or rats, and have been transplanted successively many times in these animals. The authors find that cells from tumours of the lines give rise to colonies which are fully transformed, partly transformed or non-transformed. Furthermore, they have isolated transformed colonies, and find that on recloning, the cells persist in segregating colonies of all three types. Most impressively, they have repeated the cloning of cells from transformed colonies six successive times, and find that they continue to segregate less-transformed and even non-transformed colonies. Cells from the latter have lost the ability to initiate tumours in syngeneic hosts. Most of them have also lost the capacity to multiply to very high density. The non-transformed segregants are not the rare beasts that one might expect from a back mutation, but involve a considerable fraction, in some cases a majority of the clones. The frequency of reversion varies from tumour to tumour and from clone to clone. Some of these clones give rise only to transformed clones, but this is certainly not the general case. Similar results have been found in my own laboratory (unpublished data). We can say without equivocation that "spontaneous" transformation to the malignant state is basically an epigenetic process. It would seem that chemical carcinogenesis of the liver [11], and the natural occurrence of human melanomas [12] are also epigenetic. The question arises, of course, whether the underlying process in all tumours is fundamentally epigenetic in character. I know of no experiments that rule out this possibility. This is not to say that multiple mutations cannot transform cells—clearly they can—but it implies that such mutations may be epiphenomena, or at best accessory to the development of cancer.

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This leaves us with the larger question of what kind of change is occurring in the transformed cells. Given the level of molecular complexity of cells and the immense number of reactions all running near reversibility, it has been argued that biological phenomena like cancer and differentiation are inherently irreducible to a complete chain of molecular causality [14]. This conclusion is reinforced by the great variety of degrees and types of transformation in tumours. It has been proposed that the appropriate level of description and understanding is that of the intact living cell and above [11]. The concept of progressive state selection has been introduced to represent the process of change. This concept assumes that physiological constraints can select among the ever-fluctuating physiological states in cells, and that repeated state selections result in heritability of those states. These considerations focus attention on the living cell and its neighbours which provide the immediate environment for selection, and ultimately on the whole organism. Constraints include the multifarious physiological effects of aging. It is noteworthy that this dynamic epigenetic or adaptive view of cancer development provides us with more rational approaches to the prevention of cancer than does the genetic view, and may suggest some new ideas about cure.

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Tumour Markers for Ovarian Cancer

THE SITE AND PATTERN of spread of ovarian cancer make it so difficult to detect and monitor that a circulating serum or urine marker is potentially very valuable. Great interest was generated by the discovery of the serum marker CA-125 in the early 1980s [1], but we are only now appreciating to what extent the measurement of serum CA-125 affects patient management. This marker for ovarian cancer is far less sensitive or specific than human chorionic gonadotropin (HCG) is for trophoblastic tumours, yet has become the benchmark for comparison of other markers. The advent of commercial assay kits for CA-125 has made it a widely available, though expensive test, and several quality control schemes have now been set up to monitor the accuracy of the different laboratories and kits.

The observation that serum CA-125 is only elevated in about 50% of patients with stage I ovarian cancer immediately suggests that about half of all these potentially curable tumours will not be detected by CA-125-based screening. A study at the Royal London Hospital measured serum CA-125 in 20 000 postmenopausal women attending a screening clinic [2]. By performing an ultrasound in all those with CA-125 levels over 30 U/ml, they obtained an overall specificity of almost 100% and detected 11 ovarian carcinomas. Only three of these screen detected cancers were stage I. We do not know yet whether stage I ovarian carcinomas detected by an elevated CA-125 level are cured by surgery and only time will tell how many tumours develop in women who are negative on screening. This led the UK Co-ordinating Committee on Cancer Research in 1989 to recommend that screening for ovarian cancer should not yet be offered to women outside a clinical trial.

When using serum CA-125 to help in the diagnosis of ovarian carcinoma, it should be remembered that its level is above 35 U/ml in a number of conditions. These include 1% of healthy controls, the first trimester of pregnancy, endometriosis, cirrhosis especially if ascites is present, a variety of other benign conditions and over 40% of patients with advanced intra-abdominal (non-ovarian) malignancy [1, 3]. In a patient suspected of having ovarian cancer, an elevated CA-125 level increases the likelihood of finding cancer and should prompt the surgeon either to refer the patient to a gynaecological

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