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Differentiation and Cancer

M.D. Mason

NORMAL DIFFERENTIATION AND STEM CELLS

NORMAL TISSUE growth and renewal depends on cellular differentiation from a pool of stem cells, undifferentiated cells which are the origin of the mature cells that characterise an individual tissue. This system certainly exists in the haemopoietic system, in most common epithelia, and may even exist in some other non-epithelial tissues although this is not established [1]. Similarly, malignant tumours may also, as a 'tissue', be based on a stem cell system [2]. In some tumours, the stem cell population will predominate and no differentiated elements will be apparent under light microscopy. In others, mixtures of undifferentiated cells and more mature cells may give a malignant tumour its characteristic morphology.

The term determination refers to a heritable undertaking by a cell, usually during embryonic development, to follow a particular pathway of specialised development at some stage in the future. Differentiation implies that a cell acquires certain structural and functional characteristics that endow it with the ability to undertake a specialised task, e.g. to carry oxygen, or to absorb nutrients. Differentiation may involve, for example, the secretion of certain specialised molecules that are not produced by the undifferentiated cell, and therefore determination must by definition precede differentiation. Commitment implies that a cell has entered the programme of differentiation, though it has not yet completed the process. According to classical dogma, once a cell is committed it will remain faithful to one lineage pathway of differentiation only—a committed intestinal epithelial precursor cell will not turn into a red blood cell,

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for example. However, although this dogma is fundamentally almost certainly correct, certain features of malignant cells imply that early on in differentiation a cell may keep its options open with regard to which of several possible lineage pathways it may choose to follow, and primitive cells may carry markers characteristic of more than one lineage (referred to as 'lineage promiscuity' by Greaves, [3]). Indeed, there is evidence that the system can sometimes be flexible to the point that 'impossible' transitions, such as from epithelial cell to fibroblastoid cell, do in fact occur [4], but phenomena like these must be seen as exceptions and not the rule.

In an organised tissue stem cells may only be evident by their effects on tissue growth and renewal. Thus, although the existence of a bone marrow primordial stem cell was suggested many years ago [5], its formal identification has been difficult [1].

Stem cells are unique in that when they divide their two daughter cells have fundamentally different characteristics (i.e. cell division is asymmetric). One, identical to the parent stem cell, replaces it in the tissue pool, to maintain the overall number of stem cells. The other is committed, and will eventually give rise to a mature cell whose phenotype is characteristic of the tissue in which it arises. What governs the decision by a daughter cell to self-renew or to differentiate is one of the most fascinating questions in developmental biology [1]. In order to renew as a tissue, differentiation from undifferentiated stem cells must occur. In general, fully mature cells are post-mitotic, that is, they are non-proliferating and destined to die; this is as true of terminally differentiated cells arising from malignant stem cells as it is of post-mitotic cells from normal stem cells.

Stem cells as the target for carcinogenesis

It was Pierce, working on teratocarcinomas, who postulated that malignant tumours arose from stem cells, and not from the mature end-cells which give a tissue its morphology [6]. He viewed cancer as a caricature of normal differentiation in which malignant stem cells gave rise by self renewal to a larger pool of similar malignant stem cells, and sometimes also to committed cells that had a variable capacity for differentiation. It is implicit in this classical model that differentiation is a one-way process; normal mature post-mitotic cells do not revert to undifferentiated stem cells and give rise to cancers by 'de-differentiation', a term used loosely by clinicians which should be expunged from their vocabulary for ever! Also implicit in the stem cell model is that in normal tisues, between the undifferentiated stem cell and the post-mitotic end cell are a number of stages during which a committed cell may have limited proliferative capacity but not be completely undifferentiated. The phenotypic diversity of these intermediate cells could go some way towards explaining the corresponding diversity of tumours that can arise from a single tissue, if such tumours were caricatures of normal differentiation. The evolution of a low-grade tumour into a high-grade tumour results from a shift in emphasis from differentiation to self-renewal [2].

The genetic control of differentiation

Much of our understanding of normal differentation comes from studies of embryonic development, and comparatively little is known about normal adult cell differentiation. It may seem surprising to the oncologists that non-mammalian systems are relevant to human cancer, but such is indeed the case. Mutations of *Drosophila* have identified genes that are of crucial importance in normal development, whose products, as for oncogenes (see this volume), may be divided into DNA binding proteins, cytoplasmic molecules, and cell surface molecules. The DNA-binding proteins include the products of the highly conserved homeobox genes [7]. Several homologues of *Drosophila* genes are found in both normal and malignant mammalian cells. For example, the *gli* oncogene found in childhood sarcomas is closely related to the *kruppel* gene in *Drosophila* [8]. The wingless gene product in *Drosophila* is homologous to the product of the oncogene *int*-1 [9]. *Decapentaplegic* codes for a protein which is homologous to TGF-β [10]. The *Drosophila* gene *notch* codes for a transmembrane receptor with an Epidermal Growth Factor repeat motif [11].

At first sight these examples of homology are confusing because they do not present a clear picture of the relationship between normal differentiation and malignancy. However, they serve to illustrate two points. First, that many molecules which regulate growth and differentation have multiple and possibly diverse functions [12]. Second, that in order to understand how a factor influences carcinogenesis or the behaviour of malignant cells, we may first have to understand its functions in normal embryological development. It is noteworthy in this context that the action of retinoic acid (RA), a potent inducer of differentiation in many tumour cell systems *in vitro*, appears to involve the activation of several homeobox genes [13].

DIFFERENTIATION IN SPECIFIC TUMOUR TYPES

It is clear that cells from many common tumours are capable of partial, or even terminal differentiation. It was shown 20 years ago that dividing cells in a squamous cell carcinoma were the source of the terminally differentiated squamous 'pearls' within that tumour [14]. Differentiation *in vitro* of malignant cells into more mature cells which may or may not be terminally differentiated has been observed in tumours as diverse as colorectal carcinoma, leukaemia, and gliomas [15–17]. Differentiation *in vivo* of a neuroblastoma into benign ganglioneuroma was described over 60 years ago [18]. It is beyond the scope of this article to review in detail all of the known examples of cancer cell differentiation, but two tumour types stand out as being particularly important models of this phenomenon.

Germ cell tumours

Germ cell tumours provide the most dramatic model of cancer cell differentiation. The diversity of cell types present in such tumours can be understood in the light of evidence that the embryonal carcinoma (EC) stem cell in non-seminomas is very similar to a pluripotent embryonic stem cell, i.e. it is capable of differentiating along multiple lineage pathways. In the mouse, EC probably corresponds to cells of the inner cell mass of the pre-implantation embryo. Germ cell tumours are caricatures of normal development in which somatic differentiation gives rise to the embryo and subsequently to the animal itself, and extra-embryonic differentiation gives rise to supporting tissues such as yolk sac and trophoblast, which do not form the animal proper. By understanding what sort of cell the malignant teratoma stem cell thinks it is supposed to be, we can begin to understand certain aspects of its behaviour.

It was the experiments of Kleinsmith and Pierce [19] that demonstrated that malignant teratoma cells could differentiate into 'benign' tissues. They found that it was possible to inject a single teratocarcinoma cell into a mouse, and generate a tumour comprised of both malignant teratoma and also of benign differentiated tissue. Even more dramatic were the experiments by Brinster, in which a malignant teratoma cell was introduced

into a normal mouse blastocyst which was then transferred to a foster mother. The healthy offspring resulting from this blastocyst was a chimeric mouse, in which its tissues were derived partly from normal mouse embryonic cells, and partly from teratocarcinoma cells [20]. The molecular processes favouring the survival of malignant teratocarcinoma stem cells are, indeed, 'subservient to the normal differentiation programme' [3]. In one chimera the cells derived from the teratoma included those of the germinal epithelium, and it was possible to propagate a second generation of apparently normal mice whose 'father' was, in a sense, a teratocarcinoma cell! It is clearly incorrect to say that 'once a cancer cell, always a cancer cell'. Conversely, the transplantation of a normal mouse blastocyst into an extra-uterine site led to the formation of a malignant teratocarcinoma [20]. Although it is not possible to perform such an experiment in human teratomas, it is clear that, as in the mouse, human EC cells are pluripotent.

When human EC cells of the cell line NTERA-2 are treated *in vitro* with RA they undergo somatic differentiation into neurone-like cells. By contrast, cells of the mouse EC cell line F9 treated with RA undergo extra-embryonic differentiation into parietal endoderm. Treatment of NTERA-2 cells with another differentiation-inducing agent, hexamethylene bisacetamide (HMBA) induces them to differentiate into a large, flat cell type with entirely different phenotypic markers to the RA-treated cells [21]. The outcome of an interaction between a cell and a differentiation-promoting agent clearly varies according to the cell and according to the stimulus. Whether the differentiated cells produced in this context are truly benign and post-mitotic is open to question; the clinical phenomenon of sarcomatous degeneration of differentiated testicular teratoma deposits argues that such tissues are not always stable.

Haematopoietic malignancies

Human lymphoid malignancies provided the most comprehensive model of the relationship between normal differentiation and the phenotype of cancer cells. The phenotypic characteristics of normal lymphoid precursor cells along their cell lineage pathways of differentiation from the primordial stem cell have been elaborated to a high degree of sophistication. What is striking about lymphoid malignancies is that their cellular phenotype is often remarkably similar to that of normal cells somewhere in the pathway between primordial stem cell and mature cell [3]. The real problem in haemopoietic malignancies and perhaps in other cancers may not be that abnormal differentiation is taking palce, but rather, that normal differentiation is frozen at an intermediate stage leading to the clonal expansion of a cell type which is usually transient and therefore extremely rare [3]. The motive of carcinogenesis in this setting would be the stabilization of this transient cell type. Some specific genomic abnormalities may merely be the method whereby this is achieved. For example, the t(14;18) translocation in lymphomas involving the *bcl-2* gene may be important because *bcl-2* codes for a protein that is an inhibitor of programmed cell death [22], and see Brada, (270–272). The t(15;17) translocation seen in acute promyelocytic leukaemia results in the fusion of a retinoic acid receptor gene to a different site, which is interesting given that RA promotes the differentiation of acute promyelocytic leukaemia cells [23].

In haemopoietic malignancies, the block in normal differentiation can be reversed; injection of leukaemia cells into the placentae of mouse embryos sometimes resulted in normal mice whose haemopoietic tissues were chimeric. Therefore, all of the leukaemia cells injected into these mice were able to participate in normal differentiation [24]. Differentiation of erythroleukaemia cells can also be induced by HMBA [25].

CONCLUSIONS; PROSPECTS FOR DIFFERENTIATION THERAPY OF CANCER

The only hope that some form of differentiation therapy might work in cancer is if the block to terminal differentiation that occurs in malignancy is relative and not absolute. That this might be so is suggested by the effect of agents such as RA and HMBA which induce differentiation in malignant stem cells that would normally remain undifferentiated, and clinical trials incorporating these and other agents are underway. If the nature of the 'block' in differentiation is such that in an individual dividing cancer cell the probability of self-renewal is much higher than that of differentiation, the real goal of differentiation therapy is to tilt the scales in the opposite direction. Let us be optimistic in that by understanding normal differentiation such a goal may yet be attained in the common malignancies.

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Approaches to Proto-oncogene and Tumour Suppressor Gene Identification

Helen Patterson

INTRODUCTION

THE PAST decade has seen dramatic advances in our understanding of tumour development at the molecular level and it is now clear that the malignant phenotype arises as a result of the accumulation of genetic mutations in two classes of cellular genes, proto-oncogenes and tumour suppressor genes. Genetic alteration of proto-oncogenes causes their conversion into dominantly acting oncogenes and results in the altered expression of the oncogene or the production of an abnormal oncoprotein product. Although the demonstration in tumour cells of rearrangement, amplification or point mutation in a gene sequence is often taken as evidence that this sequence is acting as an oncogene, unequivocal proof can only be obtained by demonstrating transforming effects following expression of the cloned gene in, or introduction of the oncoprotein product, into the appropriate non-transformed cells. The considerable effort directed towards determining the function of these genes has clearly shown they are involved in the pathways by which growth factors promote normal cellular proliferation.

A substantial body of evidence now indicates that suppressor genes may also be involved in tumourigenesis. Although original accounts of these types of gene were restricted to rare inherited tumours, such as retinoblastoma and Wilms' tumour, it is now apparent that alterations of suppressor genes occur in most major classes of malignancy. In contrast to dominant oncogenes, where it is the presence of an abnormal gene product that is required for transformation, alteration of suppressor genes results in loss of genes that are involved in controlling cell growth and differentiation. In this case the complete removal of gene function requires inactivation of both copies of the gene. Proof that a gene has suppressor function can only be obtained from experiments in which tumour cells are reverted to a normal phenotype following introduction and expression of the normal cloned gene.

Following the original identification of oncogenes as the transforming sequences of acutely transforming retroviruses several different strategies have been used to detect activated genes that can contribute to tumour development. These methods, which will be reviewed below, have proven extremely successful and over 60 oncogenes and their corresponding proto-oncogenes have now been characterised. Although the identification of tumour suppressor genes has proven more difficult, a handful of genes, all of which have been implicated in human malignancy, have now been characterised and the strategies used in their isolation will also be considered.

PROTO-ONCOGENES

Retroviral oncogenes and integration sites

Two types of tumour-inducing retrovirus can be distinguished. One group (the acutely transforming retroviruses), which harbour viral oncogenes, is characterised by the ability to efficiently induce tumours in animals and to rapidly transform cells in culture. Over 20 retroviruses containing distinct oncogenic sequences have now been characterised and it is clear that in each case the viral oncogene (v-*onc*) was derived by transduction from a cellular gene, which is referred to as the cellular proto-oncogene (c-*onc*). Sequences originally identified as viral oncogenes include v-*myc*, v-H-*ras*, v-K-*ras*, v-*abl*, v-*src*, v-*erb*A and v-*erb*B [1] (Table 1). The v-*onc* and corresponding c-*onc* usually differ in their level of expression as v-*onc* sequences are under viral transcriptional control and may also be truncated or contain point mutations.

Chronically transforming retroviruses, so called because they induce tumours in animals with long latency, lack oncogenes but act via proviral integration in the host genome to disrupt cellular proto-oncogene sequences and their transcriptional control. Sequence analysis of viral integration sites in avian leukosis virus induced tumours has identified sequences previously cloned from acutely transforming retroviruses, e.g. c-*myc* [2] and c-*erb*B1 [3]. Sequences not previously cloned as viral oncogenes include *int*1 [4] and *int*2 [5], the common integration sites in

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