The Metastatic Cell

Behaviour and biochemistry

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To the thousands of authors I have read, for their results and ideas which form the basis of this book, I offer my greatest thanks. Of course, I have not always been in agreement with published work, but then controversy is a major part of scientific progress and there is no reason why it should not play its role in this book as elsewhere.

Numerous colleagues have assisted me in bringing this book into fruition, particularly during its final stages. These have included Professor Ray Ralph, Professor Stan Bullivant and Dr Warren Judd in Auckland, Dr Corrado D'Arrigo, Dr David Sinclair and Dr Gordon McPhate in St Andrews, and Professor Adam Curtis in Glasgow. I am especially indebted to Dr Clare Ward (Auckland) for her sterling efforts in reviewing the entire manuscript, and to Mr Simon Evans (Auckland) for his assistance with compiling the reference list. It goes without saying that whatever gremlins have crept into the text originate solely from my word processor.

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Finally, completion of this book has not been without a considerable burden on my family. To Barbara and Simon, for their endurance, patience and forbearance, I offer my most sincere thanks.

Preface

This book provides a basis for approaching the problem of cancer through examination of the biochemistry and behaviour of malignant cells. It stems from my experiences with medical students who, despite a solid grounding in general pathology and now also in molecular and cellular biology, nevertheless remain relatively ignorant of a large body of research in fundamental aspects of cancer biology. Its purpose is to redress this imbalance, providing a framework for critical analysis. It should also prove comprehensive enough to offer a basis for science students contemplating a career in cancer research. It must be emphasized, however, that this topic is exceedingly vast and this book reflects only a few selected areas, particularly those which fall within the sphere of my own research interests.

I do not apologize for the fact that this book offers no answers to the problem of cancer. If it serves only to make the reader ask more questions, then in many ways it will have achieved its purpose. It contains a wealth of experimental results from which a plethora of hypotheses relating to cancer may be proposed. In dealing with this information it is important to keep in perspective that cancer is not a single disease, and that there is unlikely to be any universal answer to the cancer problem. For this reason, sweeping generalizations based on single model systems are to be avoided at all costs. As the reader will find out, scientists who have causes to champion and an insatiable desire for funding do not think twice before launching into the most improbable of generalizations. I have endeavoured to keep these in perspective, but readers contemplating a career in cancer research should nevertheless appreciate the fundamental importance of hypotheses to scientific enquiry.

Contents

1 The tumour cell phenotype

1.1 WHAT IS A TUMOUR?

Strictly speaking, the term tumour (Latin *tumor*) refers to a swelling. and many of us will recall learning that Celsus recognized *tumor*, alor with *rubor* (redness), *calor* (heat) and *dolor* (pain), as the cardinal sig of acute (non-persistent) inflammation. However, it is now common tc use the term tumour as a synonym for **neoplasm**, meaning a new growth. This new growth results from an inheritable change in a cell (or cells) which allows them to escape from many of the normal homeostatic mechanisms which control proliferation. It seems likely that this change can occur in any cell capable of dividing. When it has taken place, the cell is said to be **transformed**, although this term was originally used when the cell change occurred in an *in vitro* experimental system. There are a number of provisos in using the term 'transformed' to describe tumour cells under both *in vitro* and *in vivo* conditions, the most important being that *in vitro* transformed cells do not necessarily form tumours *in vivo*. Cells transformed experimentally by certain viruses, for example, may not grow when injected into syngeneic hosts. The most likely reason for the failure of such cells to form tumours is that they are rejected by the host as a consequence of its defence systems recognizing viral determinants on the cell surface. Experimentally, we may suppress the defence systems of animals in order to allow such cells to grow *in vivo*. This satisfies the main criterion for neoplastic change, since in practical terms confirmation of *in vitro* transformation is best obtained by witnessing the *in vivo* growth of a tumour from the corresponding cells.

It is of some importance at this early stage to differentiate between cell proliferation of normal tissue (even when extensive as seen in wound healing, for example) and cell proliferation associated with

transformation. In the former case, proliferation is under some form of control and ceases when a regulated end point (e.g healing) is reached; transformed cells, however, usually continue to proliferate to the detriment of the host. We shall see later that even under *in vitro* conditions, normal cells respond to proliferation signals in a manner which is usually distinct from that of transformed cells, although many specific aspects of their responses share common molecular pathways.

The escape of tumours from many of the normal regulatory control mechanisms of the body also clearly distinguishes them from other tissue swellings such as may be seen, for example, in a lymph node draining an inflammed site. Despite the origins of the term 'tumour', it should be noted that not every tumour will necessarily result in a swelling. Leukaemias, for example, may extend diffusely in the bone marrow and circulate in the blood without producing an overt swelling. Very occasionally tumours may be seen to regress, which might suggest that in certain cases some tumours may not have fully lost, or may be able to regain, sensitivity to the normal proliferation regulatory mechanisms.

Finally, it is worth noting that what is clinically called a tumour is more than just a mass of transformed cells. First, as will be decribed in due course, a tumour is heterogeneic in that over time its cells are likely to change. Some tumours may do this at different rates or along different paths from others, resulting in a phenotypically mixed bag of cells. Second, as described by Woodruff (1980), a tumour may be viewed as a highly complex ecosystem which contains not only transformed cells but normal host cells as well. These are likely to include macrophages, lymphocytes and fibroblasts to varying degrees, and the tumour will also contain blood vessels and other stromal elements. There is usually also a good deal of necrotic material, particularly in large tumours. It may be expected that there will be interplay between these various components and that the ultimate pattern of tumour growth will depend as much on this interplay as on other contributions from the host such as the supply of nutrients and removal of waste products.

1.2 GENERAL ASPECTS OF CELL GROWTH

Before discussing aspects of tumour cell growth further it is pertinent to review some general aspects of cell division.

1.2.1 The cell cycle

The cell division process is best described as a cycle from which cells

may enter or leave via a 'resting' phase (G_0). As illustrated in Fig. 1.1, cells enter the cycle in a 'gap' known as G_1 prior to their commencing DNA synthesis in S phase. This phase is followed by another 'gap' (G_2) which leads into the mitotic or M phase. The 'gaps' are thought to represent preparatory phases prior to DNA synthesis and mitosis: protein and histone synthesis occur in G_1, for example, and these are essential for progression through the cell cycle. The daughter cells arising from mitotic division enter G_1 from which they may continue to cycle or they may enter the resting phase in which they could take on specific functions (**differentiate**). Some normal cells, such as the polymorphonuclear neutrophil leukocytes (PMNs), are thought not to leave G_0 and therefore they are not capable of division. Tumour cells may also enter G_0 and this may be the basis of dormancy in which tumour cells seem to be in a non-proliferative state.

The regulated movement of cells to and from and within the cycle controls cell division. Control is thought to be mediated by various types of intra- and intercellular signals acting at certain positions in the cell cycle called control points. Detailed studies of the responses of confluent, serum-starved BALB/c 3T3 cells to fresh serum or growth factors has lead to the demonstration of several such points (reviewed in Denhardt, Edwards and Parfett, 1986). The addition of certain growth factors such as platelet-derived growth factor (PDGF) acts on cells in G_0 to induce a state referred to as **competence** from which the cells can progress through cell division following the addition of other factors (Pledger *et al.*, 1978). About halfway through G_1 there is a control point referred to as the V point beyond which cells will not progress unless they have been treated with appropriate factors such as somatomedin C, also known as insulin-like growth factor 1 (IGF–1). Later in G_1, about 2 h before the commencement of S phase, there is a restriction point (R) where cells are thought to commit themselves to DNA synthesis. Another control point at which cells may be arrested, the W point, exists immediately before S phase. As far as transformed cells are concerned, some are thought to lack the requirement for a competence-inducing signal and some may not display an obvious restriction point. It should be noted that the details of cell cycle events worked out for one particular cell type in response to particular growth factors need not fully describe the processes involved in other cells, and that the functions of control points in tumour growth are far from being understood fully. Experimental studies, particularly from research on the development of chemically induced squamous carcinomas in the hamster cheek pouch, suggest that transformation is associated with an increase in the rate of cell proliferation, but as yet there is little reliable data from human studies.

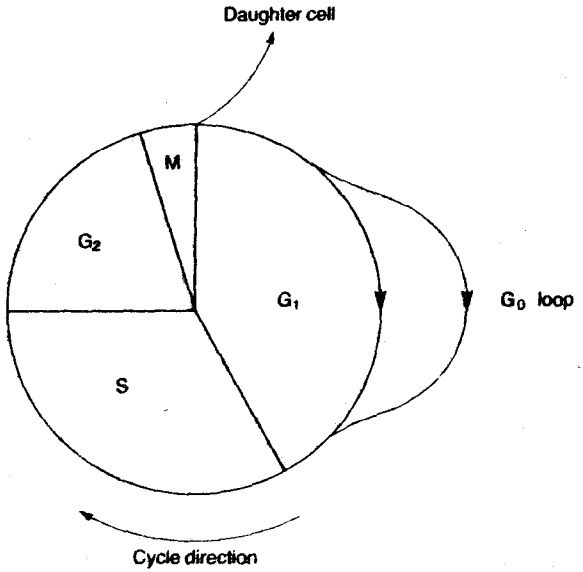

Figure 1.1 The cell cycle. Each cell can be considered to enter the cycle at G_1 after the mitotic division of its parent. Under appropriate conditions, DNA synthesis commences and the cell proceeds into M phase via G_2. Some cells may enter a 'resting' phase (G_0) usually leading to differentiation, and from which they may or may not re-enter the cycle. A typical mammalian cell in culture has a cycle (generation) time of around 18 h, with about 8 h being spent in G_1, 6 h in S, 4 h in G_2 and less than one hour in M phase.

1.2.2 Growth control

Different organs and cells may respond to growth controls in different ways. Generally speaking, however, we may hypothesize a steady state under normal circumstances in which cells are either stabilized, having reached an appropriate population size, or they turn over to match the number of cells lost for one reason or another. This steady state may be perturbed under various circumstances. The removal of one kidney, for example, may lead to compensatory growth in the other; the application of exogenous hormones may lead to target organ growth as seen in the adrenal gland following treatment with adrenocorticotrophic hormone (ACTH); and wounding of organs such as the skin may lead to reparative growth. These growth responses are nevertheless still under some form of control, and it is only following transformation that this appears to break down.

Studies of normal renewing cell populations, such as that of the epidermis and bone marrow, have led to the formulation of a **stem cell model** of tissue maintenance. There are many refined versions of this model, but all of them are based on a hierarchical level of organization (Fig. 1.2). Generally speaking, the loss of normal differentiated tissue cells is maintained by a steady state balance in which new cells are supplied from a small pool of stem cells. Although their progeny may enter the differentiating pathway, stem cells are the only cell type capable of self renewal. As shown in Fig. 1.2, stem cells may also shift in and out of a non-proliferating state (G_0). The stem cell progeny which enter the differentiation pathway (**transitional cells**) have a finite proliferative ability, but the divisions they do undergo result in considerable expansion of the original clones. The transitional cells can assume varied differentiation characterisitics which are determined for each tissue. Progression along the differentiating pathway results in a population of **end cells**, which are fully differentiated but non-dividing. The stem cell concept has been applied to neoplastic tissues and this is discussed further in section 1.6.3.

What mechanisms can be proposed to control the steady state of cell growth? It must be admitted at the outset that there is no single, widely accepted biological model of growth control. It is possible to envisage a steady state of growth under the influence of factors which stimulate and/or inhibit cell division and a considerable amount of research is proceeding in this direction. What controls these possible factors, however, is even more of an enigma. A summary of various growth factors implicated in the control of cell division is provided in section 1.7.1. At this stage it is worth pointing out that there are three main pathways by which factors might influence cell division:

(a) *The endocrine pathway*

This is the pathway reflected in the activities of the typical hormones, which are secreted by one cell type and are carried in the blood to influence the functioning of another cell type some distance away.

(b) *The paracrine pathway*

Factors produced by one cell type diffuse locally to influence another cell type nearby.

(c) *The autocrine pathway*

This pathway is characterized by the synthesis of a factor for which the

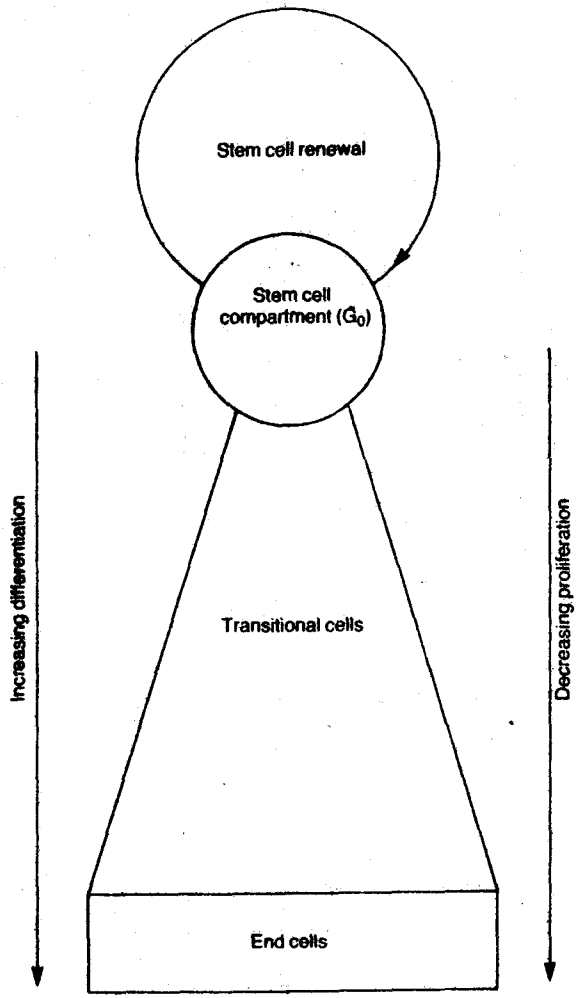

Figure 1.2 The stem cell model of cell renewal and differentiation. In this model, stem cells are the only cell type capable of self renewal. Under appropriate conditions, stem cell progeny enter the differentiation pathway passing via transitional cells into a terminal end cell stage. Clonal expansion of the original stem cell progeny occurs during differentiation, but the cells become progressively restricted in their division potential (after Buick and Pollack, 1984).

producing cell also has receptors (Sporn and Todaro, 1980). Although autocrine secretion is a normal physiological process (occurring in embryonic development and wound healing, for example), its unregulated activity has been reported in a number of tumours.

1.3 HOW ARE TUMOURS CLASSIFIED?

Tumours are classified with reference to a number of criteria including their behaviour, their appearances, and their origins. Clinical experience indicates that there are two fundamental types of tumours, **benign** and **malignant**, which behave in different ways. Benign tumours remain localized and do not spread to different parts of the body. Malignant tumours, on the other hand, usually invade and destroy host tissue and may spread throughout the body. The term **cancer** is used to mean a malignant tumour. A summary of the major differences between benign and malignant cells is provided in Table 1.1.

1.3.1 Behaviour

There are two major behavioural traits of tumours which assist in their classification, namely their growth and their invasive and metastatic behaviour.

(a) *Growth*

Unlike the growth of normal tissue, the growth of a tumour seems not to be under control by the normal regulatory systems of the body. This

Table 1.1 Major differences between benign and malignant cells*

Feature	*Benign*	*Malignant*
Cytoplasm	slight basophilia	marked basophilia
Mitotic figures	few and normal	many and abnormal
Nucleus	predominantly normal	pleomorphic
Nucleoli	little altered	often swollen
Tissue structure	usually normal	dysplastic/anaplastic
Functions	usually normal	lost or deranged
Capsule	usually intact	often lacking
Metastasis	never	often
Local invasion	rare	common
Fatalities	rare	often

* Note that benign lesions never metastasize, but exceptions exist for every other feature listed.

is not to say that the growth of a tumour is entirely autonomous, since it is still dependent on the host for the supply of nutrients and the removal of waste products. Although uncontrolled growth is taken as the hallmark of a tumour, tumours vary in their individual rate of growth, an observation which suggests that this behavioural trait may not be particularly reliable as a basis for classification. Indeed, in classifying tumours it should be borne in mind that there are few hard and fast rules and that there are many exceptions to the generalized features used in classification. Thus, although many (but certainly not all) malignant tumours have a higher mitotic rate than benign tumours (often as judged by the presence of more numerous mitotic cells), some tumours of either type may not be growing actively at all. In addition to having generally a relatively high mitotic rate, malignant cells often have nuclei which are more variable and irregular (**pleomorphic**) when compared with the more typical nuclei of benign cells. Furthermore, the nucleoli of malignant cells may be relatively larger and more prominent than those in benign cells, and their cytoplasm may be more markedly basophilic indicating high levels of cytoplasmic mRNA which is typical of active cells. It should also be noted that benign tumours seldom kill the host unless they grow to press on some vital structure such as a major artery or an airway. According to impeccable sources (*The Guiness Book of Records* quoted in *The Times*!), the largest benign tumour ever removed from a human (an ovarian cyst, which usually contains a high fluid content) weighed a remarkable 328lb (about 149kg).

(b) *Invasion and metastasis*

Cells of benign tumours are often enclosed in a fibrous capsule and they usually remain at their site of origin. Malignant tumours, on the other hand, rarely have a complete capsule and they frequently invade the local tissue. Although invasiveness is a characteristic feature of malignant cells it is not peculiar to them. Normal cells in the embryo can be invasive as seen, for example, in the invasion of the endometrium by trophoblasts. In adults, leukocytes such as the polymorphonuclear neutrophils (PMNs) display invasive behaviour when they exudate from the blood towards inflammatory foci. Superimposed on their locally invasive character, malignant tumours often spread to remote sites after gaining access to the lymphatic or blood systems or to body cavities. The spread of malignant tumours culminating in the establishment of one or more secondary tumours at a remote site is known as **metastasis**. Although trauma may occasionally result in the implantation of a benign tumour cell at a