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5 Some Mathematical Modelling Challenges and Approaches in Cancer

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5.1 Introduction

Over the past several decades an intense, primarily experimental, scientific effort has yielded remarkable increases in our understanding of tumour biology. In 2003 alone over 22 000 articles on cancer were published in the world literature (Gatenby and Maini, 2003). However, the impressive scientific contributions contained within individual articles are often fragmented and isolated due to the absence of comprehensive conceptual frameworks that allow data to be organized and integrated. Furthermore, many extant conceptual models are linear, narrowly focused and non-quantitative, and thus of limited value in a disease such as cancer, which is a multiscale process (microns to centimetres) dominated by non-linear system dynamics.

Recently, the limited impact of these efforts on the personal and societal burden of human cancer has led to interest in new multidisciplinary approaches that synthesize biological data and hypotheses with mathematical modelling. In fact, there seems to be an emerging consensus that mathematical approaches are necessary to develop a coherent framework for understanding the complex intra- and extracellular dynamics that govern tumour biology.

As in the physical sciences, mathematical models serve to organize and integrate the extant data within tumour biology by formulating relevant biological hypotheses in terms of ordinary or partial differential equations, or other mathematical constructions.

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Analytical expressions and numerical simulations developed from these models predict system dynamics that can be tested experimentally. A good model is capable of providing a virtual 'laboratory' in which system parameters and hypotheses can be varied systematically and tested in ways that would not be feasible experimentally.

Michelson (1996) has noted that 'modeling is a process rather than a technique'. Many specific mathematical methods have been employed in modelling cancer, including ordinary differential equations, partial differential equations and cellular automata approaches. This wide range of methods reflects the complexity of the task. Tumours exhibit marked heterogeneity in a wide range of temporal and spatial scales. For example, accumulating genetic mutations are characteristically found in cancer cells, which typically exhibit a total number of mutations that ranges from hundreds to hundreds of thousands. In addition there are a large number of unmutated genes that exhibit marked variations in expression when cancer cells are compared with their normal progenitors. These changes are typically time dependent as multiple populations arise, proliferate and regress during the stepwise evolution of tumours from normal through multiple preneoplastic lesions to invasive cancer. In fact most individual tumours consist of a mosaic of multiple phenotypically and genotypically distinct subpopulations, each capable of further evolution with time.

In addition to this genotypic and phenotypic diversity, the complex tumour–host interaction results in considerable spatial and temporal heterogeneity. Thus, tumours often contain areas of hypoxia and acidosis due to inadequate vascular density, or diminished blood flow due to spasm, thrombosis or vascular shunting. Furthermore, the host immune response antigens on the transformed cells may result in tumour infiltration by a wide range of anti-bodies, macrophages, lymphoctes and associated biological modifiers, with variable effects on both the tumour cells and their environment.

Tumour therapy adds further complexity with the death of some tumour cells, evolution of resistant phenotypes and therapy-induced alterations in the microenvironment.

An important component of mathematical modelling of biological processes is bioinformatics, which employs sophisticated statistical and computational approaches to evaluate the enormous data sets obtained from molecular biological methodologies, particularly genomics and proteomics. In general, bioinformatics is focused on the analysis of molecular scale data, which is then correlated to larger scale structures such as tumour growth rates, metastatic potential, etc. Such data and its inferences can be used to inform the mathematical models describing biological processes at the molecular, subcellular, cellular, tissue, organism or population scale.

5.2 Multiscale modelling

One of the fundamental difficulties in deriving a mathematical model for tumour growth is the implicit multiscale nature of the process, ranging from subcellular (molecular) processes to those that act on the tissue length scale. Cancer growth is only one example in biology where this problem arises. Indeed, it is intrinsic to any situation in which

interactions on a local scale determine and are modified by global dynamics. In established areas of modelling, e.g. materials science, one can use homogenization and averaging techniques. These rely on a certain microscopic regularity in the material being modelled. However, the diverse non-homogeneous nature of biological systems means that this approach cannot be applied easily to problems in the life sciences.

It is now thought that the complexities of cancer are understandable in terms of a small number of underlying hallmark properties, namely, self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of programmed cell death (apoptosis), limitless replication potential, sustained angiogenesis, tissue invasion and metastasis (Hanahan and Weinberg, 2000). Hence, any modelling approach must be developed with these traits in mind. To date, many mathematical models have focused on one or two aspects of tumour dynamics occurring at a particular scale. In fact there is now quite an extensive mathematical modelling literature in relation to cancer growth but a review is beyond the scope of this chapter. We refer the reader to the reviews of Adam (1996) and Araujo and McElwain (2004). Mantzaris, Webb and Othmer (2004) present a very detailed review of tumour angiogenesis, whereas Bellomo, Bellouguid and De Angelis (2003) review interactions with the immune system. The paper by Jain (2001) reviews several models for drug delivery.

More detailed models using the cellular automaton (CA) approach have been proposed. The discretized quality of CA models allows individual cells and their life history to be examined and are thus ideal for small, heterogeneous populations that cannot be described accurately with ordinary or partial differential equations. However, traditional CA models have the disadvantage of not including continuous, time-dependent biological processes such as the gradients of substrate or growth signals. For this reason, modified CA models have been developed (see, for example, Patel *et al.*, 2001) in which a tissue is described by an $n \times n$ CA lattice in which each cell corresponds to a physical cell but is also described by a state vector that includes such things as concentrations of substrate or growth factors. Over time these molecules are produced, consumed and diffuse, allowing for spatial and temporal heterogeneity. The rules of the cellular interactions then can be linked explicitly to these concentrations so that the proliferation, invasion and regression of populations can be observed. Thus, the emerging area of hybrid models combines single cell-level phenomena with continuum equations for macromolecular transport. There are now a number of such models in the literature. For example, the model by Ferreira, Martins and Vilela (1998, 1999) uses a two-dimensional hybrid CA to model cancer and normal cell movement, and it calculates growth factor concentrations from a continuous model. The influence of the cell on growth factor concentrations is via delta function source/sink terms in the continuum model for growth factor concentration. In addition, average nutrient levels influence cell proliferation probability. Although most analyses of the modified CA models have been in two dimensions, there is increasing appreciation for the need to observe whole volumes of tumour. The most recent three-dimensional CA model appears to be that of Kansal *et al.* (2000). This model does not explicitly include nutrients or mechanical interaction between cells, but mimics their effects in a phenomenological way and can produce three-dimensional structures resembling tumours with different clonal subpopulations similar to those observed experimentally.

Here, we review the results of some recent work in which we explore different aspects of tumour dynamics.

5.3 Tumour vascular modelling

Extensive work by Folkman (2003) and others has clearly demonstrated the critical role of angiogenesis in the development of invasive cancers. In the absence of in-growth of new vessels, proliferation of tumour populations is limited by substrate availability, which must diffuse from adjacent normal tissue. Diffusion-reaction mathematical models and empirical studies have clearly demonstrated that cell viability due to diffusion of substrate from a blood vessel is limited to 100–160 microns. Thus, proliferation and in-growth of new vessels are required for any sizeable tumour population. Indeed, empirical studies have demonstrated that avascular growth will produce a tumour no more than a few cubic millimetres in volume and acquisition of the angiogenic phenotype corresponds to the development of an invasive cancer.

Given the importance of angiogenesis in tumour biology, it is not surprising that intensive modelling efforts have been employed to understand the underlying molecular, cellular and tissue dynamics. Our approach to modelling vascular tumours is to represent tissue level signals (e.g. nutrient concentrations, growth factors, etc.) by systems of non-linear partial differential equations. These signals are 'read' by cells, represented by cellular automata units that respond accordingly. The response, to begin with, is represented by a phenomenological set of rules, but as the model becomes more sophisticated these rules will be replaced by ordinary differential equation models that describe the evolution of chemicals/proteins, etc. within the cell.

For example, in Alarcón, Byrne and Maini (2003) an idealized hexagonal network of blood vessels is considered. The radii of the vessels within this network are modified by the mechanical stimulus of flow (wall shear stress) and tissue demand (following Pries, Secomb and Gaehtgens, 1998), resulting in a heterogeneous network. This then provides the source of nutrient (in this case oxygen), which is modelled by a reaction diffusion system in which nutrient diffuses across the blood vessel walls into the tissue, in which it diffuses in a Fickian way and is taken up by cells. In turn, the cells divide if the nutrient concentration is above a certain threshold value whereas if it is below this threshold value the cells will either die (normal cells) or fall quiescient (cancer cells). These threshold values are set arbitrarily for each type of cell. Furthermore, cell–cell interaction is also taken into account by increasing the level of this threshold if a cell is surrounded by neighbours of a different type (this is a highly phenomenological way to model the type of cell–cell competition mentioned below).

This simple model allows us to explore the effects of heterogeneity of oxygen concentration on the growth dynamics of cells and we show that this has a profound effect (Figure 5.1), namely, that nutrient heterogeneity appears to reduce greatly the tumour tissue's ability to grow.

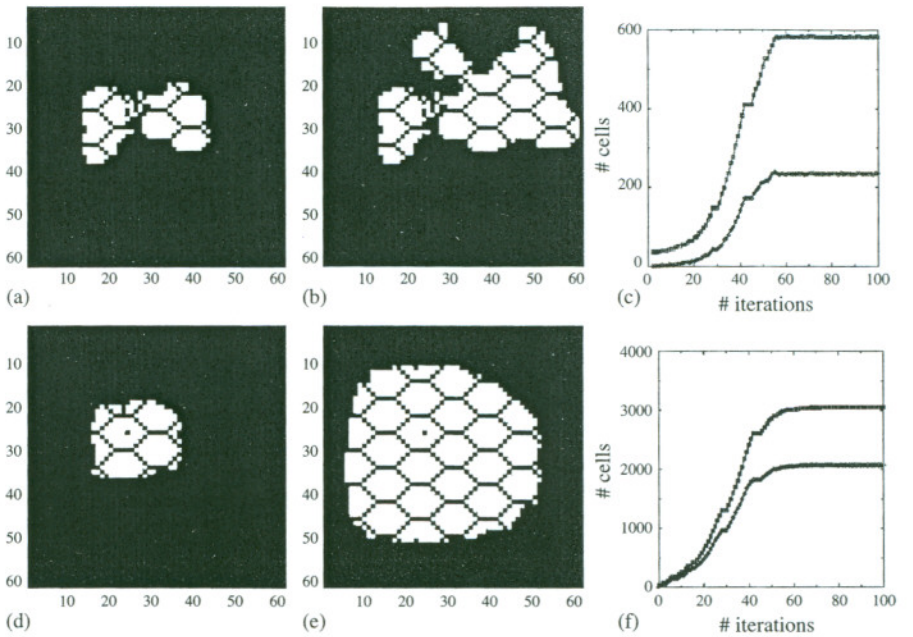

Figure 5.1 Results from a cellular automaton model in which a hexagonal array of blood vessels (partially visible) carries nutrients into a tissue composed of cancer cells (white spaces) and tissue void of cells (black). Graphs (c) and (f) show the time evolution of the total number of cancer cells (proliferating + quiescent; upper curve) and the total number of quiescent cells (lower curve). Two cases are shown: (a–c) heterogeneous oxygen concentration determined by structural adaptation of the vasculature; (d–f) homogeneous oxygen distribution. Notice the order of magnitude difference in the cell number (cf. (c) and (f)). For full details see Alarcón, Byrne and Maini (2003). Figure reproduced from Alarcón, Byrne and Maini (2004a) with permission from Elsevier

This model provides a basic framework that can be developed to increasing levels of sophistication. For example, the model predicts that there are large regions of tissue in which there are low oxygen levels. In actual tumours, such hypoxia results in the cancer cells secreting growth factors (e.g. VEGF) to promote angiogenesis. Therefore, the model needs to be modified. The level and detail of modification should be determined by the question that one is trying to answer. For example, if one wants to know the effect of blocking a specific pathway in, say, HIF-1 (hypoxia-induced factor-1) dynamics, then one must develop a very detailed model for this pathway. On the other hand, if one wants to investigate the effect on the growth dynamics of spatially varying oxygen concentration, then one can simply include a rule in the CA that says that if oxygen levels breach a certain threshold value then cancer cells begin to produce VEGF. The spatiotemporal dynamics of VEGF then can be modelled by a partial differential equation.

With VEGF causing angiogenesis there is again a choice. One can either bring in models for sprouting or modify the structural adaptation rules for the existing blood

vessels to include a VEGF-concentration-dependent radius. Employing the latter approach, Alarcón, Byrne and Maini (2004a) showed that cancer tumour levels increased in number but did not reach the levels for the homogeneous case.

A key determinant of the above model dynamics is the different behaviour of cells in hypoxic conditions. This leads to the controversial question: why do normal cells undergo hypoxia-induced cell cycle arrest (eventually leading to apoptosis) whereas cancer cells undergo hypoxia-induced quiescence? Alarcón, Byrne and Maini (2004b), using the results on the effects of p27 from Gardner *et al.* (2001), have shown that if one assumes that p27 inhibits the cyclin–CDK complexes and that the growth of p27 is regulated by cell size in normal cells, but that this cell-size control is lost in cancer cells, then one can reproduce several of the behaviours observed under hypoxia. Specifically, it is consistent with the observation that low expression of p27 is a poor prognostic indicator (Kirla *et al.*, 2003).

The above model framework can be used to investigate drug delivery protocols. For example, protocols for Doxorubicin treatment for non-Hodgkin's lymphomas (NHL) were analysed by including, in the Alarcón, Byrne and Maini (2003) modelling framework, vessel maturity, NHL cell-cycle kinetics and Doxorubicin pharmacokinetics and pharmacodynamics (Ribba *et al.*, 2005). This allowed for comparison between treatment efficacy for different grades of NHL.

5.4 Population models

The general functions of cancer cells can be divided into proliferation and invasion. The former is the result of some combination of cellular changes that includes loss of growth inhibition pathways (e.g. tumour suppressor gene mutations), upregulation of growth promotion pathways (e.g. gain of function mutations in oncogenes), decreased cell death due to loss of apoptosis (through p53 mutations) and senescence (e.g. increased telomerase activity) pathways and escape from normal tissue defences such as the immune response (Yokota, 2000). The latter requires increased cellular mobility, loss of anchorage dependence on basement membranes, extracellular matrix breakdown (among others) and destruction of normal peritumoural cells, which may function as a relative barrier to tumour invasion.

It appears that acquisition of the malignant phenotype is a multistep process that occurs over a long period of time as the above changes accumulate through a sequence of genomic events coincident with a progressive drift from normal tissue through premalignant lesions to invasive cancer (Fearon and Vogelstein, 1990). In fact, carcinogenesis is often described as 'somatic evolution' because it appears to be formally analogous to the Darwinian dynamics in nature as individuals and species (phenotypes) compete for dominance in a given environment (Nowell, 1976).

Healthy functioning tissue in a multicellular organism, on the other hand, is the antithesis of a Darwinian environment because multiple cellular populations coexist in a cooperative, non-competitive microenvironment, i.e. normal cells repress their

proliferative capacity to maintain a stable multicellular society necessary for the formation of functioning organs. Tumour cells typically progressively lose this social sense during the steps of carcinogenesis (Maynard Smith called them 'selfish cheats': see Parker, 1978) and increasingly act as individuals with the goal of maximizing their own proliferation. Thus, to the evolving tumour cells, other normal and neoplastic cell populations are competitors rather than colleagues. Because of this, tumours can be considered a microecology dominated by Darwinian competition and subject to mathematical models employed in population biology.

A critical clinical consequence of these evolutionary dynamics is significant heterogeneity in tumour populations and their environment. Thus, invasive cancers typically exhibit marked spatial and temporal phenotypic and genotypic heterogeneity (Lengauer, Kinzler and Vogelstein, 1998). This variation among individuals within the population leads to significant variability in response to various anti-tumour therapies and is most likely a major factor in the limited success of modern oncology in eradicating most human cancers.

Many mathematical approaches in *in vivo* tumour behaviour formalize the concept of carcinogenesis as somatic evolution by applying models derived from population biology and evolutionary dynamics (Michelson *et al.*, 1987). This approach can be both descriptive and mechanistic, with the latter identifying specific population interactions critical for the development of invasive cancer, focusing on: competition among different tumour populations for dominance, particularly during the multistep process of carcinogenesis; and competition between tumour and normal cells at the tumour/host interface.

These models typically are of the general form:

$$\frac{\mathrm{d}p_n}{\mathrm{d}t} = p_n(w_n - \langle w \rangle), \text{ where } t \gg \Delta t, \quad \langle w \rangle \equiv \sum_n w_n p_n, \; w_n \equiv w_n(t) \tag{5.1}$$

where p_n is the probability that any observed cell in the sample tissue will be a member of population n, w_n is the fitness of population n and $< w >$ is the mean fitness of all extant populations. Clearly this somatic ecosystem will favour populations that achieve maximal fitness within the local tissue landscape. So how does a tumour cell evolve to a state of fitness that allows it to dominate the local somatic ecosystem and drive most or all of the competing populations to extinction? That is, what properties confer a proliferative advantage on a cell sufficient to allow it to form an invasive cancer? Interestingly, in some ways we already know the answer: if carcinogenesis is the result of somatic evolution, then phenotypic properties commonly found in invasive cancers *must* arise as adaptive mechanisms to proliferative constraints within the microenvironmental fitness landscape. Conversely, the common appearance of a phenotypic property in cancer populations is presumptive evidence that it confers a selective growth advantage (Gatenby and Vincent, 2003). In other words, typical properties of the malignant phenotype are neither random nor accidental. Rather, they arise from the evolutionary dynamics of carcinogenesis and must confer a selective growth advantage. Thus, in many ways we already know the phenotypic properties

that turn a cell into a cancer cell – they are those that are consistently observed in malignant cells. The task is to identify how these properties confer an evolutionary advantage and thus contribute to the development of an invasive cancer.

As an example, we will focus on a curious but common property of primary and metastatic cancers: altered glucose metabolism. Glycolysis – literally the lysis of glucose – first requires the conversion of glucose to pyruvate and then to the waste product lactic acid. In most mammalian cells, glycolysis is inhibited by the presence of oxygen, which allows mitochondria to oxidize pyruvate to CO_2 and H_2O. This inhibition is termed the 'Pasteur effect' after Louis Pasteur, who first demonstrated that glucose flux was reduced by the presence of oxygen (Racker, 1974). This metabolic versatility of mammalian cells is essential for maintenance of energy production throughout a range of oxygen concentrations. Conversion of glucose to lactic acid in the presence of oxygen is known as aerobic glycolysis, or the 'Warburg effect' (Warburg, 1930). Increased aerobic glycolysis is uniquely observed in cancers. This phenomenon was first reported by Warburg in the 1920s, leading him to the hypothesis that cancer results from impaired mitochondrial metabolism. Although the 'Warburg hypothesis' has proved to be incorrect, the experimental observations of increased glycolysis in tumours, even in the presence of oxygen, have been repeatedly verified experimentally (Semenza, 2001).

It is now clear that this altered tumour metabolism is more than simply a laboratory oddity. Widespread clinical application of the imaging technique – positron emission tomography (PET) using the glucose analogue tracer^{18} fluoro-deoxyglucose (FdG) – in thousands of oncology patients has demonstrated unequivocally that the vast majority of primary and metastatic human cancers exhibit significantly increased glucose uptake (Czernin and Phelps, 2002).

For many cancers the specificity and sensitivity of FdG PET to identify primary and metastatic lesions is near 90 per cent. Sensitivity is lowered because FdG PET has difficulty resolving lesions of $<1\ cm^3$, and specificity is lowered because other tissues, notably immune cells, also avidly trap FdG. When these limitations are accounted for, it can be reasonably surmised that virtually all invasive cancers avidly trap FdG. Interestingly, cultured tumour cells maintained in normoxic conditions continue to use glycolytic pathways for energy production. Furthermore, a number of clinical studies have demonstrated that increased glucose uptake correlates directly with increased tumour aggressiveness and poor prognosis (Burt *et al.*, 2001).

At first glance, this consistent metabolic shift seems at odds with an evolutionary model of carcinogenesis, because the proliferative advantage of the glycolytic phenotype is not immediately apparent. First, anaerobic metabolism of glucose is inefficient because it produces only 2 ATP/glucose, whereas complete oxidation produces 38 ATP/glucose. Second, the metabolic products of glycolysis, such as hydrogen ions (H^+), cause a spatially heterogeneous but consistent acidification of the extracellular space (Bhujwalla *et al.*, 2002). This results in significant cellular toxicity because normal mammalian cells typically undergo apoptosis due to increased caspase activity when exposed to acidic extracellular environments. Intuitively, it would seem that the Darwinian forces prevailing during the somatic evolution of invasive cancers would select *against* a metabolic phenotype that is more than an order of magnitude less

efficient than its competitors and environmentally poisonous. In other words, the accepted tenet of 'survival of the fittest' would appear generally to favour populations with a more efficient and sophisticated substrate metabolism. So, why do tumour populations consistently evolve to the inefficient and potentially toxic glycolytic phenotype?

In fact, mathematical models of the tumour/host interface using coupled systems of ordinary differential equations, partial differential equations and modified CA techniques appear to resolve this conundrum (Gatenby and Gawlinski, 1996; Patel *et al.*, 2001). Analysis of early tumour growth suggests consitutive upregulation of glycolysis is a required adaptation to the intermittent hypoxia observed in premalignant lesions (Gatenby and Gillies, 2004). The resulting acification of the environment requires further evolution to adaptive phenotypes that are resistant to acid-induced toxicity. We find that cell populations emerging from this evolutionary sequence possess a remarkable proliferative growth advantage because they alter the environment (through increased acid production) in a way that is fatal to their competing populations but harmless to themselves. Furthermore, the models demonstrate that the acid produced by tumours will flow down concentration gradients into the peritumoural normal tissue. This will produce consisent morphological features in peritumoural normal tissue (Figure 5.2) resulting from normal cell apoptosis, extracellular matrix degradation, blunting of immune response and promotion of angiogenesis. These results, termed the acid-mediated invasion model, are consistent with numerous experimental and clinical observations (Gatenby and Gawlinski, 1996, 2003).

5.5 Conclusion

Cancer growth is a complex multiscale process dominated by constantly evolving non-linear dynamics. Increasingly, cancer therapy is being designed to interrupt key components of critical pathways within this complex system. For example, a number of drugs target cells in a certain part of their cell cycle. Other drugs aim to stifle the angiogenesis process so that the cancer 'suffocates' from lack of critical substrate. By creating virtual tumours with appropriate quantitiative methods, mathematical modelling can organize extant data into an integrative theoretical framework that can clarify the underlying dynamics that govern invasive cancers and potential therapeutic interventions that may interrupt its growth. For example, the acid-mediated tumour invasion hypothesis proposed by Gatenby and co-workers suggests novel and, at times, counter-intuitive possible therapies such as, for example, increasing systemic acidity so that the tumour is poisoned.

Mathematical modelling of processes occurring on one particular spatial scale is an essential first step in understanding the dynamics of this disease but a full understanding requires a multiscale approach. Moreover, this then allows one also to investigate combination drug therapies that may consist of drugs acting on processes occurring on different length scales. Here we have highlighted one particular approach, that of hybrid cellular automata. Such an approach has been used very effectively

already in other areas of the life sciences. For example, Dallon and Othmer (1997) investigated pattern formation in the slime mould *Dictyostelium discoideum* using such an approach to model signal transduction of the chemoattractant cyclic AMP and cell motion in response to the signal. This type of modelling approach was adapted to investigate scar tissue formation during dermal wound healing. In this case, it is widely believed that matrix orientation plays a crucial role in determining the severity of scar tissue after dermal wounding. Dallon, Sherratt and Maini (1999) developed a multiscale modelling framework to examine the interaction of many of the factors involved in orientation and alignment. Briefly, the model considers a fibrin clot into which cells (modelled as discrete objects) move, degrading the clot and laying down collagen. The fibrin and the collagen matrix are modelled as continuous vector fields whose direction and length represent, respectively, the predominant orientation of

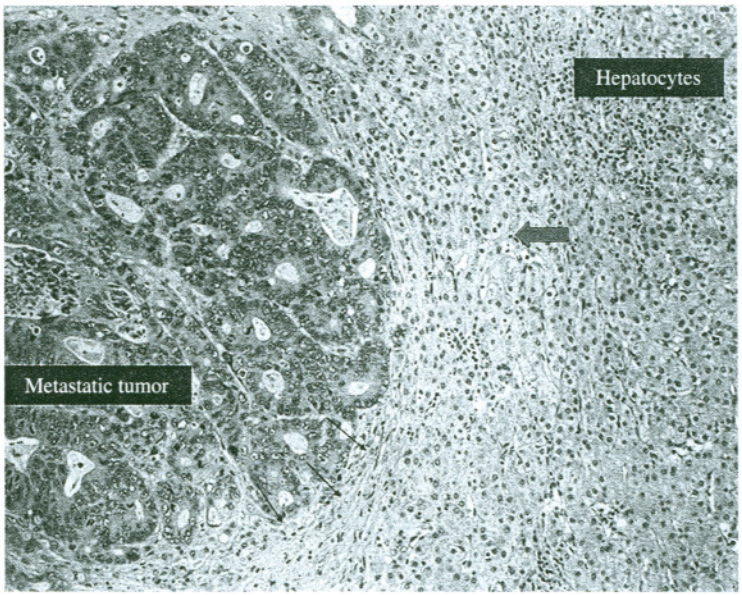

Figure 5.2 Haematoxylin- and eosin-stained section of a colon cancer metastasis to the liver to illustrate the tumour-host interface morphology. Note that the hepatocytes closest to the tumour edge exhibit diminished numbers with expansion of the interstitial spaces and less nuclear and cytoplasmic staining than those cells more distant from the edge (the open arrow demarcates the approximate boundary). These morphological features are predicted by the acid-mediated tumour invasion model, which demonstrates that acid will diffuse into normal tissue adjacent to the tumour edge, resulting in loss of normal cell integrity due to apoptosis mediated by caspase and p53 pathways, as well as increased extracellular matrix degradation and loss in intercellular gap junctions. The models also predict that the normal tissue immediately adjacent to the tumour-host interface often will become acellular (small arrows). This complete loss of normal tissue integrity provides space for expansion of the tumour cells, which remain viable even under extreme microenvironmental conditions, providing a mechanism for tumour invasion

fibres and the density. They showed that this model predicts patterns of alignment on a macroscopic length scale that are lost in a continuum model of cell population (Olsen *et al.*, 1999) and have used the model to investigate several factors that influence the alignment of collagen. Specifically, they were able to relate the model to current anti-scarring therapies using transforming growth factor β and made predictions as to which were the crucial factors influencing alignment and hence scarring (Dallon *et al.*, 2000; Dallon, Sherratt and Maini, 2001).

The rapid advances in biotechnology have resulted in a huge increase in biological data and generated very important insights into, for example, causes and possible cures for certain diseases. These data have the potential to elevate our understanding of complex biological systems to a new level. To achieve its full potential, it is now widely recognized that there is an urgent need to develop new theoretical tools for the analysis and synthesis of detailed low-level information into comprehensive, integrative and quantitative descriptions that span a wide range of spatiotemporal scales. This new research area is viewed as the next grand challenge in the life sciences and is often referred to as the 'Physiome Project' (http://www.physiome.org/). This worldwide effort aims to describe biological function, based on genomic and proteomic mechanisms and their interaction, using qualitative mathematical models. It is an inherently interdisciplinary effort, with experimentalists and theoreticians working closely together, iterating between experiment and modelling. The ultimate goal is to meet the key post-genomic aim of transforming the wealth of data generated into a detailed understanding of biological function, and hence of the complex biological systems that together form the basis of living organisms. Here we have illustrated some approaches to this problem in the context of cancer dynamics and have outlined some of the challenges that must be surmounted by theoreticians over the coming years if mathematical modelling is to be an integral part of the fight against cancer.

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