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WORKSHOP REPORT

Mathematical modelling of cancer invasion and metastasis: an interdisciplinary workshop held at the University of Warwick, Coventry, UK

11–13 September 1996

Introduction by Dr Jonathan A. Sherratt, Mathematics Institute, University of Warwick, Coventry CV4 7AL, UK

The twin revolutions in molecular biology and nonlinear mathematics over the last two decades have changed mathematical modelling of cancer out of all recognition from the simple fitting of experimental data to Gompertzian growth curves. A workshop at the University of Warwick in September brought together theoreticians, experimentalists, and clinicians, to identify the state of the art and future potential for modelling in the area of metastasis and invasion. In this article, I will describe the background to the area and highlight the key themes emerging from the workshop, as an introduction to the abstracts which follow.

Mathematical models have been in use for biological prediction since the early part of this century, initially in ecology and embryology. These early models were phenomenological, i.e. they acted as a convenient way to express and explore theories, but did not represent particular postulated mechanisms. The classic modelling by Hodgkin and Huxley [1] of electrical signalling in the squid axon was also mainly phenomenological. However, the ability to isolate biological mechanisms at the molecular level has led to a new type of mathematical model, which represents specific low-level mechanisms, either known or hypothesized. This use of mathematical modelling was pioneered during the late 1970s and early 1980s by James Murray at the University of Oxford, mainly in applications to developmental biology. A classic success story is the determination of the link between cAMP kinetics and the spiral wave signalling that leads to cellular aggregation in the starvation response of the slime mould *Dictyostelium discoideum* [2].

The Workshop on the Mathematical Modelling of Cancer Invasion and Metastasis was held on 11–13 September 1996 at the University of Warwick, and was supported by the Applied Nonlinear Mathematics initiative of the EPSRC.

A pioneering example of a medical application of theoretical models was the work of Michael Mackey and Leon Glass at McGill University in the 1970s [3], which related feedback delays to physiological oscillations, for example of leucocyte count in leukemia and breathing patterns in Cheyne–Stokes respiration. More recently, there has been extensive work in my own group and elsewhere on models of wound healing, linking data on biochemical regulation of cell behaviour to observed healing rates and wound quality [4, 5]. HIV progression has also been modelled extensively, with simulations being used to test and refine alternative explanations for the long lag between infection and the onset of AIDS [6, 7]. In addition, there is a large body of theoretical work on the epidemiology of infectious diseases [8].

Within cancer, the scope for mechanistic, predictive modelling is immense. One valuable contribution is to stimulate the effects of particular oncogenic mutations, such as elevated cell division [9, 10], reduced apoptosis [11], or altered cellular metabolism [12]. The growth constraints on tumours prior to vascularization have been modelled extensively, leading to predicted relationships between cellular parameters and size limitation (Ward and King). Tumour immunology has also been considered by a number of theoreticians, initially studying the 'immune surveillance hypothesis' [13], and more recently addressing the role of the immune system in regulating tumour composition and morphology (Owen and Sherratt). Mathematics has also been used to stimulate cancer therapies, comparing different chemotherapy strategies [14, 15] and their spatial limitations [16], and using PET data to analyse drug dynamics (Matthews). The aim of the Warwick workshop was to identify the way in which this modelling expertise could be most effectively channelled to answer current questions in invasion and metastasis, and I will summarize the key points

within three convenient, though somewhat arbitrary, subject divisions.

Fractals and chaos in metastasis

Perhaps the most unifying aspect of all mathematical models of biological systems is that they are intrinsically nonlinear. That is, the implications of a change in the expression of a particular growth factor within a tumour, or in the number of individuals in an animal herd, cannot be written down *a priori*, since they depend on many other aspects of the system. This nonlinearity finds perhaps its most pronounced expression in the chaotic behaviour and fractal morphologies characteristic of many biological systems, including cancer. An understanding of the spatial and temporal chaos underlying metastasis is crucial for key issues such as reliable markers for the success of particular therapies; nonlinear mathematical models are the natural vehicle for this understanding (Goddard *et al.*, and Vaidya and Baum). Within a single tumour mass, nonlinearities are manifested in the irregular shape of the boundary between the tumour and the surrounding tissue. Detailed data on the fractal nature of this boundary is now emerging for a range of human tumours, and is a crucial yardstick for theoretical models (Cross); moreover, local and global fractal dimensions can be a valuable prognostic indicator of invasion (Landini).

Tumour–host interactions: invasion and intervention

The recent discovery of many molecular mechanisms responsible for cancer invasion makes this a prime area for mathematical models to act as a link between microscopic and macroscopic data. The final aim of this work is a single, verified model for the invasive cascade, but an essential precursor to this is the separate study of the contributing factors. Thus the mechanism, such as imbalance between proteolytic enzymes and their inhibitors, or changes in cell–cell adhesion, can initially be modelled separately before being combined into a single model framework (Jiang and Perumpanani *et al.*). Similarly, the role of a pH gradient at the tumour–host interface has been studied mathematically, predicting a relationship between morphology and growth rate (Gatenby and Gawlinski). An important theme emerging from the workshop, particularly in models of tumour–host interactions, is the importance of modelling approach. Mathematical modelling is not a single discipline: there are many different ways of representing biological systems theoretically, depending on the available inputs and the form of desired predictions. Differential equations provide the most efficient way of deriving formulae relating cellular parameters to macroscopic observables such as tumour shape [17] (Byrne). Such models are formulated in terms of 'densities' of cell populations, but alternative approaches simulate the behaviour of individual cells, following specified rules and in response to the local environment (Shonkwiler, and Drasdo). Appropriate choice of modelling framework is a key issue in the effective application of mathematics.

Tumour angiogenesis

Mathematical modelling of tumour angiogenesis has been studied for a number of years, focusing initially on the basic mechanisms by which angiogenesis factors can stimulate vascular ingrowth [18]. Recent modelling achievements include prediction of the ways in which the extracellular matrix can modulate the cellular response to angiogenesis factors (Burn and Sleeman), and a highlighting of the delicate balance between contra-acting chemotactic and haptotactic gradients in endothelial cell movement (Anderson and Chaplain). Recently, detailed data have become available on specific angiogenic mechanisms, which raise many challenges for future modelling. Specific examples emerging from the workshop included the role of angiogenic hotspots (Bicknell) and the heterogeneous expression of the membrane glycoprotein CD105 (Kumar *et al.*). Another key player in the angiogenic process is the macrophage, whose numbers have been proposed as a prognostic indicator in breast cancer [19]. Mathematical models for the macrophage invasion of tumours have recently been developed (Owen and Sherratt), and recent data on the spatial distribution of endothelial cells and macrophages within tumours are an important resource for future modelling (Lewis).

Cancer invasion and metastasis is a prime example of an area in which mathematical modelling can make a real contribution. There is an increasingly large volume of data available at both the microscopic (cellular and molecular) and macroscopic levels, and much of the modelling expertise for exploiting this data is already in place. With all these key ingredients assembled, mathematical modelling has a huge potential role to play over the next few years.

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ABSTRACTS

Mathematical modelling of avascular tumour growth

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Prior to vascularization, developing solid tumours tend to grow as spheroids consisting of a rim of proliferating cells together with a core of quiescent and dead cells. Two mathematical models for the nutrient-driven growth of avascular tumour spheroids are presented. Numerical simulations of the first, simpler, model (described in [1]) show that the main features of early avascular growth (notably 'exponential' and 'linear' growth regimes) are captured. Furthermore, the heterogeneity within the spheroid is demonstrated. Exploiting the physically realistic limits of small nutrient consumption rate and slow death rate, asymptotic methods are used to describe the initial 'exponential' phase and two subsequent phases of growth retardation, the first of which is due to nutrient diffusion limitations and the second to necrosis through absence of nutrient in the core. Under these limits the maximum attainable growth speed can be expressed in terms of the model parameters. The second model is a development of the first which incorporates two additional mechanisms which result in the depletion of the necrotic material. This material (such as proteins and nucleic acids) diffuses through the spheroid where its leakage to the outside medium, together with its utilization by dividing cells, leads to a loss of volume, and eventually the growth of the spheroid may saturate. Numerical solutions of the resulting system of partial differential equations reveal that growth may ultimately cease or may become linear, depending on the parameter values. The travelling wave and steady-state limits of the model are also investigated, details being given in [2].

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The macrophage invasion of tumours: effects on growth, composition, and spatial structure

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Many solid tumours contain a high proportion of macrophages, which can have a variety of effects upon the tumour, leading to a delicate balance between growth promotion and inhibition. I will discuss the development of a temporal model for the early, avascular growth of a tumour, which concentrates on the chemotactically induced invasion of macrophages, and the inhibitory effect of their ability to recognize and kill cancer cells. The model consists of macrophages, mutant cells and normal cells, together with a generic regulatory chemical and a macrophage–mutant cell complex which is a key step in mutant cell lysis. A simple scheme is considered for this interaction between macrophages and mutant cells, and it is shown that such an immune response is not sufficient to prevent tumour growth, due to it being a second-order process with respect to the density of tumour cells present. A variety of more complex formulations for this interaction all lead to the same conclusion. However, the presence of macrophages does have important effects on tumour composition, and I will describe a detailed bifurcation analysis of the model which clarifies this. Having discussed this temporal model, I will consider an improvement incorporating cell movement and chemical diffusion. Tumours are certainly not homogeneous in structure and the inclusion of these motility terms leads to the formation of regular spatial patterns behind an invasive wave of tumour cells. Such patterns are explained by the rapid diffusion of chemical regulator from regions of high mutant cell density, suppressing local macrophage recruitment and activation, and allowing the mutant population to grow still further. The inclusion of macrophage chemotaxis, using parameters estimated from experimental data, can give rise to spatiotemporally irregular solutions behind the invading wave front. These results suggest that tumour heterogeneity may arise in part as a natural consequence of the macrophage infiltration.

Modelling tumour drug dynamics using positron emission tomography

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By labelling molecules with positron emitting isotopes, compounds such as anticancer drugs can be tracked in human patients, using positron emission tomography (PET). Presently, this approach is being used to assess the tumour and normal tissue pharmacokinetics of conventional and new anticancer agents, and to assess tumour characteristics using radiolabelled compounds which provide measures of metabolic activity, proliferation and blood flow. Existing methodology has analysed the temporal and spatial data using a region of interest approach and compartmental modelling. This relies on a clinician, drawing by hand a region on an image template, with the mean measurement used to create representative time–activity curves of the concentration of label. Compartmental modelling, together with arterial sampling to provide input functions, can then be used to extract useful parameters such as uptake rate constants. However, the use of this approach is problematic for all but the simplest question, due to contamination from labelled metabolites, heterogeneity within tumours and the difficulty in fitting complex models to the data. As a result new methods of analysis are being sought. Possible areas for investigation are modelling at a pixel level (aiding the heterogeneity problem) and simultaneously fitting population datasets, which may also enable more complex models to be fitted to the data.

Applications of non-linear mathematical techniques in medicine: examples, suggestions and questions

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As knowledge has progressed in medicine it has become clear that the simple linear model of one

disease per patient and one cause per disease is inaccurate. Patients may have multiple diseases and each 'disease' could have multiple causes. The multiplicity of causes has meant that the pathogenesis of the more complex conditions, such as malignancy, has remained a mystery. This is changing with the advent of molecular level medicine but even still it is very hard to piece together all the disparate factors that cause disease in an individual or even in a population. The geneticists may like to tell us that the somatotype is the result of programming by the genotype. It seems more likely that the phenotype is the result of interaction between the genetic programming and the physical and chemical laws. So why does the body grow the way it does and what are the mathematical patterns of the body? Some structures can be described by fractals. Good examples are the surface of the brain, the Haversian canals of the bone, the branching vascular systems and the branching bronchi. On very close examination the overall pattern is statistically self-similar but the exact rendering of the pattern is never encountered twice due to local minor differences which are explicable due to small differences in local growing conditions. Fractal patterns and dimensions can also be seen in neoplasia. Benign tumours may have a very regular repeating fractal structure but malignant lesions follow a much more random and chaotic pattern. Measurement of the fractal dimensions may be a guide to the nature of a lesion. Understanding the mathematical patterns of anatomy and pathology may help in determining the mathematics of morphogenesis and pathogenesis. Can chaos mathematics be used to model outcome in patient groups? Questions include why there is such an enormous variety of response to cytotoxic chemotherapy? Which patients will develop metastatic disease? Which patients will develop local recurrence? Why do some patients with the risk factors develop disease but others with exactly the same risk factors do not? As a specific example: If cancer of the breast is due to build-up of risk factors why is the second breast only affected in 10% of cases?

The importance of mathematical models for hypothesis generation in biology

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The conventional model of breast cancer suggests that it starts in a single cell; the cancer cell grows into a clinical tumour, gains access to the lymphovascular system, spreads via blood and lymphatics to the rest of the body and overcomes the body's resources to be ultimately fatal according to the principles of linear mathematics. However, biology does not render itself to simple order. It is governed by a systematic disorder and requires mathematics of disorder or chaos to reflect reality. Many clinical dilemmas justify this approach. (1) Formation of the primary tumour from putative single cells does not follow a linear path in either time or space. (2) Clinical response of primary tumours to systemic agents has no reliable predictive factors. (3) Risk of spread to distant sites does not rise linearly within the natural history of primary tumour. (4) Clinical appearance of distant metastasis after primary treatment is not linear in time with hazard peaks at 3 years and between 7 and 10 years. (5) Distant metastasis to specific organs also follows non-linear dynamics. (6) Clinical behaviour of even similar disease is non-linear in time. Therefore, the opening sentence above should read '. . . the cancer cell *may* grow into a clinical tumour, *may* gain access to the lymphovascular system, *may* spread via blood and lymphatics to the rest of the body and *may* overcome the body's resources to be ultimately fatal'. The clinical datum is available at only a cross-section in time at some of these events. The probabilities of each of these *'may's* need to be computed using suitable mathematical models. In order to construct a clinically testable hypothesis, biology without the appropriate mathematical model is insufficient.

Fractal characteristics of human tumours and their blood supply

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Fractal objects can be generated by simple iterative algorithms to produce complex objects with space-filling properties that are better described by fractal dimensions than integer-dimensional parameters. Human tumours, such as colorectal carcinomas, show scaling self-similarity over a limited range of scales which suggests that there may be a fractal element to their structure. Using an implementation of the box-counting dimensions on a digital image analysis system we have confirmed a fractal element of structure in neoplastic colorectal polyps, colorectal carcinomas, cutaneous malignant melanomas and cultured human breast carcinoma cells. The fractal dimension in these tumours correlated with the subjective interpretation of 'infiltrative' patterns, those with higher fractal dimensions having a less smooth boundary with infiltrating columns of tumour cells. Such infiltrative tumours will have a higher probability of accessing vascular channels and metastasing so measurement of the fractal dimension of tumours may be of prognostic value. We have also measured the fractal dimension of the arterial blood vessels in human renal cell carcinomas and have shown a much higher fractal dimension for the vessels in the tumours than the background kidney. All these observations show that models of tumour growth should produce structures with fractal elements and that simple iterative processes may be involved.

Complexity in tumour shape: from simulation to objective quantification using fractal geometry

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In histopathological diagnosis of epithelial neoplasms, the shape of the limit between tumour and normal surrounding stromal tissues is an important morphological feature that indicates the degree of infiltration or local aggressiveness of the epithelial growth. Complex tumour profiles were simulated using a 'cell pushing' model, which showed how contact inhibition may play an important role in the formation of irregular profiles. This, however, poses a further and more fundamental question of how such complexity could be assessed successfully. In routine histopathology, the tissue profiles are systematically assessed in a subjective manner which shows poor reproducibility. As fractal geometry provides a formal approach to the quantification of complexity, it was used to investigate the extent to which fractal methods were able to characterize the limits between stroma and epithelium in normal mucosa, epithelial dysplasia (a premalignant condition) and squamous cell carcinoma of the mouth floor. The global characterization (via the yardstick and box counting), the local and local-connected dimension (via the mass radius relation) of tissue profiles were investigated as quantitative morphological parameters to be used in diagnosis. Certain parameters of the distribution of the local-connected dimensions of the profiles classified the cases belonging to the three types of histopathological diagnosis considered (normal, dysplasia and carcinoma) with 85% accuracy by means of discriminant analysis. Other approaches (global characterization and local fractal dimension) achieved less accurate classification. The values of the local-connected fractal dimensions were also used to produce colour-coded dimensional images of the profiles to highlight locations with higher irregularity that may correlate with locally invasive 'higher risk' areas.

Molecular and cellular mechanisms of cancer invasion/metastasis and intervention

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Invasion and metastasis are perhaps the most life-threatening consequences for patients with cancer. The process, now referred to as the metastatic cascade, is composed of a number of separate but highly related steps. The cascade is initiated by the dissociation of tumour cells from the primary site. Abnormal cell–cell adhesion mechanisms (i.e.

E-cadherin reduction and mutation) are implicated in this step. Tumour cells will subsequently adhere and invade the basement membrane and extracellular matrix. The abnormal expression of integrins, focal adhesion kinase pathways, the imbalance between proteolytic enzymes and their inhibitors are associated with matrix adhesion and invasion. After tumour cells enter the circulation, their survival relies on the balance between cytotoxic immune cells and those host cells which shield the tumour cells from being recognized by immune cells. A final and perhaps one of the most important parameters determining the successful formation of a metastasis is angiogenesis, which is controlled by angiogenic factors produced by both normal and tumour cells. Options to combat these steps have been explored. There are now means to safely increase cell–cell adhesion, decrease the level of proteolytic enzymes, effectively reduce cell–matrix adhesion, maintain the balance of immune function to anti-invasiveness, and inhibit angiogenesis. Strategies to combine these novel approaches with traditional chemotherapy are also tested. Although the anti-invasion and anti-metastasis options are at an early stage, successful development of these strategies will lead to better control of the disease.

Modelling matrix degradation during tumour invasion

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Malignant invasion occurs consequent to the accumulation of a series of mutations in a normal cell which confer it with the invasive potential. This invasive potential comprises alterations in cellular adhesion, cell motility and alterations in the protease–antiprotease axis. Each of these changes represent a set of phenotypes which may be variably manifested in an invading cell. Changes in adhesion may either be homotypic or heterotypic. Alterations in motility comprise both changes in directed (chemotaxis and haptotaxis) and random motility. Proteolysis seen in malignant invasion may result either from increased protease production or decreased anti-proteases. The authors have derived a detailed model incorporating all the above-mentioned phenotypic changes in a fashion where the effects of these changes can be studied either individually or collectively. The model comprises a system of mixed hyperbolic–parabolic partial differential equations which describes the interactions of normal and malignant cells with the extracellular matrix in which gradients are created by the action of proteases. The authors have at first studied individual submodels focusing on single pairs of interactions. This approach has enabled detailed analytical work to be carried out and also helped define the nature of these individual interactions in a fashion that would not be experimentally possible. Initially the authors studied the interaction of haptotaxis and proteolysis with detailed descriptions of the effects of receptor modulation of haptotaxis. The model was then expanded to study the effects of protease diffusion and the generation of a chemotactic gradient by the proteolysed extracellular matrix. The convective effect of an expanding neoplasm on surrounding connective tissue and the resulting encapsulation was modelled. Finally the authors solved the whole system numerically demonstrating the interplay of the features studied separately in the whole system. The modelling resulted in a number of novel mathematical and biological results. Mathematically the occurrence of a singular barrier was shown to dominate the behaviour of the system in the absence of diffusion where the invasive cells used a combination of haptotaxis and proteolysis simulating urokinase plasminogen mediated invasion. The incorporation of protease diffusion, simulating the effects of collagenases, produced a mixed hyperbolic–parabolic system where the system was best studied by the method of hyperbolic–parabolic separation. The interaction of chemotaxis with haptotaxis produced oscillatory waves whose profile and velocity varied cyclically. Biologically a new mechanism for the parabolic dependence of invasion on protease production was demonstrated in which cells move up fixed and soluble gradients of extracellular matrix. These results were expanded to explain the regional variations in invasion seen in different loci. The authors then used the results of the modelling to design a series of experiments to study the interaction of directed motility with protease production. Work has now begun at the Institute of Molecular Medicine in Oxford to experimentally verify these predictions.

A reaction–diffusion model of acid-mediated invasion of normal tissue by neoplastic tissue

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We present mathematical analyses, experimental data, and clinical observations which support our novel hypothesis that tumor-induced alteration of microenvironmental pH is a simple but complete mechanism controlling cancer invasion. A reaction–diffusion model describing the spatial distribution and temporal development of tumor tissue, normal tissue and excess H+ ion concentration is presented. The model predicts a pH gradient extending from the tumor–host interface which is confirmed by re-analysis of existing experimental data. Investigation of the structure and dynamics of the tumor–host interaction within the context of the model demonstrates a transition from benign to malignant growth analogous to the adenoma–carcinoma sequence. The effect of biological parameters critical to controlling this transition are supported by experimental and clinical observations. Tumor wave front velocities determined via a marginal stability analysis of the model equations are consistent with *in vivo* tumor growth rates. The model predicts a previously unrecognized hypocellular interstitial gap at the tumor–host interface which we demonstrate both *in vivo* and *in vitro*. A direct correlation between the interfacial morphology and tumor wavefront velocity provides an explicit, testable, clinically important prediction.

Approaches to modelling solid tumour growth

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In this presentation a brief review of existing models of avascular solid tumour growth is discussed, in which the tumour's development depends on the balance between cell proliferation and cell death. Under assumptions of radially-symmetric growth, these models accurately reproduce the well-defined layered structure observed when multicellular spheroids are cultured in the laboratory: proliferating cells are restricted to the nutrient-rich outer rim of the tumour, necrotic cells are localized at its nutrient-starved centre, and non-proliferating, hypoxic cells are sandwiched between the two regions. We also discuss two extensions of the original models. First we investigate how the expression of different growth factors influences the tumour's growth dynamics. For example we show that growth factors which enhance natural cell death can have a beneficial effect, either reducing the tumour's size or eradicating it completely. Conversely growth factors which inhibit apoptosis can dramatically alter the tumour's growth characteristics. In the second case we assume that nutrients consumed at the tumour boundary provide the energy needed to maintain the tumour's compact structure. The amount of energy that is consumed characterizes the degree to which the cells adhere to each other. We show that as the amount of energy needed to maintain the tumour's integrity increases, the size of the equilibrium configuration diminishes until, eventually, the system cannot support a tumour. We also use this mechanism to assess the tumour's ability to invade the host tissue. In particular we show that as the amount of energy needed to maintain its structure increases the tumour becomes more stable to asymmetric fluctuations and, hence, less prone to invasion. We conclude that this mechanism may provide clinicians with a useful tool for assessing a tumour's potential for invasion.

Computer simulation of angiogenesis and invasion

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We undertook to design a computer cancer model for the following potential benefits: it can support or deny theories about mechanisms, it reduces the workings of the disease to a set of parameters, a model is quantitative, not just qualitative, it allows 'what if' questions to be investigated, it makes predictions that are inaccessible or difficult to ordinary measurement such as long-term predictions or group predictions, it allows meaningful cost-effective studies. Our simulation began with a

certain number of normal, primary tissue cells which are at risk for seven possible mutations: (1) lethal, (2) divide rate (DIV), (3) death rate (DEA), (4) angiogenesis (ANG), (5) invasion (INV), (6) mutator (MUT), and (7) metastasis (MET). The subsequent fate of the cells depends upon the specific pattern of mutations suffered. In brief, if a cell has accumulated the DIV, ANG, INV, and MET mutations, then it can take up residence and grow in a new tissue beyond the primary site. This metastatic event greatly increases the number of cells in the simulation and, with additional metastasis events now occurring, the cell growth rate becomes exponential. At this point the simulation ends. To make use of the simulation, major challenges remain. Due to the limitations of the computer, the number of computer cells is far less than their biological counterpart; it must be learned how a single computer cell can accurately stand in for a large number of real cells. But most of all the several parameters of the model must be determined from biological observations.

Applications of a Monte Carlo model to growing solid tumors

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Cancer is one of the most frequent causes of death in industrial countries despite the rapid progress in medicine. The enormous increase of computing power during recent years facilitates a deeper understanding of underlying mechanisms by appropriate theories as well as detailed modelling of growing tumors. Based on a Monte Carlo approach which seems extremely suitable to stimulate growing cell populations such as solid tumors [1], results on many biologically interesting quantities, such as, for example, the number of cells as a function of time $N(t)$, the subclone statistics, cluster size distributions, cluster masses and boundary lengths, density profiles and space-filling profiles, statistics of interaction partners, age distributions, cell cycle length distribution, generation statistics and cluster correlation functions are presented. The model considers each cell as a discrete unit with average shape. A stochastic dynamics is assumed for cell movement, growth and division (including a mitotic cycle consisting of interphase and mitosis) applying the Metropolis algorithm [2]. An interaction potential between neighbor cells is defined consisting of a repulsive part (confined compressibility of a cell) and a cell type-dependent attractive part (due to adhesive interaction of cell adhesion molecules in the membranes of the cells). The simulation results show that the behavior of all measured quantities can be classified due to the growth regimes of the growth law $N(t)$. In correspondence with analytical investigations [2] the growth law $N(t)$ is found in the computer simulations (in $d = 2,3$, where d is spatial dimension) to cross over from an initially exponential growth over a transient regime into a power-law growth with $N \sim t^d$ for sufficiently large times. Initial and asymptotic behavior are believed to be universal while only in the transient regime do the quantities depend on the model parameters growth rate, interaction strength and interaction range. In the transient as in the asymptotic regime, some quantities, for example, the subclone statistics, obtained when marking the subclone of each cell and displaying the number of offspring as function of the cell number in chronological order, show remarkable large fluctuations. Thus, differentiation in fast-growing cell populations such as in tumors will never succeed in forming reproducable differentiated tissue without additional regulation mechanisms. This suggests that fluctuations, especially for fast-growing cell populations, also have to be taken into account when manipulating them, for example, at the development of radiation strategies for tumors to prevent the formation of metastasis. The model is extremely flexible and can be enlarged to much more complicated situations, which are barely accessible to analytical investigations. This can be illustrated by the example of a two-dimensional simulation of a photodynamic therapy of a solid tumor, where intercellular diffusion of chemicals as well as intracellular chemical reactions which influence the growth, movement and death of the cells have to be taken into account (examinations in three dimensions are under investigation).

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Mathematical models of angiogenesis

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Solid tumours are known to progress through two distinct phases of growth, namely an avascular phase and a vascular phase. The transition from the dormant avascular state to the vascular state, wherein the tumour acquires the ability to invade surrounding tissue and metastasise to distant parts of the body, depends on its ability to induce new blood vessels from the surrounding tissue to migrate towards and gradually penetrate the tumour, thus providing it with an adequate blood supply and microcirculation. In order for vascularization to be accomplished, it is now well-established that tumours secrete diffusible chemical compounds known as tumour angiogenesis factors (TAF) into the surrounding tissue and the extracellular matrix (ECM). The first events of angiogenesis are the rearrangements and migration of endothelial cells (EC) situated in nearby vessels. In response to the angiogenic stimulus, EC in the neighbouring normal capillaries which do not possess a muscular sheath are activated to stimulate proteases and collagenases. The EC destroy their own basal lamina and begin to migrate into the ECM. Small capillary sprouts are formed by accumulation of EC which are recruited from the parent vessel. These sprouts grow in length by migration of the endothelial cells. At some distance from the tip of the sprout the EC divide and proliferate to contribute to the number of migrating EC. Solid strands of EC are formed in the ECM. Laminae develop within these strands and mitosis continues. Initially, the sprouts arising from the parent vessel grow in a more or less parallel fashion to each other. They tend to incline toward each other at a definite distance from the limbus when neighbouring sprouts run into one another and fuse to form loops or anastomoses. Both tip-to-tip and tip-to-branch anastomosis occur and the first signs of circulation can be recognized. From the primary loops, new buds and sprouts emerge and the process continues until the tumour is eventually penetrated.

Modelling vascular tumour growth and invasion

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For a tumour to become vascularized it requires a capillary network to supply blood and remove waste products. In order to do this the tumour secretes certain chemicals (tumour angiogenesis factors, TAFs), which diffuse into the tissue surrounding the tumour. Endothelial cells (EC) react to the TAF, through a combination of chemotaxis and haptotaxis, and migrate towards the tumour forming capillary sprouts. The sprouts then begin to branch and form loops (anastomosis) which eventually circulate blood. However, it has been shown experimentally that in the absence of EC proliferation, the EC do not migrate sufficiently to achieve tumour vascularization. A mathematical model for EC migration during tumour-induced angiogenesis is presented. We consider a possible mechanism, in terms of the desensitization of endothelial cell-surface receptors responsible for the identification of TAF. Results in both one-space and two-space dimensions are discussed.

Tumour angiogenesis, vascular density and metastasis

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Much interest has recently centred on the observation by many groups that the vascular density of primary human tumours correlates with metastasis and survival. These studies have shown that the presence of areas of high vascular density (known as hot spots) correlates with metastasis. In model systems the angiogenic peptide vascular endothelial growth factor-121 ($VEGF_{121}$) was transfected into human MCF-7 breast carcinoma cells with constitutive high level expression. Xenografts of these cells in athymic

mice showed that $VEGF_{121}$ expression increased tumour growth and vascular density. Despite the appearance of vascular hot spots similar to those present in primary human tumours, no evidence of metastasis was seen. Clearly, other factors are involved in determining metastasis such as the presence of lymphatics or of intrinsic inhibitors of metastasis such as angiostatins. Transfection of MCF-7 cells with several other angiogenic peptides gave rise to the presence of vascular hot spots only when the factor was freely diffusible from the cells that secrete it, e.g. $VEGF_{121}$. In addition to polypeptide mediators of angiogenesis, several low molecular weight species are known to have activity. Notable among these are 2-deoxyribose. We have found 2-deoxyribose to be strongly angiogenic in the chick chorioallantoic assay. 2-Deoxyribose mediates the angiogenic activity of thymidine phosphorylase that is also known as platelet-derived endothelial cell growth factor. The exceptionally strong expression of TP/PDECGF in many solid human tumours combined with thymidine fluxes arising from breakdown of DNA in apoptosing cells points to TP/PDECGF as playing a key role in tumour angiogenesis.

CD105 and angiogenesis

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One of our monoclonal antibodies, E9, recognizes an antigen which has been assigned to CD105. CD105 is a homodimeric glycoprotein that is predominantly expressed in human vascular EC, both *in vitro* and *in vivo*. The gene for CD105 is mutated in patients with hereditary haemorrhagic telangiectasia. CD105 binds to TGF-β1 and TGF-β3 but not TGF-β2. The lack of TGF-β2 receptors on EC strongly suggests isoform-specific functions. However, further studies are needed to localize the TGF-β binding regions of CD105 and to produce antibodies which could interfere with the interaction of TGF-β with its receptor complex. *In vivo* CD105 was more strongly and frequently expressed in EC of tissues undergoing angiogenesis, viz tumours, healing wounds, embryonic and stroke tissues. When the reactivity of mAb E9 in normal and tumour tissues was compared with a number of standard pan-EC markers (such as CD31, von Willebrand factor), blood vessels in and around tumour tissues were stained strongly by mAb E9, whereas the same blood vessels in serial tissue sections were either weakly positive or were not stained by the pan-EC antibodies. By contrast, in several normal tissues, mAb E9 stained only a proportion of blood vessels which were decorated by pan-EC markers. In another study when brains from stroke patients were stained, the number of blood vessels and intensity of staining with mAb E9 was far greater in the penumbra around infarcted areas compared with the contra-lateral normal hemisphere. The same EC were also positive for V-CAM, a marker of EC activation. These results further confirmed that mAb E9 has greater affinity for activated EC. In normal healing wounds, inflammatory and psoriatic skin lesions, EC were stained more strongly than uninvolved normal tissues. Paradoxically, in placenta, only the syncytiotrophoblast of chorionic villi and decidua stained by the expected staining of blood vessels within villi was lacking. Since both experimental and therapeutic irradiation can profoundly affect the expression, synthesis and secretion of a number of molecules by EC, the observed upregulation of CD105 in irradiated EC was not surprising. That the increased occurrence of CD105 in irradiated tissues or body fluids from patients is of clinical relevance is already appearing to be the case. The use of a sensitive quantitative ELISA assay has demonstrated significantly raised levels of CD105 in the sera of patients with many different types of vascular disorders, e.g. cancer, atherosclerosis and diabetic retinopathy.

References

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Regulation of tumour angiogenesis by macrophages: role of hypoxia and angiogenic factor production

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Solid malignant tumours are often a heterogeneous mixture of cells, including the neoplastic cell population and such stromal cells as macrophages, fibroblasts, T and B lymphocytes, mast cells, neutrophils and endothelial cells. Macrophages enter such tissues as monocytes from the systemic circulation and then differentiate to form tumour-associated macrophages (TAMs) which can comprise up to 50% of the total tumour cell mass in some tumours. Our data indicate that a high intratumoral density of TAMs is significantly correlated with increased tumour angiogenesis and reduced survival in breast cancer [1]. TAMs were seen to congregate in avascular, relatively hypoxic sites in these tumours, suggesting that hypoxic tumour cells may produce chemoattractants to attract TAMs into such sites. Indeed, when the degree of necrosis was examined in 90 malignant breast lesions, this was seen to significantly correlate with macrophage infiltration with large numbers of TAMs situated in or around necrotic foci in these tumours. This is supported by our recent *in vitro* experiments co-culturing human macrophages with multicellular spheroids of MCF-7 breast cancer cells. This resulted in a more marked infiltration of macrophages into the hypoxic/necrotic centre of spheroids than either the outer layers of relatively normoxic cells in these same spheroid preparations or the centre of spheroids lacking visible signs of hypoxia or necrosis. We and others have shown recently that TAMs release soluble factors that stimulate angiogenesis both *in vitro* and *in vivo*. These include such pro-angiogenic factors as VEGF, TNFa, EGF and bFGF, the secretion of which by macrophages is upregulated by experimental hypoxia. TAMs may be drawn into hypoxic-necrotic areas in solid tumours and then become activated by the low oxygen levels present in these sites to produce pro-angiogenic factors. This would then serve to encourage the ingrowth of blood vessels to these tumour sites to restore the oxygen and nutrient supply for tumour cells.

References

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