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Modelling in tumour biology part 1: modelling concepts and structures

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Our strategies for the treatment of cancer are constrained by our incomplete understanding of tumour biology and behaviour, and by the enormous complexity and resilience to therapeutic perturbation found in the biological world. We are obliged to simplify this complexity through the use of models and mechanistic explanations. In the first of these papers, we consider the nature of modelling mechanisms available to clinical researchers and the extent to which we rely upon them in our understanding of the nature and behaviour of tumours. In the second part, we will consider specifically how models help us to develop more effective strategies for cancer therapy.

Introduction

The living world is infinitely complex. Models represent reality, but they are always approximations. The complexity and heterogeneity of biological systems, including human tumours, obliges us to build models in order to understand their structure and function. This essay considers the nature, range and the limitations of model systems in tumour biology. A further article will consider the opportunities and problems in modelling cancer treatment strategies and clinical trials.

Models aim to represent reality in a form comprehensible to the prepared human mind. They are dictated by the capacity of the brain for data processing, by our preference to describe new concepts in terms of familiar structures and by our communication needs. Models may take many forms. They may exist as drawings on paper, or may be static physical forms of wood, metal, plastic or paper. They may be dynamic physical models with working parts. They may be virtual, existing within mathematical equations; as concepts in the mind, such as Darwin's great model of speciation through survival of the fittest; or as programs within the electronic computer. They are tools for creation and for analysis. Creative modelling involves design and testing, as for example in an engineering model, or the use of a cell line or animal model for the study of physiology, pathology or therapeutics of tumours.

Models may vary from the simple to the complex, and may be two, three or multi-dimensional. Complexity is imparted by the number of parameters or concepts to be represented within the single model and by the volume and character of the data inputs. Clearly, the smaller the number of factors in the design of the model, the simpler the model is to construct and to interpret. Conversely, the greater the number of parameters included in the model, the more it may approximate to reality. To make the model manageable, constraints thus need to be placed upon it, on the degrees of freedom of the elements and upon its dimensions in space and time.

Successful models shed new light on problems and make the world intelligible to us, from the structure of the atom to the expansion of the Universe. They explain the structure of the organelle, cell, tissue, organ, body and population, and the physiological processes which make them work. The best models become widely adopted into scientific thinking and artistic expression. They allow the generation and testing of hypotheses, and indeed are integral to the processes of thought. Conversely, all models have limitations. Simple models may produce elegant insights and shape profound observations, but they also risk oversimplication and the oversight of critical variables. The looser the approximation of the model, the less the representation of reality. Conversely, even modelling with exquisite detail may fail to reveal the underlying truth. This is particularly the case where dynamic processes are studied with static imagery, as for example, histology or histochemistry. The belated recognition of apoptosis illustrates this conundrum, wherein a process fundamental to tissue growth and modelling was completely overlooked until the latter part of the past century.

Biological models may be used deductively or inductively. Deduction is the art of drawing conclusions from the available data. Deductive models can be constructed to interpret data which may be of varying degrees of complexity and obtained from a variety of sources. Such was the process of deducing the structure of DNA, as described by Watson & Crick,[1] wherein physical and crystallographic data was replicated in cut-out models until the true structure was identified. Inductive modelling takes an original idea, and then designs appropriate experiments to collect the necessary

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data to support or refute the hypothesis. Both deductive and inductive modelling require mental agility and manipulation of the available data. The distinction between the processes may sometimes be narrow and semantic.

The data inputs to our model may be derived from observations of the real world. These data inputs may be represented in many forms, from simple to complex data sets, may be analogue or digital, and may be derived from a range of sensors and biosensors, including the human senses and analytical instruments. Analytical models seek to deconvolute existing data. A neural network or other analytical computer algorithm would be such an application of modelling.

The choice and design of model to address a particular problem may pose many difficulties. Take, for example, the static artistic description of a single cell. What should the resolution be? How many dimensions should be represented? How will physiological processes be represented? How will molecular interactions be sign-posted? Such problems extend to the collection of the image. A single cell may be represented in experiments by a single light pulse, by a few hundred digital pixels (as in scanning techniques), or by millions of pixels, as in a microscopic or photographic image. Each technique represents reality in different ways, with different information gains. Now consider the problems imposed by dynamic events. Take a mathematical model of that single cell on a culture plate growing to two-dimensional confluence. What should the smallest unit of representation be? How will the environmental influences and forces which impinge on the cell be modelled? What mathematical rules will govern the behaviour of each component under biological conditions, as in relation to contact with other cells?

Types of models

Static and graphical models

All biological systems and structures are fluid and dynamic, changing in time and place, with the expenditure of energy. Static models seek to distill these processes of change into clear images. Line drawings and diagrams are among the simplest static models. Geographical maps are among the purest expression of such representational modelling in common use. Good diagrams and graphical models have proven to be powerful tools for guiding cancer research. One example is the Cell-Cycle model[2] (Fig. 1), which reveals the behaviour of proliferating cells, and provides the basis for experimentation in cell-cycle control systems.

Simple models may also be physical, as for example architectural models or molecular structures. Molecular biology is crucially dependent upon graphical models, because the components are too small to be visualized, and the rate of molecular processes is too rapid to be captured. The description of the architecture of the DNA helix created an enormously valuable and durable model.[2] It explained DNA duplication and the linear model of base sequences which comprise the genome, which in turn explained transcription and translation, and the structure of chromosomes. An enormous range of graphical modelling techniques have since been developed in molecular biology, usually based on computer graphics, which display the three-dimensional structure of proteins and nucleic acids, and to study the interactions of molecules.

Dynamic models

Dynamic models offer functional insights into the real world. Living experimental systems are dynamic models, as are clinical studies and trials. The tools of dynamic modelling may be conceptual, founded in and derived from our intellectual and knowledge base. Dynamic experimental models allow hypotheses to be tested. They will generally be governed by a scientific hypothesis and will have specific objectives and end points. They may be mathematical, for example algebraic, statistical or epidemiological. They may exist within computers, as spreadsheets, data sets or interactive, graphical models, in which case the basic element of representation in the model may be as small as a digit of binary data.

Models of stable and unstable systems

Many models work well because stability is a characteristic of dynamic biological systems. Cells have structural stability despite molecular fluidity and lack of rigidity. Evolution has imposed profound stability on biological systems, as in embryogenesis, whereby the developmental processes are so stable that similar organisms are reliably reproduced over thousands of generations. The normal tissue, such as bone marrow or the mucosal crypt, is precisely stable in the steady state, despite being highly proliferative, because cell accretion is perfectly balanced with cell loss.

Conversely, tumours are unstable sytems. Genetic instability is evident in aneuploidy, while growth instability is self-evident. It is the temporal instability (volume increase, invasion or metastasis) rather than the existence of the tumour which kills. The restoration of stability or steady state symbiosis of a tumour with its neighbouring tissues is an important objective. Tumour biology thus imposes an additional dimension to our modelling of cancer structures and behaviours.

Mathematical and computer modelling

Mathematical and engineering concepts can be helpful in representing the biological world in various ways. These include linear and non-linear systems, chaos theory,[3] fractal geometry, neural networks and risk analysis. Computers allow fast mathematical iteration and the creation and interrogation of concepts, shapes, form, structure and content of digital models at will. Mathematical, statistical and computer models are interdependent. They allow a wide range of statistical, two and three-dimensional modelling. Molecular models in computers can be used to describe protein structures,[W1] to design drugs, to test receptor function and to model biochemical processes and combinatorial drug chemistry.[3] Such modelling of genetic systems and substructures currently finds advanced expression in the current tools of molecular biology. For example, oncochips offer the constrained surface of a silicon

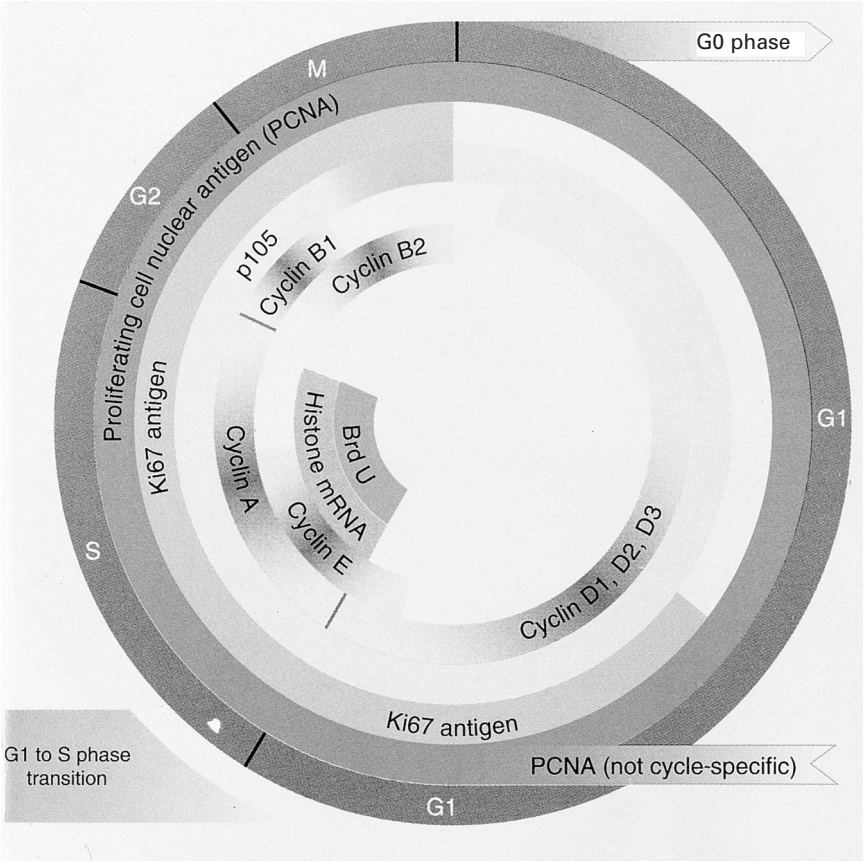

Fig. 1. The cell-cycle model. This elegant model, originating from Howard and Pelc's 1951 paper,[2] describes the sequential phases of the cell-cycle from the diploid, G0/G1 resting phase, though DNA synthesis (the S phase) to the tetraploid, unstable G2 phase which precedes mitosis. The model provides a simple framework for describing the expression of various cell-cycle regulatory proteins, such as the cyclin family; S phase specific markers, such as bromodeoxyuridine (BrdU); and the points of action of the various regulatory signals, such as the switch to S phase.

chip on which are built huge numbers of peptide sequences to be tested at will. They also facilitate the study of base sequences in normal and mutated genes, and of individual genotypes.

Powerful computers allow sufficient data iterations to produce close simulations of real events, be they weather models, nuclear explosions, fluid dynamics, aircraft design or genetic evolution. The computational capability and power of computers is further enhanced by networking of machines, and by linkage across the Internet. Computers thus allow the modelling of hypothetical biological worlds and states. 'What if?' questions allow us to refine and test our hypotheses in virtual time and space before committing laboratory and clinical resources. Spread-sheets offer a common example of exploratory data modelling.

Artificial neural networks

Artificial neural networks (ANNs) illustrate the development and use of modelling tools to analyse complexity. They are mathematical cellular systems which usually exist within a digital computer and which may be used to model biological processes.[W2] They are designed to acquire, store and utilize experiential knowledge. ANNs attempt to create artificial intelligence by mimicking brain structure and function. They are composed of many simple processors (units or elements), each with the possibility of local memory, equivalent to but much less sophisticated than living neurons. Units are connected by communication channels carrying numeric data encoded by various means. Each unit functions in parallel, and can only operate on local data inputs, and is governed by behavioural and training rules concerning permissible responses to various data conditions.

The function of ANNs is determined by network structure, connection strengths (known as synaptic weights) and the processing performed at nodal computing elements. The network thus acts as a data processor which can store experiential data and make use of it. ANNs can thus 'learn' and can be used to draw generalizations. Each unit operates asynchronously and there is no overall system clock regulating the elements.

ANNs can perform any computable function as a normal digital computer, but they are most useful for addressing problems which are imprecise, which have sufficient modelling data and for which expert system rules are difficult to apply. Fundamental mathematical considerations constrain the utility of ANNs to simple or clearly defined

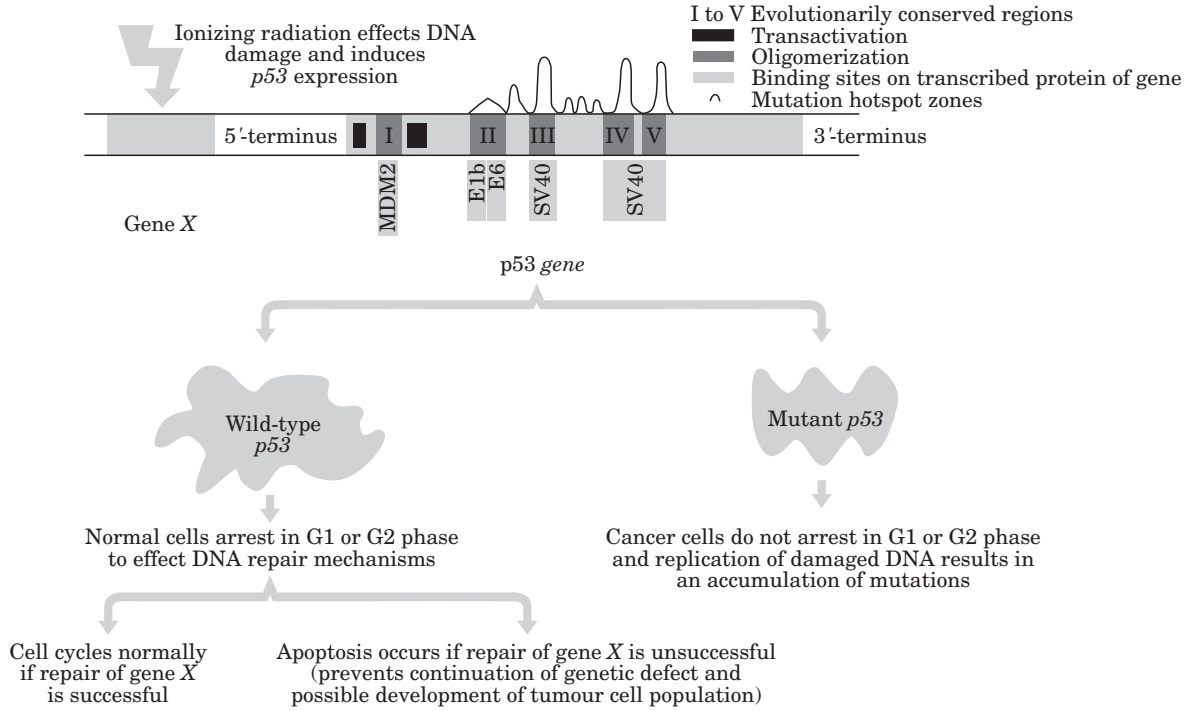

Fig. 2. The p53 gene. This elegant diagram illustrates how complex information about the human genome, mutational frequencies and gene products can be expressed with great clarity using simple linear models and line diagrams.

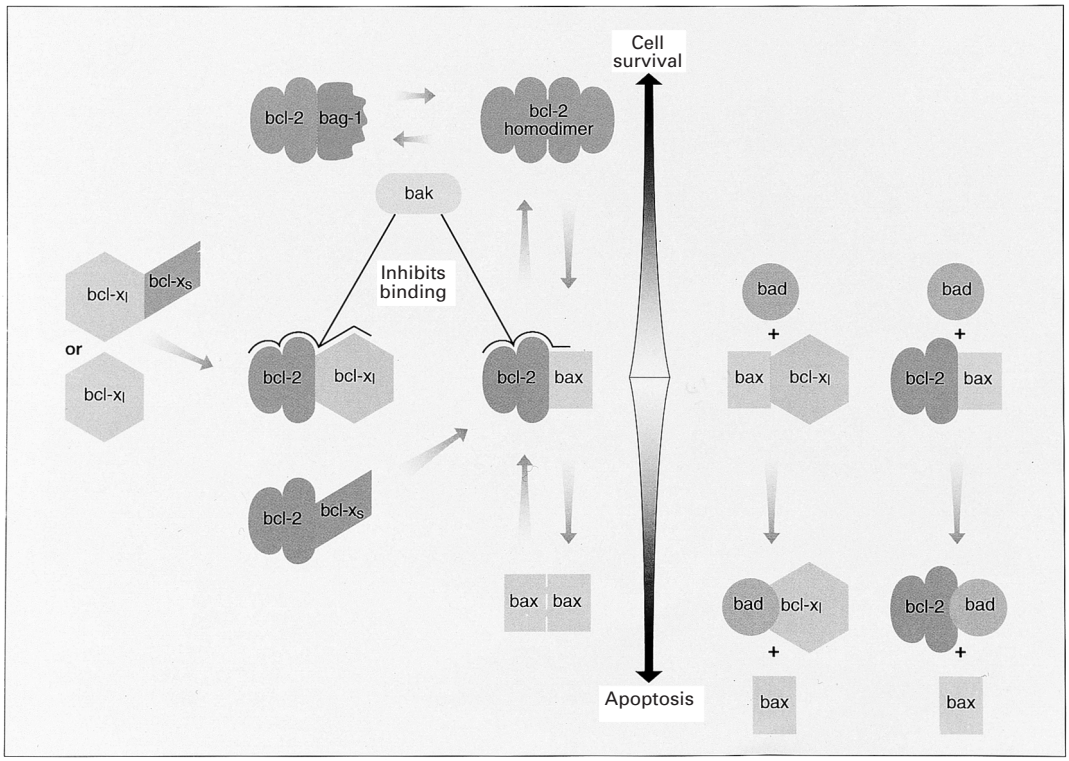

Fig. 3. Apoptosis or cell survival. This model diagram illustrates with great clarity the qualitative relationship between many proteins which regulate the balance between cell survival and apoptosis. The model has great utility as a vehicle for describing hypotheses; because of its flexibility as new proteins are discovered; and because it provides a basis for computer modelling when quantitative data about protein concentrations and kinetics are introduced.

problems. ANNs overlap with statistical techniques, as results are often probablistic.

Fuzzy logic is a form of logic often used in association with ANNs. It uses soft linguistic system variables (such as large, small) and continuous values in the range (0,1) rather than binary, true/false decisions. It is used where a system such as a human expert system is difficult to model precisely. A fuzzy logic system will comprise a rule base, membership functions and an inference procedure. Examples of practical uses of ANNs and fuzzy logic can be found in molecular biology, where they can be used to calculate three-dimensional protein structure from two-dimensional amino acid sequences, and in the interpretation of nucleotide sequences.[W3] They have also been applied to a number of problems in oncology, including the interpretation of mammograms,[W4] modelling the prognosis of renal cell cancer,[W5] the automation of cytological screening,[W6] and skin cancer diagnosis.[W7]

Fractal geometry and chaos

The concepts of chaos and fractal geometry have been discussed in a previous essay.[4] Fractal geometry models complex and self-replicating images and structures with relatively simple formulae. It is a tool of the computer age. The complexity in natural systems may be modelled from relatively simple rules and mathematical descriptions. For example, solid tumour boundaries and morphologies may be described by fractals,[W8] as may the modelling of the segmentation of microcalcifications in mammograms.[W9]

Modelling the future, prediction and risk analysis

Risk analysis seeks to predict future outcomes and to develop strategies for understanding and compensating for a variety of possible outcomes. The likelihood of individual survival to the end of the day, the decade or the century, conditions present behaviour. The modelling of the behaviour of whole human populations in health and disease from census data and life table analysis conditions much of human activity, from the provision of health services and of insurance, to the development of strategies to treat disease. Modelling risk is thus a crucial component of modern life, health and social organization.[5]

Algorithmic modelling

Clinical algorithms model the decision making process in diagnosis and treatment. They reduce expert knowledge to a series of logical decision steps. They grew out of the application of Boolean logic (using decision switches such as AND, OR and NOT) to computer software. They are a form of graphic model of a complex intellectual process. They do not supplant the expert nor do they model all aspects of the clinical process, but they can offer significant help to the less knowledgeable or experienced within a field of knowledge, and are useful in reverse as a tool to clarify the processes of clinical decision making.

Model systems in tumour biology

Biological and laboratory experiments endeavour to model reality. Living systems are among the most useful dynamic models of tumour biology. Thus, for example, controllable experimental systems such as the cell in culture, the nematode worm or the fruit fly, may be used to deduce the behaviour of complex biological elements including the gene, the chromosome, the cell, the tumour, the individual and the epidemiological population. Samples of a tumour itself may be used to model the behaviour of the entire tumour. There is considerable interest in the development of experimental models which avoid the use of living systems, and considerable progress has been made in the use of *in vitro* bioassays and virtual reality modelling, for example in drug development. However, these cannot yet wholly substitute for *in vivo* testing.

Cell lines

Cell lines are among the simplest biological systems to manipulate in cancer research. They retain the immense complexity of biological functions at the cellular level, while offering control of the ambient environment, relative simplicity in maintenance and propagation, selectability for desirable features and ease of access for environmental manipulation, such as the application of drugs or growth factors. They nevertheless have considerable limitations as models. They do not represent the complexity of human tumour systems, for example the three-dimensional structure, cell heterogeneity, nutrient and oxygen gradients, and immunological influences.

Animal models

Animal models offer a number of conceptual advantages over cell lines in tumour modelling. The animals can be bred for specific genetic characteristics and reasonable numbers can be studied economically for statistical purposes. As living organisms, they allow tumour implants to grow in three dimensions, while subjected to the normal physiological, immunological and vascular responses of the host animal.

There remain considerable biological limitations to the use of animal models, not withstanding issues of ethics. Rodent physiology and immunology are often very different to the human condition, while the discrepancies of animal host and tumour size from human equivalents are not simple problems of scale. Animal models may not be representative for many other reasons, including the portal of access, the mode of induction of neoplasia and the timescale of tumour growth.

Human tumour models are usually the most appropriate for studies of cancer biology and therapy. They include isolated cells *in vitro*, which may be cultured for colony sensitivity assays; tumour explants grown in animals; and human tumour studies *in vivo*. The latter suffer the disadvantages of unpredictability, heterogeneity, sample size and the constraints of safety and ethical practice. *In vivo* studies are nevertheless essential for modelling, as for example, the study of new drug dynamics in Phase I and

Phase II clinical trials. Indeed, the clinical trial can itself be considered as a form of experimental model.

There remain many unknowns about the mechanisms of cancer aetiology, epidemiology, growth, dissemination and treatment. The basic unit of cancer modelling may be the gene, the chromosome, the cell, the tissue, the tumour, the host organ, the growth of tumours (cell proliferation, cell loss and angiogenesis), the behaviour of tumours (invasion, metastasis), the individual patient, or the epidemiological population, according to the hypothesis posed.

Genetic processes, gene mutations and genetic disorder

The science of genetics is profoundly dependent upon models which describe the structure of DNA, nucleotide base (CGAT) sequences and gene maps. These are critical to the understanding of molecular biology and to the reduction to comprehension of massive linear data sets. Models of the genome and the process of transcription have shown how certain dominant and recessive genes can function in oncogenesis as tumour promoters or suppressors, following single base pair mutations or the loss or gain of specific chromosomal alleles.[6–9]

A single abnormal cell can grow into a lethal tumour. The mechanism has been modelled as a staged progression of genetic and functional changes in the cell which may be accelerated by environmental interactions and by hereditary genetic changes. This progression has been modelled as a cascade of molecular events which help us to understand the nature of the process, and to correlate form (the mucosa–adenoma–tumour sequence) with genetic dysfunction in colorectal cancer, for example.[6]

Darwinian evolutionary competition may provide a model to explain genetic heterogeneity and malignant change.[10] Cancers display genetic differences between the parent genotype and the tumour which vary with time, growth and site within the tumour. Genetic instability may lead to many different cell lines, or clones, which may have better or worse survival characteristics. The emergence of malignancy through cumulative genetic damage may thus be in part an unavoidable intrinsic process, given the huge numbers of cells produced by proliferative tissues. Models of cell division (meiosis and mitosis) also help us to understand the genesis of chromosomal abnormalities, which may manifest as aneuploidy in cancerous cells.[11]

Chaos may be a useful model for gene function and dysfunction. Minor sequence changes in the genome may have profound, or 'chaotic' consequences for cancer development. The p53 protein system[12,13] is one example of structural genetic disorder at the level of single base pair mutations leading to regulatory protein dysfunction and to malignancy, which may be intrinsically chaotic. Normal p53 prevents progression through the cell cycle of genetically damaged cells. It is a transcription factor which is known to modulate the expression of at least seven key genes. It may then act as a common pathway or 'gatekeeper' in the coordination of damage repair, cell cycle arrest in G1 and apoptosis. Some single base mutations of the p53 gene, of which there are many variants,[14] allow uncontrolled cell cycle progression and impairs apoptosis. Many key mutations of p53 in cancer cells affect p53 binding to DNA. Damage to one gene may thus provide a cascade of dysfunction throughout the cell.

The cell as a collection of molecular machines

Natural selection pressures refined over aeons have determined the emergence of stable, heavily conserved biological processes within cell, tissue and organ. These ensure the continuity of life, reproduction and species survival, of which the relationships between are elegantly modelled in the various trees of life which deduce interspecies relationships. Key processes have been so heavily conserved through evolution that many simple biological systems can be used as study models for higher organisms.

Molecular modelling has yielded one of the most interesting developments in cell biology in the past 5 years. We have long known that many genes and proteins with key regulatory functions are conserved across species. It has recently been recognized that functional groups of gene products are also highly conserved. Many activities in cells in widely differing tissues are conducted by interlocking groups of such proteins, representing the functional building blocks of biology, and working as engines and assembly lines within cells.[15] Thus, for example, the nucleotide binding domain (NBD) for handling energy transfer molecules such as ATP and GTP, is found in organisms as disparate as bacteria and man.[16]

Modelling time in tumour biology

Time is a key variable in biology. All biological systems change over short and long periods of time, and the biology of tumours changes continuously both in space and time. Over longer time periods, (days to weeks), the physical and functional characteristics of a tumour change significantly. The passage of time may be modelled as a molecular clock, which times the cell-cycle, cell replication and the passage of cell generations. The cell-cycle models time accurately, with consistent phase lengths. The loss of repetitive DNA from the telomeres at the ends of chromosomes each time a cell divides provides a dynamic model for the aging of cells and for the immortalization of tumour cells.[17] On much longer time scales, evolution itself can also be modelled as a molecular clock, using gene mutation maps to determine the rate of change of speciation.

Modelling the cell-cycle and its control

Cell proliferation and tumour cell population dynamics are clarified by the cell-cycle model, the phases of which have precise, species specific durations. The expression of many proteins fluctuates through the cell-cycle with DNA synthesis, cell division and quiescence. The study of the cell-cycle in organisms such as yeasts reveals complex but precise and highly conserved molecular processes[18–21] which impose a logical and temporal discipline on the cell-cycle. The fidelity of proliferation is so important to the survival of the normal organism that extremely reliable, heavily conserved controls and failsafe mechanisms have evolved to achieve this end. For example, the cyclin (A–H) and cyclin dependent protein kinases (cdk) go through a similar,

complex sequence of synthesis, association and degradation as the cell-cycle progresses in yeasts and mammalian cells. Signal transduction, subcellular localisation of proteins and specific check points all ensure normal cell division (Fig. 1). The cell cycle model has many consequences, such as allowing cell production rates to be calculated *in vivo*.[22,23]

Modelling cell accretion and cell loss

Tumour growth is the product of an imbalance between cell production and cell loss, which varies considerably with time and changing environmental factors. We may represent the complex imbalances between tumour growth and decay by a simple model. In the bathtub analogy, water running in and out represents cell accretion and loss. The water level (or tumour volume) will be determined by the relative inflow from the taps and the outflow from the waste pipe. We can thus calculate the many conditions which will meet the steady state, water accrual or water loss.

Cell loss is a key process in tumour biology. It may be inadvertent, through hypoxia, immunological response, therapeutic action, exfoliation or shedding, or deliberately induced. Apoptosis, or programmed cell death, is now recognized as a critical biological process, for which many regulatory mechanisms have been detected (Fig. 3). It is now seen to be the main cause of cell loss in growing and remodelling tissues, including embryogenesis, and in maintaining the steady state balance in mature tissues.[24–26]

Modelling the behaviour of tumours

Solid tumours are frequently characterized by biological aggressiveness, which manifests as deregulated growth, abnormal angiogenesis, local invasion, metastasis, local damage (e.g. obstruction) and systemic effects (e.g. anorexia and weight loss). New vessel formation is an important process both for primary and metastatic tumour growth. Modern models of tumour growth recognize the importance of new vessel development and provide greater realism than avascular tumour growth models. A number of regulatory mechanisms have been identified which permit more accurate modelling. These include angiostatin[27] and several families of protein tyrosine kinases (PTKs), such as vascular endothelial cell growth factor (VEGF) and fibroblast growth factors (FGFs). Many solid tumours are known to produce VEGF in response to hypoxia.[28,29] It is possible to model new vessel development using fractal patterns and to incorporate growth factor gradients, stromal and extracellular matrix interactions, the role of macrophages[W7] and angiogenic hot spots. Such an approach has recently been modelled by Chaplain *et al*.,[30] in relation to breast cancer angiogenesis.

The study of cell adhesion, signalling and membrane interactions has been an important aspect of cancer research in recent years. Models of the cell membrane, of intercellular junctions, and of signal receptor mechanisms, are fundamental to this process.[31,32] The interaction between the growing face of a tumour and the surrounding tissues may also be modelled by computer, creating wave fronts driven by directed cell movement, perhaps along growth factor gradients, and regarding cells as automata.[W7]

Metastasis is the process which above all determines the aggressiveness of the tumour and the clinical outcome. It is thus an important subject for modelling.[33] Metastasis encompasses a number of processes, including detachment of viable cells from the primary tumour; survival during transmission and migration to the metastatic site; adhesion and establishment at the metastatic site; and proliferation and angiogenesis at the new site.

In many cases, metastasis is not an entirely random process. Many classes of tumour can be defined in part by their relatively ordered patterns of metastasis. Relative predictability is a feature of squamous aerodigestive and colorectal tumours. The latter consistently progress via the loco-regional lymph nodes to the liver, while ovarian tumours spread trans-peritoneally. The presence of such order implies regulatory mechanisms which may be under specific genetic controls, such as the nm23 gene.[34]

Relative unpredictability is a feature of other tumour classes, including malignant melanoma and breast tumours. Another curious feature of metastasis in some tumour classes, such as breast and melanoma, is the dormancy period following seeding of metastases, which may last for many years. Some breast cancers develop and spread in a 'logical' manner, staging through the regional nodes, while others appear to metastasize much more unpredictably. We have as yet no reliable data inputs from the primary tumour which we can use to model and predict behaviour, and which we could use to refine treatment strategies.

Modelling in clinical oncology practice

What does this all mean for the practising surgical oncologist? We now are obliged to adopt much more systematic approaches to secure optimal outcomes than has been the case in the past. Clinical and oncological practice has often been subserved by tradition, surgical personality, anecdote and generalization, manifest as clinical judgment. The advanced tools of data management and analysis, of information dissemination and computer aided modelling have not hitherto been available to us. We are now able to model likely outcomes for interventional treatment strategies, to a much greater degree than ever, and on an individual patient basis. We can input data from clinical trials, from retrospective and historical data sets, and from concurrent national and international practice by real time computer communication using tools which are familiar to many commercial firms. We thus need to create and adopt new models of information based practice within which to work, and to understand the processes by which these models are created, and their limitations.

There are also many unsolved problems in cancer biology which oblige us to seek new strategies for therapy and disease control, and in original ways. We have at our disposal a vast range of products of twentieth century technology with which to generate, test and illustrate our hypotheses and models, and with which to gather the necessary data. By modelling the critical points in regulation of neoplastic change, of growth, development, causation and behaviour of tumours, and of real patients and populations, we may hope to obtain new insights into the treatment of cancers. In the next article, we will consider

in greater detail the role of modelling in the development and improvement of cancer therapy.

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