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Advances in cell kinetics

Better understanding of the cell cycle could improve treatments for cancer

Abnormal proliferation of cells is an important feature of many diseases, particularly malignancy. Knowledge of the proliferative characteristics, or cell kinetics, of tumours may help to unravel the disease process and to optimise the timing of treatment.

Proliferating cells pass through a series of discrete phases in their life cycle. Howard and Pelc, responsible for the modern concept of the cell cycle, described five phases.[1] From the G1 (gap 1) phase cells enter either the G0 (resting) or S (DNA synthesis) phase. From the S phase they enter the G2 (gap 2) phase, from which they pass into the M (mitosis) phase and then back into G1. Chromosomes are duplicated during the S phase. The timing of the S, G2, and M phases is relatively constant for any one cell type, maximum variation being found in the G1 phase. The importance of this model of the cell cycle is that complex populations of cells become amenable to experimental and mathematical analysis.

The dissection of the cell cycle requires the labelling of newly synthesised DNA with a detectable precursor molecule. Until recently, tritiated thymidine was the mainstay of research into cell kinetics.[2] This restricted the study of the proliferation of human tumours to in vitro incubation of freshly excised tumour biopsy specimens with ^{3}H thymidine. Autoradiography allowed the estimation of the labelling index, but time dependent measurements—such as the cell cycle time—could be estimated only if multiple, serial biopsy specimens were taken. This was rarely feasible in clinical practice.

Bromodeoxyuridine (BrdUrd) and iododeoxyuridine (IdUrd) are halogenated analogues of thymidine which have long been in clinical use as radiosensitisers. They are reliably incorporated into the DNA of proliferating cells. In 1982 Gratzner[3] and colleagues[4] in the United States developed a family of monoclonal antibodies that detected these analogues bound within DNA. Proliferating cells could therefore be identified by immunohistochemical examination.

Developments in flow cytometry have greatly enhanced the use of this technique.[5] In a flow cytometer laser light illuminates a stream of suspended cells or nuclei. If these are labelled with monoclonal antibodies and fluorescent dyes, then simultaneous quantitative measurement of multiple factors—for example, the DNA content and the content of bromodeoxyuridine—is possible in each of thousands of cells.

In 1985 Begg and colleagues described a flow cytometric technique by which a cohort of tumour cells labelled with bromodeoxyuridine could be followed through their cell cycle.[6,7] From this the tumour labelling index, the duration of the S phase, and the potential doubling time of the tumour could be estimated. The method has been shown to be applicable to human tumours in vivo.[8,9] The derivation of time dependent measures of proliferation from a single biopsy specimen of a tumour or tissue pulse labelled in vivo is now possible. Bromodeoxyuridine can be given as a 250 mg intravenous bolus before surgery or biopsy for cancer. Bromodeoxyuridine shares a small risk of mutagenicity with all cytotoxic agents and ionising radiations, and prudence suggests that currently its use should be confined to adults above reproductive age with proved malignancy.

Considerable work is still needed to standardise analytical protocols and to assess the effect of tumour heterogeneity on the interpretation of results. Nevertheless, the technique marks a noteworthy advance in oncological research.[10,11] Proliferation indices are currently being assessed both as predictors of clinical outcome[12,13] and as guides to the fractionation of adjuvant treatment for cancer. For example, rapid proliferation of cells and tumour repopulation could

lead to the failure of conventionally fractionated radiotherapy. Clinical trials are in progress to evaluate hyperfractionated treatment of human tumours, supported by measurements of the potential doubling time.[14]

Labelling with bromodeoxyuridine and iododeoxyuridine can be combined with the study of other markers—such as oncogene proteins—to provide more information on the control of proliferation.[15] The spread of flow cytometers to many clinical laboratories and their increasing ease of use have opened up important new opportunities for the study of human tumours and their treatment. The wheels of the cycle are turning again.

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