Probing the cell biology of cancers

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Tumour biology was a significant theme of the 18th biennial meeting of the International Society for Analytical Cytology (ISAC), held in April 1996 in Rimini, Italy.

Cancer genetics

Considerable progress has been made in the understanding of tumour genesis and genetics, particularly in relation to breast cancer. Dr Fred Waldman (University of California, San Francisco) showed how breast cancer, like colorectal cancer, result from progressive and cumulative genetic aberrations that affect the processes of tumour initiation, progression and metastasis. Microdissection of individual human tumour samples containing foci of normal tissue, ductal carcinoma-in-situ (DCIS) and invasive tumour, can be used to demonstrate increasing clonal abnormalities and genetic variance as tumours become more aggressive. Multiple phenotypes arise within each tumour through a seemingly random process of selection, so metastasis might be a stochastic, or chance process, rather than being under the specific control of genes such as nm23.

Dr Cees Cornilisse (Leiden, Belgium) reported on the role of tumour suppressor genes in hereditary and sporadic breast cancer. He said that both copies of such genes had to be inactivated for cancer to develop — the two-hit hypothesis.

Familial studies, such as those looking at Li Fraumeni syndrome, have elucidated the breast cancer (BRCA) 1 gene, on chromosome 17q21, for which more than 100 mutations are now recognised. BRCA1 mutations often produce an incomplete protein product, facilitating BRCA1 detection through the protein truncation test. This can be performed on cDNA amplified from a candidate patient's leukocytes. Heterogeneity is found for BRCA1 in 45 per cent of hereditary breast cancer and 85 per cent of hereditary breast and ovarian cancer cases, but not in male members of such families.

Ashkenasy Jewish families are particularly at risk of transmission of one mutant of the BRCA1 gene — with AG base deletion at position 185. More recently, an international group headed by Dr Mike Stratton (Cancer Research Campaign, Sutton, UK) has identified the larger BRCA2 gene.

In sporadic breast cancer, a gene that encodes for the protein E-cadherin is commonly found to be mutated in lobular carcinoma. E-cadherin functions in the junctional complexes of epithelial cells, and its dysfunction may find physical expression in the specific histomorphology of lobular carcinomas.

Drugs and genetic dysfunction may both cause aneuploidy. Dr Z Darzynciewcz (New York, US) reported that the cytotoxic compound staurosporine impaired cytokinesis and dramatically increased aneuploidy in cell line models.

Dr Thea Tlsty (Parnassus, California) showed how chromosome fragment amplification could produce aneuploidy, and illustrated this dramatically with colour images of chromosome smears labelled by fluorescence in situ hybridisation (FISH). Amplification is a recessive trait, and it appears that normal regulation of gene structure must be lost from both chromosomes before it can occur.

Cell cycle control

The regulation of the cell cycle, which underpins normal and malignant cell proliferation, is complex but precise and evolutionarily conserved. Dr Jonathan Pines (Cambridge, UK) described how members of the cyclin (A-H) and cyclin-dependent protein kinase (cdk) families of proteins go through a complex sequence of synthesis, association and degradation as the cell cycle progresses.

Specific checkpoints at various points in the cell cycle make sure that all criteria for normal cell division, such as precise alignment of chromosomes during mitotic metaphase, are met before cell division can proceed.

Dr Darzynkiewcz and colleagues have used flow cytometry to demonstrate the cell-cycle specific expression of cyclins and associated proteins in tumour cell line models. Signal transduction around the cell is an important feature of cell cycle regulation, and the cyclins localise in different sites in the cell. For example, cyclin C is closely associated with spindle microtubules during mitosis, while cyclin B resides in the Golgi apparatus.

Other proteins, including pl5, p16, p18, p19, p21, p27 and ubiquitin, function in the inhibition, degradation and proteolysis of these regulatory switches. The cyclin system ensures secure and efficient mitotic reproduction of normal cells.

The telomerase enzyme may play a key role in the immortalisation of cancer cells. In her keynote address, Dr Sylvia Bacchetti (Hamilton, Ontario, Canada) showed that chromosomes shorten with each replication in normal cells, with the loss of short repeat sequences of DNA primer fragments from the telomeric complexes at each end of the chromosomes.

Telomere shortening appears to confer a biological clock, a proliferative lifespan, and senescence on normal cells. Telomerase, which does not appear to be expressed in normal human somatic cells, restores the length of telomeres and transforms cells so that they acquire an extended proliferative lifespan. At the limit this results in immortalisation in neoplasia. Evidence for this has been derived from cell line experiments, and from ovarian, retinoblastoma, haemato-

genous and other tumours. In a cohort of colorectal tumours and tissues, telomerase was not found to be expressed in biopsies of normal tissue or in adenomas, but was expressed in virtually all tumour samples. The indirect telomerase assay of terminal restriction fragment length is still a laboratory technique, but clinical applications can be foreseen.

Knowledge of the causes of disordered or deregulated cell proliferation in cancer may identify new targets for drug therapy. Professor Paul Smith (Cardiff, UK) reviewed the mechanisms of cytotoxic drug action in cells, and showed that cytometric techniques identify the specific point of action of various agents within the cell cycle.

Drug efficacy is a balance between damage induction and damage limitation, and relates to cell cycle controls and check points. For example, specific topoisomerase inhibition by agents such as ICRF193 at the G2 checkpoint produces disorganised chromosome segregation in G2, while mitotic spindle poisons such as the Taxol derivatives disrupt cell division.

Apoptosis — programmed cell death — continues to attract considerable research effort. A number of genes, including BCL2, protect against apoptosis. BCL2 protein is found on internal cell membranes, including endoplasmic reticulum and mitochondria.

Dr David Hedley (Toronto, Canada) reported that flow cytometric studies could link specific gene expression to microphysiological phenomena. In his model, BCL2 protein may inhibit reactive oxygen generation and ionised calcium release within cells.

Drug resistance

Intrinsic multidrug resistance (MDR) mechanisms continue to confound simple strategies for anticancer chemotherapy. Drug resistance results from passive tissue mechanisms, including aberrant vascular perfusion, tissue acidity and hypoxia, and active molecular mechanisms within cells. The membrane-bound, ATP-dependent pl7O MDR glycoprotein pump appears to be only one of many MDR mechanisms.

Dr Marcel Flens (Amsterdam, The Netherlands) reported two more such molecules. The multi-resistance protein (MRP) is one of a family of ATP-dependent multispecific organic anion transporters in epithelial, endocrine cells and macrophages. The lung resistance protein (LRP) is associated with RNA in protein pores or vaults on nuclear and endoplasmic reticulum membranes. LRP may be an important component of RNA transport between cell compartments and of the expulsion of unwanted substances from cells in exocytic vesicles.

Gene therapy

In a keynote address, Professor Karol Sikora (London, UK) reviewed the progress and potential of gene therapy in cancer which may be targeted at somatic or germline cells.

The criteria for research into gene therapy for any diseases are that the disease process should be life-threatening but potentially reversible, that the gene should have been cloned, that a delivery system should be feasible and that there should be a measurable end point to demonstrate therapeutic gain. Candidate diseases include immune deficiency syndromes, cystic fibrosis, the haemoglobinopathies, metabolic disorders, and various forms of cancer.

A variety of gene therapy techniques have been considered, including immune enhancement, the vectoring of biological agents such as tissue-specific viruses, tissue protection (eg bone marrow), selective drug activation by specific genes, and the correction of somatic defects. In the field of cancer, genetic marking may allow the detection of minimal residual disease, the characterisation of a source of recurrence and the monitoring of bone marrow purging.

Associated new technologies include genetic prodrug activation therapy and differential display polymerase chain reaction, which allows catalogues of gene expression to be prepared for individual tumours.

Gene therapy has yet to find proven applications in daily use, but early clinical trials are in progress. Dr Leszek Borysiewicz (Cardiff, UK) reported preliminary data from a study of immunotherapy for cervical cancer using a recombinant vaccine encoding human papilloma virus genes E6 and E7 to generate specific cytotoxic T cells in vivo.

Instrumentation

The technology of cell and subcellular analysis is advancing at a prodigious rate. Flow cytometry (flow microfluorimetry) is now a mature tool for rapid quantitative analysis of complex populations of cells, but is still evolving as a tool for chromosome sorting, where it is making considerable contributions to the human genome project and cytogenetic analysis.

Laser-based confocal microscopy and computer-assisted image analysis are also evolving as tools for subcellular and molecular biological analysis.

Dr Steven Fodor (Affymetrics Corp, California) demonstrated the technology of light-directed chemical synthesis of oligonucleotide arrays on a silicon chip. He explained that hybridisation sequencing of genes and fragments could be undertaken on a single sliver of glass on which up to 256,000 different but precisely defined oligonucleotides, each up to 20 bases long, were bound.

This matrix of defined DNA or RNA sequences has so far allowed the study of mycobacterium variants, HIV drug resistance, the p53, MSH1 and MLH1 genes in the colorectal cancer cascade, and the BRCA1 familial breast cancer gene. Future developments may see up to 5,000 genes encoded on a single 'oncochip', for rapid and accurate assessment of mutations in cancer studies.

Dr Richard Mathies (University of California, Berkeley, USA) described capillary array electrophoresis, which allows rapid gene sequencing when used in combination with base-specific fluorochromes and laser light excitation.

Such developments are allowing rapid progress to be made in the Human Genome Project, which in turn will be the basis of substantial progress in the biological and clinical sciences.

Other topics

The conference also addressed immunology, haematopoiesis, HIV infection and biological dosimetry, including the measurement of radiation damage from events such as the Chernobyl disaster.

■ For further information, refer to the conference proceedings and abstracts published in *Cytometry* 1996; **23**, supplement 8. ISAC also publishes a range of information on the worldwide web at http://nucleus.immunol.washington.edu/ISAC.html.