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Review

Significance of aneuploidy

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Aneuploidy is a state of abnormal and highly variable DNA and chromosome content found in both hereditary disorders and human malignancy. For two decades flow cytometry has allowed a wide-ranging survey of aneuploidy in clinicopathological series. Although up to 75 per cent of all tumours analysed display aneuploidy, its value as a clinical marker of biological aggressiveness is still uncertain. New technologies promise to reveal more precisely the genetic and subchromosomal changes that constitute aneuploidy and contribute to the malignant phenotype in human tumours.

Aneuploidy is a state of abnormal chromosome complement of cells or individuals, such that their total DNA content differs from that characteristic of their species (the karyotype) by the addition or loss of varying numbers of chromosomes or chromosome fragments. Aneuploidy is a phenomenon that has long been recognized[1] but which has come to prominence only in the past two decades, when studies of DNA content, ploidy and aneuploidy have had a major place in oncological research[2]. This growth has been driven by the availability of the flow cytometer and, particularly, by the facility to analyse archival pathological material[3] to correlate DNA content in tumours with clinical behaviour and outcome. Despite the plethora of published papers many questions about the biological and clinical significance of aneuploidy remain.

Definitions

Aneuploidy is best considered in relation to the state of normal DNA content in non-germline cells, known as diploidy. Diploidy in humans is the possession of the normal species-specific complement of two pairs of haploid ($n = 23$) chromosome sets. Even this definition of diploidy poses problems, because it presupposes a precise knowledge of the total DNA content in normal human cells of various lineages. Given the economy and efficiency found in nature, diploidy may be expected to represent the minimal DNA content compatible with effective cell function. However, it is not known whether there is variation in DNA content from individual to individual as defined by the absolute number of base pairs present and, if so, what the range of this variation might be. As the sensitivity and accuracy of quantitative DNA analysis improves, such questions should have clearer answers.

Diploidy does not imply precise chromosomal structure or arrangements. Where chromosome fragment (allelic) loss occurs at one site, it may be balanced by either translocations or DNA gains elsewhere in the chromosomal make-up. Such a state is pseudodiploidy. Hypoploidy results from allelic and regulatory gene loss and imbalance, and is a recognized feature of malignancy[4]. Measurable but subchromosomal variations are known as 'near-diploidy'. True hypoploidy is a relatively uncommon finding.

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Aneuploidy can also arise from the gain of complete chromosomal sets. Exact multiples of the haploid number are described by specific terms, for example the triploid (69 chromosomes) or tetraploid (92) states. The measurable ratio of abnormal to normal cellular DNA content is the DNA index of the cell, tissue or tumour. At present, where measured by flow cytometry, aneuploidy may be defined as a change in the DNA content of cells greater than the limit of measurement (DNA index more than 1·05). Hyperploidy and tetraploidy describe cell lines that are characterized by almost complete or complete duplication of diploid DNA content respectively. DNA indices of up to 3·0 (hexaploidy) have been reported. The maximum DNA content that is compatible with survival and multiplication within a human cancer cell is not known. In multiploid tumours more than one aneuploid cell line is detectable, each having a distinct DNA index.

Biological significance of aneuploidy

The existence of aneuploidy raises fascinating questions about the nature of normality in cells. How can cells with disordered DNA content survive and proliferate? To what extent does this occur in normal tissue in the absence of malignant change? Are there differences in behaviour between malignant diploid and aneuploid cells? Why are proliferation and cell division independent of the precise genetic content or diploidy within the cell?

Because a huge number of mitoses occur during the growth, development and turnover of normal cells, it is conceivable that aneuploidy arises frequently but transiently throughout the lifespan of normal tissues, particularly in highly proliferative tissue such as bone marrow and intestinal mucosa. It may be assumed that mechanisms exist to destroy the majority of abnormal cells, including aneuploid cells, before they achieve uncontrolled proliferation. These mechanisms may include immunological cell functions or the regulation of apoptosis (programmed cell death)[5]. Changes in the expression of regulatory genes and oncogenes, such as c-*myc* or p53, may then confer a relative survival advantage on the aneuploid cell.

A striking feature of aneuploid tumours is that there is usually only one stable aneuploid clone or population of cells. In many published series where substantial numbers of tumours have been analysed, the distribution of DNA indices

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is bimodal[6], with a peak around 1·0 and a second peak around 1·7. During the life of a tumour one or more stable but abnormal cell lineages may survive and proliferate, giving rise to abnormal but tumour-specific DNA content. The DNA content of one aneuploid tumour may be markedly different from that of another. Why? What gives any one cell lineage in each tumour population a growth and survival advantage?

The aneuploid ratio may also change during the lifespan of the tumour or in response to adjuvant treatment. For example, this may occur in breast tumours treated with tamoxifen, where the late development of aneuploidy appears to correlate with treatment failure (M. Ormerod, personal communication).

Mechanisms of aneuploidy

True aneuploidy is only one manifestation of the genetic disturbances that occur in malignant cells. These may be classified into three groups[7]: primary and secondary abnormalities of the genome, and cytogenetic 'noise'.

Primary abnormalities of the genome, such as point mutations in regulatory genes and oncogenes, may produce critical neoplastic changes. Despite considerable genetic disorder as a result of mutation and of chromosomal fragment and gene translocations, there may be no detectable change in the total DNA content or mass in diploid cells.

Secondary abnormalities, such as gene amplification and chromosome duplication, may arise within an unstable cell genome. There are a number of different situations in which aneuploidy may be found. First, duplication of one or more whole chromosomes may occur. For example, Kamada *et al.*[8] demonstrated clonal chromosomal abnormalities in 103 of 107 patients with adult T cell leukaemia or lymphoma. Second, duplication of one or more substantial chromosome fragments may occur. Third, amplification of one or more individual genes may lead to the production of large numbers of copies. For example, Meling and co-workers[9] used a probe specific for the retinoblastoma (*Rb*) gene to study amplification in colorectal carcinoma; 29·5 per cent of the 255 tumours displayed *Rb* amplification. There was a significant correlation between this gene amplification and the presence of DNA aneuploidy. Cornilesse and colleagues[10] reported an allelic imbalance in association with aneuploidy in a series of 86 primary breast carcinomas. It is also possible to speculate on other causes of aneuploidy. For example, might there be amplification of 'junk' DNA sequences (of no discernible function) within chomosomes, but with no change in the function of normal genes? Might aneuploidy be a function in some instances of infection by viruses or bacteriophages that have become incorporated within host chromosomes?

Cytogenetic 'noise' is the third proposed state of DNA abnormality. This term relates to the many chromosomally unstable tumour cells that are temporarily detectable during tumour growth but which have no survival advantage. Chromosomal disorder and aneuploidy are most likely to arise during mitosis or meiosis, when chromosomes are being shuffled and replicated. There are a number of different points during both processes at which chromosomal aneuploidy may arise.

Possible mechanisms of aneuploidy in somatic cells

During the cell cycle the normal diploid 46-chromosome set is duplicated during the S (synthetic) phase before mitosis. The resulting pairs of chromosomes are parcelled out equally to the daughter cells[11]. Before mitosis chromosomes exist in the interphase state, during which they are unravelled and the DNA is exposed for genetic activities or for DNA duplication to proceed. Aneuploidy may arise if the precise and equal distribution in time and space of the two matched sets of chromosomes to the daughter cells does not occur. As chromosomes condense during early mitosis, there is considerable scope for entanglement in this molecular 'spaghetti'. The topoisomerase and DNA ligase families of proteins[12] may be important in preventing such DNA disruption and entanglement, which might in turn predispose to aneuploidy. Order is imposed on the distributing chromosomes by the formation of a bipolar spindle[13]. Each set of chromosomes becomes attached to one pole of the spindle by filamentous contractile protein microtubules. Microtubule elongation from tubulin subunits is initiated at a centrosome at each end of the spindle. Within each condensed chromosome are regions of microtubule attachment known as centromeres, composed of repetitive DNA sequences. The attachment is mediated through kinetochores[14,15]. Chromosome pairs become evenly aligned on the equator of the spindle before microtubule contraction and chromosome separation.

Aneuploidy in germline cells

Unlike mitosis, which may give rise to sporadic, local or metachronous aneuploidy in the individual, germline abnormalities will give rise to constitutional aneuploidy throughout the body. Meiosis differs from mitosis in that a chromosomal reorganization and reduction from the normal diploid (46) to the haploid (23) chromosome state accompanies cell division. Before meiosis in germline stem cells, DNA is replicated from 46 (diploid) to 92 (tetraploid) chromosomes. In the first phase of meiosis the 46 condensed chromosome pairs align in 23 quartets, within which each chromosome is intimately associated and accurately aligned by synapses. The formation of these synapses, or synaptonemal complexes, allows the exchange of DNA sequences to occur at chiasmata. An incomplete reduction division then occurs, so that 23 rearranged chromosome pairs migrate to each pole of the cell, as occurs in mitosis. A second reduction division then occurs, yielding four haploid germ cells.

Uneven chromosome alignment, abnormal kinetochore development and function, failure of pair separation (non-disjunction) or errors of spindle orientation and contraction may lead to uneven DNA distribution and hence to aneuploidy during both mitosis and meiosis. Meiosis provides at least two additional points at which aneuploidy may arise[16]. First, inaccurate alignment at synaptonemal complexes produces unequal chromosome lengths once cross-over has occurred. Second, non-homologous chromosomes may pair up, again producing uneven exchange of DNA.

These errors may be induced by drugs acting as mitotic poisons. For example, kinetochores can be disrupted by caffeine or mitomycin C. Demonstrable aneuploidy can be induced in experimental cell systems by a variety of mutagens. These include viruses[17,18], such as SV40 and human papillomavirus 16, cytotoxic agents, such as dimethylhydrazine[19], vincristine, diethylstilboestrol and colchicine, and ionizing radiation[20]. In human tissues the risk of DNA damage and malignant change increases with age. The prime causes of such cumulative damage may be

environmental, including background and cosmic radiation, 'man-made' radiation, and chemical substances[21,22].

Measurement of aneuploidy

Fluorescence microscopy and static cytometry are established, but laborious, methods of measuring aneuploidy in small populations of cells[23–25]. Flow cytometry has been a major impetus to clinical studies of aneuploidy. The technology and methodology of flow cytometry have been described in detail in a number of texts[26]. Flow cytometry works by illuminating a stream of biological particles, such as cells or cell nuclei, with a light beam of known wavelength, usually from a laser source. Light beyond the stream is collected by a series of photomultiplier tubes linked to a computer. The particles scatter the light such that their size and granularity may be deduced. If the particles are marked with fluorescent dyes, other characteristics may be measured. Dyes, such as propidium iodide, ethidium bromide, acridine orange and 7-aminoactinomycin, bind to DNA in proportion to the quantity present in each cell or nucleus. Typically, 5000–10000 particles from any one tumour biopsy can be analysed in a few minutes.

For each particle the computer assimilates data from each of its light collectors and presents these to the observer as a scatterplot or histogram. A fluorescent event is allocated to one of 1024 channels of fluorescence according to its light intensity. In the case of the DNA profile, this is a histogram of DNA content in the population of cells or nuclei. DNA content, being proportional to the light intensity emitted by its marker dye, is conventionally plotted on the *x* axis. The components of the DNA histogram relate directly to the phases of the cell cycle. The largest fraction of cells, usually around 70 per cent, is contained within the G0–G1 phase, wherein cells contain the diploid number of chromosomes or their abnormal aneuploid equivalent. The G2 phase merges with mitosis (M phase). G2–M is a second peak, consisting of cells or nuclei containing duplicated chromosomes, which commonly accounts for 20 per cent of cells. The continuum between the G0–G1 and G2–M peaks is the S phase, which contains cells or nuclei with chromosomes at various stages of duplication.

The DNA histogram forms one of two patterns. A diploid tumour displays a simple biphasic pattern, with a large primary peak corresponding to cells in the G0–G1 phase of the cell cycle (diploid chromosome content). The smaller peak of the biphasic pattern corresponds to cells in G2; its tetraploid chromosome content falls at twice the channel number of the G0–G1 peak (*Fig. 1*). The flat intermediate zone corresponds to S-phase cells. The DNA content in G0–G1 can be defined in relation to a reference standard such as nucleated chicken erythrocytes.

Aneuploid tumours usually comprise a diploid population, being a mix of non-malignant stromal cells (e.g. lymphocytes, endothelial cells) and diploid tumour cells, and one – or occasionally more – aneuploid population(s). The DNA profile of the aneuploid cells (G1–S–G2) is, in effect, superimposed on the diploid DNA profile, but shifted along the *x* axis to the right (denoting greater DNA content) according to the excess DNA present in the aneuploid cells (*Fig. 2*). Occasionally, multiple aneuploid populations of tumour cells are present, each with their own distinguishable G0–G1 and G2 peaks.

Flow cytometry has practical limitations that bear upon the interpretation of published clinicopathological studies of aneuploidy. Some of the more important of these problems are discussed below.

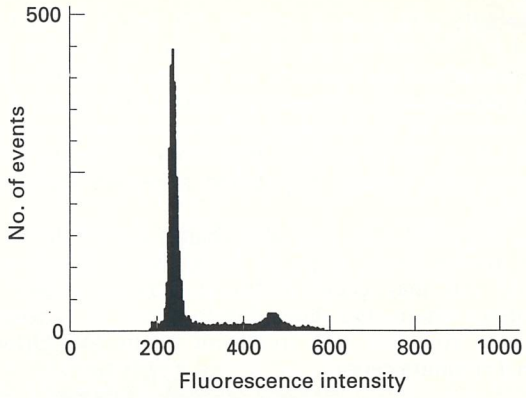

Fig. 1 DNA histogram of a diploid human colonic tumour with propidium iodide as the DNA-binding fluorochrome. The G0–G1 peak is centred on channel 240 and the G2–M peak on channel 480 on the *x* axis. The *y* axis denotes the number of nuclei. The region between channels 240 and 480, while strictly representing the S phase, is overlapped by the gaussian distributions of the G0–G1 and G2–M populations

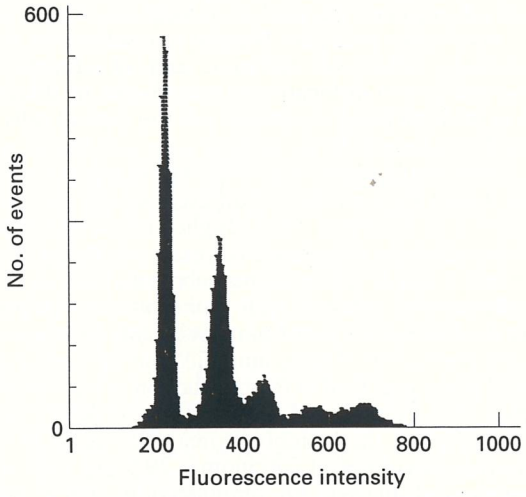

Fig. 2 DNA histogram of an aneuploid human breast carcinoma; the DNA-binding fluorochrome is propidium iodide. The G0–G1 diploid peak is centred on channel 225 and the G2–M diploid peak on channel 450. The G0–G1 aneuploid peak is centred on channel 350 and the G2–M aneuploid peak on channel 700. The DNA index of this tumour is 350/225 or 1·56. Note that the aneuploid G0–G1 peak completely overlaps the diploid S phase between channels 225 and 450, while the diploid G2–M peak completely overlaps the S phase of the aneuploid population between channels 350 and 700. This renders impractical accurate calculation of the S-phase fraction of either the diploid or aneuploid population

Particle extraction and preparation

Flow cytometric analysis depends on the preparation of single-cell or nuclear suspensions from solid tumours by enzymatic or mechanical means. Unfixed tumours and tissues are variously resistant to disaggregation according to their stromal content. Archival material, usually extracted from formalin-fixed wax-embedded blocks, is particularly useful for studies correlating DNA content with clinical features and patient survival. However, its extraction requires more drastic methods than that of fresh material[27]. Disaggregation may cause a number of problems, including considerable antigen loss and subcellular fragmentation. Nuclei are more easily obtained than whole cells, and whole-

cell extracts are rarely attainable for practical purposes from fixed archival material. Extraction artefact and technical failure may lead to rejection rates or failure of analysis in up to 40 per cent of samples in reputable series[27].

Tumour heterogeneity

Studies in which multiple samples have been analysed from individual tumours indicate that there can be considerable heterogeneity of DNA content from site to site in the tumour, for example between purely diploid and aneuploid regions. A single biopsy is unlikely to be representative of the entire tumour. In retrospective studies of archival material the researcher has little control over the representative nature of the blocks. In prospective studies, while multiple-site sampling is possible, there is no standard about how the resulting data should be analysed and presented. What weight, for example, should be given to DNA data obtained from samples from an anoxic necrosing core of a tumour, compared with those from its advancing margins?

Data analysis and observer variation

Data from all particles passing through the cytometer will be registered. This includes subcellular fragments and doublet cells. While the computer can do much to simplify analysis, for example by selecting out cell doublets, the process of analysis is not fully automated; it requires expertise and subjectivity on the part of the researcher. This often leads to considerable interobserver and interlaboratory variation in data analysis, even where standard samples are distributed[28]. In a recent review of 225 papers Wersto *et al.*[29] found frequent differences and inadequacies in the criteria used to distinguish diploidy from aneuploidy, for example in the coefficient of variation in the G0–G1 peaks. The narrowness of a peak (a low coefficient of variation) can be used as a measure of the 'cleanliness' of the extraction process. This is important because broad diploid peaks on DNA profiles may hide small aneuploid populations and lead to the erroneous classification of a tumour as diploid rather than aneuploid[30].

New technologies to study aneuploidy

Specialized flow cytometers with very high sampling rates and sensitivity allow for the automation of chromosomal sorting and karyotyping (flow cytogenetics). It is now possible to enumerate the copies of a gene or chromosome within a single cell and so better characterize the changes that have occurred to produce aneuploidy. The technology will distinguish one or more copies of a fluorescent probe bound to a specific sequence of DNA, and will exploit minute but consistent differences in size and fluorescence associated with each of the 23 pairs of chromosomes in the human genome[31]. This also facilitates the quantitation of abnormal chromosomes in tumours or in fetal karyotyping.

Molecular biological techniques are making an important contribution to the understanding of aneuploidy. Chromosome enumeration or karyotyping, which once demanded the manual study of metaphase spreads by trained observers, has been facilitated by hybridization techniques. The polymerase chain reaction is one tool that may find application in the study of aneuploidy, for example in the quantitation of sex chromosomes[32].

Non-isotopic *in situ* hybridization is another useful tool. Chromosomes and chromosome fragments can be identified with a growing library of specific DNA probes, which are single-stranded DNA fragments tailored to match predetermined naturally occurring DNA sequences. These probes can be visualized with avidin–biotin–peroxidase, digoxigenin (a component of digoxin) or a variety of fluorochromes – hence fluorescence *in situ* hybridization. Unlike earlier methods that worked only with condensed chromosomes, these probes additionally allow the identification of specified DNA fragments within the amorphous DNA mass in the nuclei of interphase (non-mitotic) cells. In any one cell the number of copies of the chromosome fragment can be enumerated directly[33].

These *in situ* hybridization techniques are now well established and reproducible, and have been the basis of direct assays of aneuploidy and aneuploidy-inducing agents in experimental cell lines, human lymphocytes[20], amniotic cells[34] and clinical tumours. Van Dekken *et al.*[35] undertook cytogenetic analysis of ten gastric tumours by *in situ* hybridization using a set of five chromosome-specific DNA probes (1, 7, 17, X and Y). Loss of the Y chromosome was a significant feature in the tumour cells of six of eight men. As probe libraries grow, so it will be possible not only to identify aneuploidy but also to specify which fragments and chromosomes constitute the amplified DNA.

Clinical manifestations of aneuploidy

Germline and hereditary disorders

Aneuploidy is not synonymous with malignant change; it is compatible with near-normal growth and development. There are a number of syndromes in which aneuploidy is a fundamental feature. These include Klinefelter's syndrome (XYY), Turner's syndrome (X0) and trisomies 13, 18 and 21 (Down's syndrome)[36]. These syndromes are associated with variable dysmorphogenesis and organ maldevelopment, but they are quite compatible with life and with the development of recognizable human form and function.

Aneuploidy is a common feature in germ cells and a common source of embryo wastage. Maternal ageing is an important cause of embryo aneuploidy[37], but is not the only factor. Benet *et al.*[38] reported a 4·0 per cent incidence of aneuploidy in a population of 505 motile human sperm complements from three normal donors. Aneuploidy can also be demonstrated in meiotically mature oöcytes[39]. Johnson and co-workers[40] found 29 aneuploid embryos in 174 pregnancies. Aneuploidy is also found commonly in aborted fetuses and in oöcytes associated with *in vitro* fertilization[41]. Aneuploidy may arise more commonly in sex chromosomes than in autosomes as a result of paternal meiotic error[42].

Aneuploidy in premalignant conditions

Some hereditary disorders that predispose to malignancy early in life also show an early tendency towards aneuploidy. These include familial adenomatous polyposis predisposing to colorectal carcinoma[43] and hereditary tyrosinaemia type 1 predisposing to hepatocellular carcinoma[44].

Sporadic malignancy may be preceded by a sequence of genetic changes in the normal tissue of origin. These genetic changes may in turn be accompanied by the emergence of aneuploidy. One example is intestinal epithelium, where genetic and regulatory changes may arise in mucosal crypt cells, leading to adenoma formation, before progressing to invasive adenocarcinoma[45]. Aneuploidy has been reported in chronic ulcerative and Crohn's colitis[46–48], and in

adenomas[49]. Caution must always be exercised in such studies to ensure that apparent aneuploidy in nominally diploid material does not merely reflect experimental artefact. However, the emergence of aneuploidy in chronic colitic mucosa may identify patients in need of early surgical intervention.

Aneuploidy in human tumours

Aneuploidy is a commonly recognized property of neoplasia, occurring in up to 75 per cent of tumours in most large series, but it is not a prerequisite for malignancy. A complete overview of the literature is beyond the scope of this paper, but many authoritative reviews of the subject have been published[26,50–52]. Original articles on this topic now run at several hundreds per year in the world literature; virtually no class of tumour has escaped analysis and a tabulation of the published results would run to many pages.

Is there any evidence that the measurement of ploidy status is clinically useful? The technical problems associated with the DNA analysis of clinical material by flow cytometry and consensus views on the prognostic value of ploidy data for common tumours have recently been compiled[53]. Conflicting views have emerged as to whether ploidy data can predict survival or usefully guide adjuvant therapy, either independently or as part of a customized prognostic index. These differences of opinion may reflect both true biological differences between series of tumours and technical differences in analysis and data collection. In general terms there has been a failure to confirm any clinical utility of ploidy measurements in virtually all classes of malignancy, including the following common surgical tumours.

Colorectal tumours. Bauer *et al.*[54] have pointed out that, although more than 20 substantial series have reported that DNA ploidy has significant prognostic value in colorectal cancer, at least eight further series do not support this view. Most studies suggest a trend to DNA aneuploidy in higher Dukes stages, and in left-sided and rectal tumours. There are no consistent correlations with other clinicopathological measures, such as tumour grade or oncoprotein expression. Wide variation in methodology and reporting standards handicaps comparisons between studies, and Dukes staging remains the single most powerful prognostic indicator.

Breast tumours. An index that could identify aggressive behaviour in node-negative breast carcinomas and select these patients for further treatment would have clinical value. Hedley and colleagues[55] concluded that the extensive literature supports the view that diploid tumours carry a favourable prognosis relative to aneuploid, multiploid and hypodiploid primary tumours. However, the DNA index does not have independent prognostic significance. While some groups have been enthusiastic proponents of DNA measurements as part of a prognostic index, the status of lymph nodes remains by far the single most powerful prognostic factor. Flow cytometric DNA analysis, while being a valuable research tool with considerable unexplored potential in chromosome analysis, has not been proven to be clinically useful outside specialist centres.

Urological tumours. DNA cytometry has not found a role in the screening of urine for primary or recurrent bladder cancer[56] because the specificity of the technique is too low. DNA analysis of cells or tumour biopsies is less informative than conventional cytology or histopathology, and a further caveat is that irradiated tissue may show tetraploid features in the absence of recurrent disease. In the case of prostatic tumours[57] DNA diploidy is again generally associated with a favourable prognosis and response to antiandrogen therapy. However, this interpretation illustrates the problems of understanding the huge literature on DNA ploidy analysis faced by the general reader. Of the 126 papers relating to ploidy in series of prostate tumours, only 32 were ultimately felt to be suitable for inclusion in an authoritative consensus review on the basis of reasonable scientific and statistical criteria.

Haematopoietic tumours. A consensus review of lymphoma, myeloma and acute leukaemia by Duque *et al.*[58] failed to establish the clinical utility of DNA analysis. Of much greater value in these tumours is flow cytometric immunophenotyping to distinguish cell lineages on the basis of their cell-surface expression of various antigens. This technique is well established in clinical laboratories.

The S-phase fraction

The S-phase fraction is the number of cells or nuclei contained between the G0–G1 and G2 peaks of the profile, as a fraction of the total cell population. This is understood to be a measure of the size of the proliferating cell population. A simple measure of the size of the proliferating compartment might be of clinical value in estimating the proliferative activity of a tumour and hence in predicting its response to adjuvant therapy. It is also important to note that the S-phase fraction, being a static measurement at a single time point in the life of the tumour, gives no indication of the rate of cell production or of tumour volume growth. In the latter case, processes of cell loss also make an important contribution. The S-phase fraction, while having no specific relevance to aneuploidy, can be estimated directly from the flow cytometric DNA profile and hence is usually reported in tandem with ploidy data. There is a large parallel literature concerning the prognostic significance of this fraction, and the constraints that apply to the interpretation of ploidy data also apply to it.

There are two further problems that affect the interpretation of data on the size of the S-phase fraction. The first lies in the definition of the S phase. Because the G0–G1, S and G2 populations are overlapping gaussian distributions (which may be unpredictably skewed), it is difficult to define where the S phase begins in relation to G0–G1 on the DNA profile, and even more so to see where it ends. A number of mathematical models have evolved to address this issue, including overlapping curves and rectangular blocks. These methods are no more than estimates of the true S-phase fraction and can produce significantly different results when applied to the same tumour. Because the S-phase fraction is typically less than 10 per cent of a tumour population, error of a few percentage points can make a large difference to the stratification of a tumour, for example as rapidly or slowly proliferating according to the median S-phase fraction of a tumour population. This problem is hugely compounded in analysing aneuploid tumours, where the large G0–G1 aneuploid population usually overlaps the diploid S phase and the diploid G2 overlaps the aneuploid S phase.

Discussion

Aneuploidy may predispose to biological aggressiveness in tumours and hence to a poor clinical prognosis but, given the complexity and subtlety of cell biology, it is not obvious why

this should be so. Malignancy reflects many properties of the cell, including the altered regulation of cell proliferation and cell loss, the metastatic potential, immunogenicity and capacity for local invasion. Any of these processes might be affected by aneuploid transformation. Aneuploidy might even be expected to disrupt and diminish rather than enhance the proliferation and spread of cancer cells. If the aneuploid component were composed entirely of 'junk' or inactivated DNA, then the aneuploid state might be entirely neutral with regard to cell function.

Conversely, the state of diploidy cannot be assumed to predispose to a biologically benign character. Many purely diploid tumours are very aggressive. All aneuploid tumours appear to be a mixture of diploid and aneuploid tumour-cell lineages, although the diploid population usually comprises a significant proportion of stromal cells. It cannot be assumed that it is the aneuploid rather than the diploid clone that is dominant in determining the biological aggressiveness of the tumour.

To summarize the clinical picture, flow cytometry has identified the phenomenon of aneuploidy as a noteworthy abnormality in many tumours, but it does not reveal information about the future clinical behaviour of any one tumour. Indeed, in no tumour class does DNA analysis match the quantity and quality of information contained within conventional histological sections. While flow cytometry can measure up to six parameters in suspensions of up to 10^5 single particles, this information must be placed in context. The human eye can take in many more dimensions of data relating to millions of cells very rapidly from a pathology slide. These dimensions include size, shape, stain intensity, intracellular and extracellular spatial relationships, and characterization, to name but a few. In the absence of molecular or flow cytometric measures of the metastatic potential of a tumour, the microscopic or macroscopic observation of tumour dissemination to lymph nodes or elsewhere remains the most powerful prognostic tool.

The primary tumour is usually removed at surgery, but it is the biological behaviour of metastases that usually determines clinical outcome. Unfortunately, research data on DNA ploidy in metastases are relatively scarce compared with the many studies performed on primary tumours. The lack of comparative ploidy data between primary tumours and metastases is another limitation on the efforts to understand the relationship between DNA content and tumour aggressiveness.

In conclusion, pure diploidy appears to be only one of many cytogenetic states compatible with cell replication and survival, although it may reasonably be assumed that it has proved to be the most efficient state in any one species through evolution. It is important to recognize that diploidy and aneuploidy are part of a continuum from subtle DNA base-pair mutations to gross chromosomal abnormalities, rather than two distinct states of existence. It is not yet known which combinations of chromosomal abnormalities confer stability on an aneuploid cell. Indeed, the apparently random distribution of DNA aneuploidy seen across a spectrum of tumours could conceivably mask very precise multiples of genes, small subsets of DNA or chromosome fragments, appended to an otherwise normal chromosome set. Paradoxically, the very variety of DNA indices found in cancer cells may indicate that a relatively simple biological phenomenon underlies the genesis of aneuploidy. Aneuploidy may yet prove to be a wholly arbitrary description. Small increments or deletions in the number of gene copies and chromosome fragments may go undetected in some tumours because of the performance limits of instruments that are insufficiently sensitive to discriminate near-diploidy.

It is not known whether there is a causal relationship between aneuploidy and cancer, nor whether aneuploidy is merely an epiphenomenon. Any one of a spectrum of abnormal states of DNA content (DNA indices) may confer specific local survival advantage to clones of cells within a tumour during malignant growth. It is important to emphasize that aneuploidy is not synonymous with malignancy, biological aggressiveness or even premalignancy because specific forms of aneuploidy, such as Down's syndrome, are compatible with near-normal growth and physical development.

What of the future? The continued publication of large series of ploidy data seems unlikely to advance accurate clinical prognostication and therapy for cancer. Ploidy is still too crude a marker for the molecular changes that determine the biological aggressiveness of any one tumour. To advance understanding it will be necessary to measure more precisely the DNA and chromosome content in individual tumours, and particularly in those at the diploid and near-diploid end of the aneuploidy spectrum. Studies of DNA content should now be focused down the chromosomal and molecular scale using the new investigative tools. This will require the study of fewer cells in much greater detail and definition, so that it may be specified which chromosomes and fragments are multiplied in aneuploid tumours, and by how much. New technologies, such as fluorescence hybridization and high-speed flow cytometric chromosome analysis, should allow considerable progress in the next decade. Once the chromosomes or gene segments that are duplicated in aneuploid states can be defined, it will be much easier to identify the culprit genes and chromosome fragments that promote mutagenesis, and to address other questions. Why are these genes and chromosomes amplified? Do the molecular mechanisms determining aneuploidy provide new targets for adjuvant cancer therapy? As yet one may only speculate about the answers to these questions, but the study of aneuploidy has posed challenging new problems for tumour biologists.

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