Review article

Clinical and biological significance of aneuploidy in human tumours

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SUMMARY Aneuploidy is a well recognised feature of human tumours, but the investigation of its biological and clinical significance has been hampered by technological constraints. Quantitative DNA analysis reflects the total chromosomal content of tumour cells and can now be determined rapidly and reliably using flow cytometry; this has resulted in renewed interest in its potential clinical applications. This article reviews the accumulating evidence that tumour ploidy reflects the biological behaviour of a large number of tumour types and that diploid tumours in particular have a relatively good prognosis. The measurement of tumour ploidy is likely to become a valuable adjunct to the clinical and histopathological assessment of cancers.

Recent advances in the field of molecular genetics suggest that a critical early step in the initiation of at least some cancers is a chromosomal rearrangement which alters the expression of certain structural genes, the so called *c-onc* genes, the result being a change in the cell's responsiveness to normal growth control mechanisms.¹⁻⁷ Karyotypic abnormalities have long been recognised in established human cancers,⁸ and here the chromosomes that are most commonly affected contain some of the genes regulating metabolism and nucleic acid synthesis.⁹⁻¹² The gains or losses of such chromosomes alter the amount of specific cell products,¹³ and if these are critical for the control of cell division and differentiation the resulting cells may achieve a proliferative advantage. Furthermore, genetic rearrangements may increase the rate of spontaneous mutation in a cell line¹⁴ and allow the more rapid progression to a highly malignant phenotype.^{15 16}

The haematological malignancies have proved to be the most productive area for studying cytogenetic abnormalities in human cancer.¹⁷⁻²¹ Major landmarks include the demonstration of a specific marker chromosome for chronic myeloid leukaemia,^{22 23} the finding that certain chromosomal

abnormalities or defects of DNA repair are associated with an increased susceptibility to acute leukaemia,^{24 25} and, more recently, the identification in some patients with Burkitt's lymphoma of an 8:14 translocation involving the homologue of a known mouse oncogene, *c-myc*.²⁶ When applied to the more common carcinomas, cytogenetics has been less successful; a major technical problem is the scarcity of assessable chromosome spreads.²⁷ A more fruitful approach has been the cytometric measurement of total cellular DNA content. With the exception of some highly specialised research methods²⁸ cytometry does not give any information about detailed chromosome structure. But it has the advantages of being applicable to interphase cells and of allowing sufficient cells to be examined for statistical analysis of cell cycle phase distribution.²⁹ Early studies using static cytometry showed a high incidence of abnormalities of cellular DNA content in the common solid tumours,³⁰ while the more recent introduction of flow cytometry has made routine examination of clinical samples a practical proposition.^{29 31} Numerous studies correlating abnormalities of cellular DNA content with clinical behaviour have either been reported or are in progress, and because this interest coincides with major advances in understanding the genetic basis of cancer it is timely to review the findings.

Material and methods

There are a number of comprehensive reviews which adequately cover the practical and technical aspects of flow cytometry,^{29,31} static cytometry,^{32,33} and chromosomal analysis.^{34,35} We will therefore cover only briefly the basic principles and advantages and disadvantages of these different techniques.

CHROMOSOMAL ANALYSIS

Chromosome studies require that the tumour is initially disaggregated by mechanical or enzymatic techniques. The resulting single cell suspension is then exposed to a mitotic inhibitor, swelled in a hypotonic solution, and fixed, and then the chromosomes are spread on glass slides. The interaction of alkylating fluorochromes (for example, quinacrine mustard) and histochemical stains (for example, Giemsa) with specific regions of chromosomes produces multiple characteristic bands which can be identified on the metaphase chromosomes.³⁶ Recent refinements using high resolution banding techniques have shown that these wide dark and light bands are composed of a number of narrower bands, which further enhance the identification of chromosomal rearrangements in cells.³⁷

The normal human somatic cell with 46 chromosomes (23 pairs) is referred to as diploid,³⁸ while a cell with fewer or more than 46 chromosomes is described as aneuploid (hypodiploid or hyperdiploid, respectively) and a cell with a balanced gain or loss of chromosomes, or with structural rearrangements, that still has 46 chromosomes is pseudodiploid. Most established tumours can be characterised by a modal chromosome number which tends to fall into either a near diploid or triploid-tetraploid mode. Within the diploid range, chromosome numbers can vary from 40 to 50, but malignant tumours can have the normal diploid component of 46 chromosomes.³⁹ High chromosome numbers may be achieved by a doubling of the complete chromosome set through several mechanisms such as non-dysjunction, endoreduplication, or cell fusion.⁴⁰ Tumours with a modal DNA number in the triploid-tetraploid range do not always have marker chromosomes present in duplicate, which suggests that chromosome loss has occurred subsequent to doubling.⁴¹

Although chromosomal analysis is applicable to leukaemias, the analysis of solid tumours poses more difficult problems and in only 10–20% of cases can interpretable chromosome spreads be obtained. For this reason there are few detailed karyotypic studies of human solid tumours, and the published data tend to be biased towards those tumours which are technically favourable—that is, those which have

a high mitotic rate and yield a proportion of well spread metaphases.⁸

QUANTITATIVE DNA ANALYSIS

Identification of individual chromosomes is possible only during metaphase, whereas cellular DNA content can be determined on interphase cells independent of the proliferative activity of the tumour.²⁹ The detection of a chromosomal abnormality by means of cellular DNA content is a function of its relative frequency within the population studied, the amount of aberrant chromosome material involved, and the resolution of the technique used. There may be a wide scattering of chromosome numbers about a modal number in certain tumours that have constant DNA packaging,⁴² but technical factors such as disruption of metaphases during chromosome spreading should also be taken into account⁴³ because a linear relation between chromosome number and DNA content has often been found.^{43–47}

Quantitative measurement of cellular DNA content has been by one of two procedures. The Feulgen-Schiff technique entails removal of the purines from DNA by acid hydrolysis and the subsequent reaction of the exposed aldehyde groups of deoxyribose sugars with aldehyde specific Schiff reagents. Such reagents may be strongly coloured, such as pararosaniline, or fluorescent, such as acriflavine.^{48,49} The second method of measuring cellular DNA content relies on the use of fluorescent dyes which bind directly to DNA. Some of these, such as ethidium bromide and propidium iodide, intercalate with double stranded nucleic acids and therefore require the removal of RNA; others, such as DAPI (4',6'-diamidino-2-phenylindol-dichloride) and mithramycin, react with specific moieties in DNA.²⁹ All these stains bind to normal DNA stoichiometrically—that is, the degree of staining is directly proportional to the amount of DNA. The DNA content of individual cells can then be measured using either static cytometry or, more recently, flow cytometry.

STATIC CYTOMETRY

Both the above staining techniques have been employed in static cytometry, in which stained single cells are visualised by a microscope based system and the amount of DNA bound dye measured directly by absorption or fluorimetric means.^{48–50} Fig. 1 shows the distribution of the cellular DNA content of a tumour obtained in this way. This technique is applicable to the analysis of histological sections of formalin fixed tissue or fixed smear preparations from tumours, where the DNA content of morphologically identified tumour cells can be determined. Leucocytes and normal epithelium pre-

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Fig. 1 DNA distribution pattern of a near diploid and hyperdiploid tumour obtained using static cytometry. The DNA values are expressed in relation to the median of the DNA values ($2n$) of the internal diploid control cells. The resolution of this technique and the number of cells analysed should be compared with the histograms obtained using flow cytometry in Fig. 2.

sent in the tissue section are used as a normal diploid control. Owing to the relatively slow data acquisition rates of this procedure, however, measurement of DNA content is usually restricted to several hundred cells in any one sample.

FLOW CYTOMETRY

In contrast to static cytometry, flow cytometry is characterised by high precision and speed of sample handling.²⁹ A single cell or nuclear suspension prepared from a sample of tumour is stained with DNA specific fluorochromes and examined at rates of about 1000 cells per second.⁵¹⁻⁵² Typical DNA histograms containing between 10 and 30 000 cells are shown in Fig. 2. In this instance chicken red blood cells were used as an internal standard to identify the diploid peak. A number of different standards such as human lymphocytes or trout erythrocytes can be used.⁵³⁻⁵⁴

CELLULAR DNA CONTENT

Most solid tumours contain a population of cells with a diploid DNA content,⁵⁵ and when this is the only cell population present the tumour is classified as diploid. Tumours which have evidence of an additional G_1 peak are classified as aneuploid, and the diploid peak is assumed to comprise host cells such as lymphocytes and stroma. In most instances this assumption holds true, although there are occasions where in aneuploid tumours the diploid component may also contain malignant cells.⁵⁶⁻⁵⁷ Most aneuploid tumours contain only a single aneuploid G_1 peak, but when there is clear evidence of more than one

Fig. 2 Representative DNA histograms obtained from solid tumours using flow cytometry. Channel number represents relative fluorescence intensity, which is directly proportional to DNA content. Numbers of cells are shown on the ordinate. Chicken red blood cells (crbc) are used as an internal biological standard to identify the diploid G_1 peak (G_{1D}) (see text). Most tumours contain a population of cells with diploid DNA content and when this is the only population present the tumour is classified as diploid (a). Tumours which have evidence of an additional G_1 peak (G_{1T}) are classified as single aneuploid (b), and when there is clear evidence of more than one tumour G_1 peak the tumour is classified as multiploid (c).

aneuploid G_1 peak the tumour is considered to be multiploid. These terms are not synonymous with chromosome number, and although in most instances a good correlation exists between chromosome number and cellular DNA content,⁴³⁻⁴⁷ the resolution using flow cytometry is such that gains or losses of one or two chromosomes would not be detected.⁵⁸

The varying degrees of aneuploidy can be quantitated by the DNA index, which represents the ratio of the DNA content of tumour G_1 cells to the diploid G_1 peak. Thus a DNA index of 1 is synonymous with a diploid ($2n$) DNA content and a DNA index of 1.5 represents cells with a triploid ($3n$) DNA content.⁵⁵

Studies in a variety of tumour types have shown a bimodal distribution of DNA content, with tumours having either a near diploid mode or a triploid-tetraploid mode⁵⁵⁻⁵⁹ (Fig. 3). The interpretation of cellular DNA content varies considerably in different studies, with some investigators classifying those tumours with a DNA index of less than 1.5 as near diploid and tumours which have a DNA index of greater than 1.5 as hyperdiploid. While this fairly crude cut off was required with static cytometry the improved resolution with flow cytometry makes it possible to determine the biological importance of diploid tumours (DNA index = 1), and tumours containing multiple cell lines with different ploidies can be better identified.

With few notable exceptions, the available data suggest that most tumours exhibit stability of ploidy both spatially and temporally.⁵⁵⁻⁵⁹⁻⁶³ Regional variations in tumour ploidy do occur in colonic cancer,⁶⁴ small cell lung cancer,⁶⁵ melanoma,⁵⁷ and brain tumours.⁶⁶ Chromosomal studies have, in general, supported these flow cytometric findings, with the finding of mosaicism in certain tumours and relative karyotypic stability in the majority of tumours.⁸ The genesis of a completely new karyotype is extremely uncommon, and when changes are evident they are usually minor with the basic karyotypic picture remaining unchanged.³⁹

There is a good correlation between the DNA content as determined with static cytometry and flow cytometry,⁶⁷⁻⁶⁸ and studies of the cellular DNA content of solid tumours determined with both methods will be reviewed. Because static cytometry is applicable to paraffin embedded tumour tissue a number of studies have been done on groups of patients in whom the outcome was known, whereas flow cytometry has been restricted to fresh tumour tissue with long term follow up necessary to determine the prognostic importance of cellular DNA content. Recently, however, a flow cytometric method for determining cellular DNA content of paraffin embedded tissue has been described⁶⁹

Fig. 3 Frequency histogram showing the bimodal distribution of tumour ploidy in breast cancer. Ploidy measurements were made using flow cytometry and are expressed as DNA index, which represents the ratio of the DNA content of tumour G_1 cells to the diploid G_1 peak. A DNA index of 1 is synonymous with a diploid ($2n$) DNA content.

which, like static cytometry, allows retrospective analysis of tumours from groups of patients whose outcome is known, but with the added advantage that large numbers of cells can be analysed rapidly.

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HAEMATOLOGICAL MALIGNANCIES

Chromosomal analysis is now recognised to be of value in the management of patients with haematological malignancies¹⁻²¹⁻⁷⁰⁻⁷¹ and is particularly useful in the diagnosis of chronic granulocytic leukaemia²²⁻²³ and Burkitt's lymphoma¹ and in the identification of different prognostic subsets within the acute leukaemias.²⁰⁻⁷⁰⁻⁷² Only the studies relating to ploidy will be presented here, however, as there is a large number of excellent and comprehensive reviews on the cytogenetics of leukaemias and lymphomas.¹⁻⁸⁻²¹⁻⁷⁰⁻⁷¹⁻⁷³

Aneuploidy occurs in up to 20% of cases of acute myelocytic leukaemia and 30% of cases of acute lymphocytic leukaemia, which is somewhat lower than the reported incidence using chromosome

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analysis and banding.^{19 70 71 74-76} Notwithstanding, the flow cytometric determination of tumour ploidy has been reported to be a useful investigation in acute leukaemia.⁷⁴⁻⁷⁸ In childhood acute lymphoblastic leukaemia there is a significant association between tumour ploidy and other recognised prognostic features such as the expression of differentiation antigens, age, sex, and initial white cell count.⁷⁶⁻⁷⁸ Patients with aneuploid acute lymphoblastic leukaemia have a better prognosis, an improved response rate to chemotherapy, and a longer duration of remission than those patients with diploid acute lymphoblastic leukaemia.^{77 78} Similar findings have been reported from chromosome studies in acute lymphoblastic leukaemia, where patients with more than 50 chromosomes detected in the marrow had a significantly improved prognosis.^{20 79} In contrast, in acute myelocytic leukaemia the presence of aneuploidy is associated with a relatively bad prognosis and a lower response to chemotherapy.^{72 80}

Aneuploidy is evident in 50-70% of patients with multiple myeloma, and preliminary evidence in relatively small numbers of patients suggests that ploidy is an independent prognostic variable: patients with diploid myelomas survive significantly longer than those with aneuploid myelomas.⁸¹⁻⁸³ The relation between ploidy and response to treatment is not clear, and the improved survival of patients with diploid tumours may relate to their relative biological indolence rather than to their inherent chemosensitivity.

There are numerous problems with the morphological diagnosis of lymphomas, and it has been proposed that the flow cytometric analysis of cellular DNA content may be a useful adjunct to their clinicopathological assessment, particularly in the case of the non-Hodgkin's lymphomas.^{19 84} There is an incidence of aneuploidy of up to 90% in the high grade lymphomas, while low grade lymphomas are more commonly diploid. A number of prospective studies are presently investigating the relation between ploidy and prognosis in the non-Hodgkin's lymphomas.^{19 75 84 85} Aneuploidy is evident in 70% of patients with mycosis fungoides and Sézary syndrome and is associated with the presence of adverse clinical features and a poor prognosis.⁴⁵ Patients with aneuploid tumours are more likely to have advanced disease and early relapse, while patients with diploid tumours have a more indolent clinical course and significantly prolonged survival.

SOLID TUMOURS

Breast cancer

The ploidy of large numbers of breast tumours has been determined with static cytometry, flow cytometry, and chromosome analysis. All techni-

ques have shown that there is a non-random distribution of ploidy with most tumours having either a near diploid or triploid-tetraploid mode, while a few have two distinct aneuploid populations (multiploidy).⁸⁶⁻⁹⁰ The general stability of cellular DNA content within different regions of a primary tumour and its metastases, regardless of location or time of occurrence,^{60 61} supports the hypothesis that breast cancer has a monoclonal origin. This finding is also of practical importance as a single estimation of ploidy is likely to be representative of the tumour as a whole. There have been no consistent chromosomal abnormalities described in breast cancer, apart from frequent changes in chromosome 1.⁸ Chromosome 1 is, however, the largest human chromosome and has a distinctive banding pattern so that changes in its structure can easily be observed, which probably accounts for why it is reported abnormal in so many human cancers.⁹¹

Depending on the technique used, aneuploidy has been reported in 50-80% of breast tumours and appears to be independent of tumour stage, size and nodal status.^{86-88 92} An association between tumour ploidy and steroid receptor status has been shown, with near diploid tumours tending to be oestrogen receptor positive, but in most studies this has not achieved statistical significance.^{87 88 92-95} It has been suggested that DNA measurements could be of value in predicting the sensitivity of breast tumours to endocrine treatment.^{93 95} There is some evidence to support this hypothesis in prostate cancer,^{96 97} but not as yet in breast cancer. It has been postulated that the prevalence of multiploidy between the ages of 40 and 60 is a possible reason for the well known refractoriness of recurrent breast cancer to endocrine treatment in perimenopausal women, as the probability of these tumours containing a hormonally insensitive subclone may be high.⁸⁸

An association between tumour ploidy and differentiation has been described, with well differentiated tumours tending to be diploid and poorly differentiated tumours aneuploid.^{87 98} It is possible that tumour ploidy may be an alternative to the currently used nuclear grading systems because it is objective and reproducible.

Benign breast tumours have a diploid DNA content,^{87 92} but tumour ploidy cannot be used as an absolute criterion to distinguish between benign and malignant tumours as the latter may also be diploid. The finding, however, of aneuploidy in so called benign breast tumours has been reported in a small number of patients who later developed invasive breast cancer^{99 100} and the value of DNA estimations in potentially premalignant breast lesions should be further studied.

There is evidence using static cytometry to show that cellular DNA content reflects the biology of

breast cancer and is of prognostic importance. Atkin showed that patients with near diploid tumours had significantly better eight year survival rates than those with high ploidy tumours.⁸⁹ Auer recently confirmed these results, showing a significant correlation between DNA content and survival in patients with stage I and II breast cancer.⁸⁶ His data, analysed by subclassifying the DNA patterns of breast tumours into four groups, showed that patients with diploid or tetraploid tumours had a significantly better long term survival than patients with either aneuploid tumours or tumours with a high proliferative fraction. In both these studies it was suggested that ploidy was an independent prognostic variable and unrelated to clinical stage or axillary nodal status.

There are no published flow cytometric studies with sufficient follow up to confirm these results. Using a recently developed flow cytometric method which can be applied to archival, paraffin embedded tissue⁶⁹ we have examined 151 primary tumours from patients with stage II—that is, axillary lymph nodes positive—breast cancer who were entered into the Ludwig Institute for Cancer Research I-IV adjuvant breast trials. Preliminary analysis shows an improved prognosis for the patients with diploid tumours, since their disease free survival curve appears to plateau at two years, whereas patients with aneuploid tumours have the continuing risk of relapse characteristic of breast cancer.¹⁰¹ If this two year plateau is real it implies that the diploid tumours are either less aggressive or more responsive to adjuvant therapy, and this important question should be resolved when the target accrual of several hundred paraffin blocks have been examined.

It appears that cellular DNA content will be a valuable adjunct in the assessment of the biology of breast cancer. Long term follow up of a number of studies is awaited, but the association of diploid/near diploid tumours with other recognised prognostic variables such as tumour grade, oestrogen receptor expression, and proliferative fraction provides circumstantial evidence of their relative biological indolence.

FEMALE GENITAL TRACT

Ovary

A number of prognostic variables have been recognised in ovarian cancer.¹⁰² Their relative importance, however, particularly in advanced disease, has not been well clarified, and treatment decisions are usually made on the basis of stage alone.¹⁰³

Recently, a consistent translocation between chromosome 6 and 14 has been reported in serous papillary cystadenocarcinoma of the ovary.¹⁰⁴ Marker chromosomes have also been found in over

70% of tumours and structural rearrangements have been shown in chromosomes 1, 3, 4, 6, 9, 10, 15, and 19, with chromosome 1 being abnormal in up to 40% of ovarian tumours^{8 105} (for review see reference 106). The significance of these chromosomal abnormalities remains uncertain.

In contrast, there is evidence that the cellular DNA content reflects the biology of ovarian epithelial tumours and provides clinically relevant information.^{107–111} There is a bimodal distribution of ploidy with a clustering of tumours about a diploid or triploid-tetraploid mode,¹⁰⁸ while about 15% of tumours are multiploidal.^{109 110} As in breast cancer, ploidy is in most cases a stable marker in ovarian cancer exhibiting consistency within different sites of the primary tumour and metastases and during subsequent tumour progression.⁶² There is an important association between stage and tumour ploidy, with early stage tumours tending to be diploid while more advanced tumours are usually aneuploid.^{108 109}

In a recent study of 91 patients with advanced ovarian cancer ploidy was determined by flow cytometric analysis of paraffin embedded tumour blocks: 69% of tumours were aneuploid and 31% were diploid.¹¹⁰ Patients with diploid tumours (mean survival = 123 weeks) had a significantly longer survival than those with aneuploid or multiploid tumours (median survival 52 and 63 weeks, respectively), and multivariate analysis showed that tumour ploidy was the major determinant of survival ($p < 0.0001$). Preliminary data suggest that this also holds true in early stage ovarian cancer,¹¹² where the ability to predict patient outcome accurately could have important therapeutic implications. Studies are underway to determine whether this influence of ploidy on prognosis is a reflection of natural history or response to treatment.

Tumour grade is believed to be an important prognostic variable, particularly in early stage ovarian cancer,¹⁰² but there are conflicting views regarding its relation to tumour ploidy.^{108 109 113} This probably reflects the inherent problems in the subjective grading of ovarian tumours, and an objective grading system based on DNA content may be more appropriate.

Borderline ovarian tumours (tumours of low malignant potential) account for 15% of ovarian tumours and as a group are characterised by an indolent biological behaviour even when spread beyond the ovaries occurs.¹¹⁴ Most long term survivors with advanced stage ovarian cancer have been shown to have had borderline tumours,¹¹⁵ and the correct diagnosis therefore has important prognostic and therapeutic implications. The diagnosis can, however, be difficult even for the experienced gynaecological pathologist, and there is evidence

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that the determination of cellular DNA content may be a useful adjunct to the histopathological diagnosis of borderline malignancy. Most borderline tumours are diploid, and the finding of aneuploidy could imply an aggressive biological behaviour.^{111 116}

UTERINE CERVIX

As with ovarian cancer, stage is generally accepted to be the most important prognostic factor in cervical cancer.¹¹⁷ Only a few attempts have been made to study cervical cancers with banding techniques and to date no consistent chromosomal abnormality has emerged.¹¹⁸ Using static cytometry, Atkin reported a worse prognosis for the near diploid group in patients with squamous cell carcinoma of the cervix,^{108 119 120} but these findings were not significant on application of multiple regression analysis and have not been confirmed. The results of a flow cytometric study by Jakobson involving 171 patients with squamous cell carcinoma of the cervix showed that patients with low ploidy tumours (DNA index <1.5) had a significantly better prognosis than those with high ploidy (DNA index >1.5) tumours.¹²¹ The distribution of ploidy was not correlated with stage, and there was a significantly higher recurrence frequency in patients with high ploidy tumours in all stages investigated. As it is particularly difficult to determine response to radiotherapy, it was not clear if the difference in recurrence rate and overall survival in this study was due to an increased radiosensitivity of low ploidy tumours.

There is a significant correlation between DNA index and the incidence of pelvic lymph node metastases in patients with stage IB and IIA tumours, with high ploidy tumours having a higher likelihood of metastasising.¹²² This finding could have important implications in terms of the choice of treatment and deserves further study in larger numbers of patients.

In a study of intraepithelial neoplasms Jakobson has shown that flow cytometry may be a useful adjunct to histopathological classification since cells exhibiting mild to moderate dysplasia were invariably diploid while areas of severe dysplasia and carcinoma in situ were usually aneuploid, with a frequency approaching that of invasive cervical cancer.¹²³ These findings are in accordance with cytogenetic investigations, which showed only 29% of cases of carcinoma in situ had a chromosome number in the diploid range.¹²⁴ The presence of aneuploidy indicates an increased likelihood of progression to invasive cancer and it has been suggested that lesions with an aneuploid pattern should be considered to be precursors of invasive cervical cancer.¹²⁵

The prognosis of adenocarcinoma of the cervix is related predominantly to clinical stage and to a lesser extent to histological type, growth pattern, and

degree of differentiation.¹²⁶ Early stage tumours and better differentiated tumours tend to be of low ploidy, but even within tumours of a similar stage and differentiation, low ploidy tumours have a considerably better prognosis than high ploidy tumours.¹²⁷ Similar studies of clear cell carcinoma of cervix and vagina have also shown that clinical stage is the over-riding prognostic factor, and although the results in a small group of patients with clinically low stage tumours suggested that the prognosis was slightly better if ploidy level was low rather than high, the finding was not statistically significant.¹²⁸

ENDOMETRIAL CANCER

There have been few studies of chromosomes or cellular DNA content in endometrial cancer, but the data that are available show that near diploid tumours have a considerably better prognosis than those with high ploidy.¹¹⁹ Most endometrial tumours are near diploid, and although the ratio of well differentiated to poorly differentiated tumours is greater in the near diploid group, the difference in prognosis cannot be solely related to the degree of differentiation.^{119 129}

PROSTATE

A characteristic feature of prostatic cancer is the great variation in biological behaviour, ranging from latent carcinoma to highly aggressive metastasising carcinoma; this wide clinical range is only partly reflected in the morphological features.¹³⁰ Detailed chromosome analyses of human prostatic cancer, either primary or metastatic, are lacking.⁸ Retrospective studies using static cytometry have shown that diploid tumours have a considerably better prognosis than aneuploid tumours^{96 131} and that diploid tumours also have a higher response probability to oestrogen treatment, which may explain their different biological behaviour.^{96 97}

The DNA content of prostatic tumours can now be estimated using fine needle aspirates, which provide material well suited to flow cytometry^{132 133}; a number of prospective studies are underway. Although well differentiated tumours tend to be diploid and poorly differentiated tumours tend to be aneuploid, about half of the moderately differentiated tumours are diploid and half aneuploid.^{133 134} This finding is of particular interest since moderately differentiated tumours are common, and are clinically recognised to be heterogeneous with regard to survival and response to treatment.

Tetraploid populations are common in prostatic carcinomas, but the clinical importance is unknown, as polyploidisation may be a normal feature in certain tissues.¹³³ The presence of multiploidy has been thought to be evidence of tumour aggressiveness, and this is supported partly by the finding that 22%

of poorly differentiated tumours have evidence of multiple cell lines while no well differentiated tumours exhibit multiploidy.¹³⁴

BLADDER CANCER

Clinical and morphological parameters alone are not sufficient to assess the possible outcome of patients with bladder tumours.¹³⁵ Attempts to identify new and objective prognostic variables have led to a number of studies of chromosomal analysis and cellular DNA content. The results of the first comprehensive study of chromosomes in bladder cancer, which appeared in 1967, indicated a relation between chromosome number, histological appearance, and invasiveness of the tumour.¹³⁶ Non-invasive tumours had a near diploid mode, while poorly differentiated tumours tended to have a modal chromosome number in the triploid-tetraploid range. These findings have been confirmed by other studies, which have also indicated that marker chromosomes have potential prognostic value and their presence in papillary tumours is associated with high likelihood of recurrence.^{137 138}

Similar findings have been reported with quantitative DNA analysis using static cytometry. Tumour ploidy has been shown to correlate well with biological behaviour, patients with diploid tumours having a survival advantage.^{96 139} A number of large prospective flow cytometric studies are currently underway, and interim findings relating ploidy to tumour grade, stage, and recurrence rate have been reported.^{135 140-142} Grade 2 (moderately differentiated) tumours constitute a heterogeneous group with respect to survival and are also heterogeneous with respect to DNA content. About 66% of grade 2 tumours are diploid and 34% are aneuploid, while grade 1 tumours are usually all diploid and grade 3 tumours invariably aneuploid.¹³⁵ The DNA pattern, histological grade, and recurrence rate have been studied in superficial bladder tumours.^{141 142} Progression occurred in tumours with a triploid DNA content, rarely in tetraploid tumours, and not at all in diploid tumours. These results show that superficial bladder tumours can be well characterised by their DNA profiles. There is a significant association with tumour ploidy and stage (TNM classification), with about 60% of T₁ tumours being diploid while T₂-T₄ tumours are usually aneuploid and are more likely to contain multiple aneuploid cell lines.¹³⁵ In a small study of 20 patients with in situ primary carcinoma of the urinary bladder aneuploidy was found in all cases. In seven of these more than one aneuploid cell line was evident and in all these cases progression occurred within two years.¹⁴⁰

Bladder tumours are often multifocal in origin and can have different histological appearances in different areas.¹⁴³ The incidence of intratumoral var-

iations of ploidy and the possible variations in different sites have not, however, been widely studied.¹⁴⁴

As the clinical management of transitional cell tumours of the urinary bladder is strongly dependent on the correct histopathological grading, which is associated with both inter- and intraindividual discrepancies,^{145 146} it is likely that determination of cellular DNA content will find a place as an adjunct to the histopathological assessment of bladder cancer.

RENAL CANCER

In renal cancer ploidy is in most cases a stable tumour marker, with little variation occurring within different sites of the primary tumour. It has been estimated that by taking two samples from different regions the probability of missing a hyperdiploid population is less than 1%.⁶³ Cell populations with an aneuploid DNA content were detected in 46% of renal carcinomas, and the comparison of ploidy with histological grade showed that most highly malignant grades are aneuploid while 45% of the hyperdiploid and 89% of diploid tumours are of a low grade. These initial results indicate that aneuploidy might provide useful prognostic information, as after follow up of six months to two years, 47% of patients with hyperdiploid tumours and only 9% of patients with diploid tumours died or developed multiple metastases.⁶³ As the tumour ploidy is not strictly correlated with nuclear morphology it has been suggested that prognosis might be determined more accurately using a grading system based on both ploidy and histological grade.^{147 148}

LUNG

Aneuploidy is common in epidermoid carcinoma, adenocarcinoma, and small cell lung cancer, and a typical bimodal distribution is common to all; ploidy levels tend to be higher in epidermoid cancer than in adenocarcinomas.^{65 149-151} A relatively high incidence of clonal heterogeneity has been reported in small cell lung cancer, with up to 30% of tumours having evidence of two aneuploid clones.⁶⁵ Unlike breast cancer and ovarian cancer, there are commonly differences in ploidy between the primary tumour and metastases. This "genetic instability" or clonal heterogeneity may account for the rapid emergence of drug resistance in a tumour type which is characterised by high response rates to chemotherapy but a low percentage of long term survivors.⁶⁵ The finding of a stable hypodiploid/near diploid clone in small cell lung cancer is associated with a better prognosis, but as these patients also tend to have limited disease the value of ploidy as an independent prognostic variable has not been shown.¹⁵⁰ The reason for the improved prognosis may reflect drug response: unimodal hypodiploid/

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diploid small cell lung cancers have been reported to have a longer duration of response and better survival than patients with hyperdiploid tumours.¹⁵² Although there are few chromosomal studies reported in lung cancers, the data that are available also suggest that hypodiploidy is a feature of some small cell lung cancers. A specific chromosomal finding (3p-) has also been noted in small cell lung cancer but its importance is unclear.¹⁵³

The assessment of tumour ploidy as an adjunct to the histopathological distinction of carcinoid tumours from small cell lung cancer may be useful as carcinoid tumours are almost invariably diploid and have an indolent behaviour.¹⁵⁰

Epidermoid carcinomas and adenocarcinomas of the lung have been reported to have a better prognosis if they exhibit a near diploid DNA content.^{154 155} But this is an area where larger numbers of patients should be studied to determine whether ploidy is an independent prognostic variable.

COLONIC CANCER

The behaviour of colonic cancer is predicted mainly on the basis of clinico-pathological staging and histological grading.¹⁵⁶ Recent studies based on a relatively small number of patients have suggested that the determination of DNA distribution in colon carcinomas may be of prognostic value.^{157 158} Patients with diploid tumours have a considerably better prognosis than those with aneuploid tumours, independent of histological grade and Dukes' stage.¹⁵⁸ Other biological differences between diploid and aneuploid colonic carcinomas have also been shown: the production of carcinoembryonic antigen is more common in aneuploid tumours while the release of the secretory component of IgA is more common in diploid tumours.¹⁵⁹ Colonic carcinomas are commonly heterogeneous with respect to DNA content, with up to 30% of tumours showing regional variations in ploidy independent of histological differentiation⁶⁴; the biological importance of this is not

MELANOMA

There are few data available relating ploidy and survival in melanoma. Aneuploidy has been reported to be present in 70–90% of malignant melanomas, with up to 30% of cases having multiple aneuploid cell populations.^{57 160} A considerable variation in chromosome numbers has similarly been found between cells within the same tumours.¹⁶¹ There is a significant correlation between aneuploidy and melanoma thickness, but no such correlation with level of invasion or histological type.¹⁶⁰ In one study of patients with localised and regional disease more patients with aneuploid tumours died than those with diploid tumours, and multiploidy was associated with a particularly high mortality. These

findings did not achieve statistical significance, however, possibly because of the small numbers of patients in the study.¹⁶⁰

BRAIN TUMOURS

DNA distribution has been studied in both malignant and benign brain tumours, but its biological importance remains unclear.^{66 162–166} There is some evidence that DNA content is not a stable marker and may vary within different regions of a tumour and change on recurrence.⁶⁶ It is tempting to speculate that the relatively common occurrence of heterogeneity of tumour ploidy within brain tumours, colonic cancer, small cell lung cancer, and melanomas reflects their biological behaviour, which is usually characterised by either intrinsic chemoresistance or by the rapid emergence of drug resistance. There are conflicting results with respect to the relation of ploidy to grade, and while diploid modal values are evident in most benign brain tumours it has been reported that poorly differentiated gliomas may also have a diploid or near diploid DNA mode.^{162 165} Aneuploidy is common in malignant brain tumours, but no obvious correlation between DNA pattern and prognosis has been found.¹⁶⁴ This finding may reflect the fact that small numbers of patients with a wide variety of brain tumours have been analysed together, and in many instances the techniques used had a low resolution in discriminating diploid tumours from aneuploid tumours.

Meningiomas are usually considered to be histologically benign and in most instances have a diploid DNA content.^{163 166} Chromosomal analysis and banding has been performed in a large number of meningiomas, and monosomy 22 is reported to be a frequent and specific finding, although the clinical importance of this is unclear.¹⁶⁷

BONE TUMOURS

A number of studies using absorption cytophotometry to determine cellular DNA content of both osteosarcomas and chondrosarcomas have shown that tumour ploidy reflects the biological behaviour of bone tumours.^{168 169} In a retrospective study of 45 patients with chondrosarcomas, patients with diploid tumours had a significantly better prognosis than patients with aneuploid tumours regardless of location, size of tumour, histological grade, or treatment.¹⁶⁹ Of patients with diploid tumours 81% survived 10 years, compared with 29% of the patients with aneuploid tumours, and it seems that ploidy determinations may complement conventional histopathological and clinical assessment of the biological behaviour of chondrosarcomas.

The histological diagnosis of osteosarcoma may sometimes present considerable difficulties, and the distinction between high grade osteosarcomas from

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Tumour type	Survival advantage for diploid tumours	Stage	Ploidy correlates		Other	References
			Histological subtype	Histological grade		
Solid tumours						
Breast	+	-	+	+	Receptor expression	86-89, 92-95, 101
Ovary*	+	+	-	±	Borderline malignancy	107-111, 113, 116
Cervix†	+	-	-	-	Lymph node spread	121-123, 127
Endometrium	+	-	-	+		119, 129
Prostate	+	-	-	+	Hormone responsiveness	96, 97, 131-135
Bladder	+	+	-	+		96, 139-142
Renal§	+	-	-	+		63, 147, 148
Lung§	+	+	-	-		149-152, 154, 155
Colon§	+	-	-	-	Carcinoembryonic antigen production	156-159
Melanoma§	-	-	-	-		57, 160
Brain§	-	-	-	-		66, 162-166
Bone	+	+	+	+		168, 169
Haematological						
Acute lymphoblastic leukaemia‡	-				Immunological phenotype	76-78
Acute myelocytic leukaemia	+					72, 80
Myeloma*	+	+	-	-		81, 83
Mycosis fungoides	+	+				45
Sezary's syndrome						
Lymphomas§	±		+	+		19, 75, 84, 85

*Ploidy shown to be an independent prognostic variable by multivariate analysis.

†Conflicting data—early study showed aneuploid tumours to have a better prognosis (although not significant).¹⁰⁸

‡Patients with aneuploid tumours have significantly better survival than those with diploid tumours.

§Intratumoural heterogeneity of ploidy relatively common.

¶Follow up too short for definite conclusions.

+ = positive correlation.

- = no correlation.

low grade indolent parosteal variants can be difficult.¹⁷⁰ The analysis of tumour ploidy may be valuable in this setting, as has been shown in a small study where parosteal variants were diploid and classic osteosarcomas were aneuploid.¹⁶⁸

PHEOCHROMOCYTOMAS

Malignant pheochromocytomas are rare tumours which are difficult to diagnose, and it has been claimed that the only absolute criterion for diagnosis is the presence of secondary deposits.¹⁷¹ Benign pheochromocytomas have a diploid DNA content¹⁷² while malignant tumours have an aneuploid DNA content, and it is likely that determination of cellular DNA content will enable the biological behaviour of this rare group of tumours to be determined.

CONCLUSIONS

The cytogenetic analysis of solid tumours has until recently found little practical application, and clinical and histological criteria have largely been relied on to assess the biological properties of a tumour and to predict the patient's outcome. It has been suggested for many years that both chromosomal analysis and quantitative DNA estimation may have a role in evaluating tumour behaviour and prognosis, but technical factors precluded them from widespread use. While the chromosomal analysis

and banding of solid tumours is still essentially confined to basic cancer research, recent technological advances have made quantitative DNA analysis and estimation of tumour ploidy relatively simple, rapid, and well suited for clinical application.

The flow cytometric analysis of DNA content appears to provide an objective assessment of the inherent malignant potential in a large number of tumour types (Table). There are, however, inadequacies in some of the reported studies as the relation between ploidy and other prognostically important features such as stage and grade are not always mentioned. In addition, the effect of tumour ploidy on response to treatment needs further elucidation. If the studies suggesting a correlation between response probability and tumour ploidy are confirmed the applications will be wide, as it is not usually possible to predict the likelihood of a long term response to chemotherapy.

The fundamental reasons behind variability in the biological behaviour of histologically similar tumours with different DNA contents still needs to be explained. Causative factors may be important as it has been shown that different aetiological agents can cause histologically identical animal tumours with different, yet consistent, karyotypic patterns.¹⁷³ Alternatively, quantitative differences in gene dosage or mutation rate may also account for the behaviour of certain tumours.¹⁷⁴ It is likely that with

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the rapid inroads being made in the field of molecular biology and cytogenetics explanations for the clinical importance of aneuploidy will be forthcoming.

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