Chromosome studies of solid tumours

N P Bown

Introduction
Cytogenetic analysis of malignant cells very often indicates acquired chromosome changes—that is, deviations from the normal constitutional 46,XX or 46,XY karyotype. Much of what we know about these changes is derived from studying leukaemias and lymphomas. Many of these abnormalities are non-random, and particular chromosome changes are consistently found in particular disease types. These abnormalities may be of chromosome structure (translocations, deletions, inversions, etc) or of chromosome number (monosomies, trisomies, etc). The recurrent finding of such specific, clonal karyotype abnormalities underlies the integral role of cytogenetics in the clinical investigation of pre-leukaemias, leukaemias, and lymphomas. Depending on the specificity of the particular chromosome abnormality, cytogenetics can provide powerful confirmatory evidence in diagnosis, and is occasionally of prime importance in defining disease type. As the disease progresses, secondary karyotype changes may appear; where such clonal evolution pathways are well characterised—for example, in chronic myeloid leukaemia—cytogenetics can be used to stage the disease and monitor malignant progression.

For both lymphoid and myeloid disorders, significant correlations have also been established between the chromosome changes seen in the bone marrow at presentation and the patient’s prognosis in terms of response to treatment and survival.

Over the past two decades, cytogenetic studies in haematological malignancies have also contributed to profound advances in the understanding of oncogenesis: the existence of recurring chromosome breakpoints implies the presence, at discrete loci, of genes that are important in the development of cancer. In several diseases molecular studies have elucidated the mechanisms whereby chromosome rearrangements result in oncogene activation or alteration.

The cytogenetic investigation of solid tumours is at a much less advanced stage. The reasons for this are largely technical: many tumours contain areas of necrosis or show low mitotic activities; and tissue cultures often fail to accumulate sufficient numbers of mitotic cells of suitable quality for chromosome analysis. Problems also arise in disaggregating the material to produce the cell suspensions required for direct harvests of metaphase cells and for short term cultures. Improvements have been reported in the yield of metaphase cells following tumour disaggregation with enzymes such as collagenase.

The cytogenetics of solid tumours is currently the subject of intense investigation, and the rate of accumulation of data now makes a single brief review impossible. What follows is heavily biased towards tumours occurring in children, reflecting the author’s research interest. It is not yet clear whether karyotype studies of solid tumours will prove, like leukaemia cytogenetics, to be of routine clinical use, but several disease specific chromosome aberrations have now been described (table). Correlations with prognosis and links with molecular changes of oncogenic importance have also been established.

Sandberg and Turc-Carel have indicated several areas in which solid tumour cytogenetics has already proved valuable:
(a) Defining subsets within a histologically homogenous tumour type—for example, those showing i(5p) or del(5q), +7, −9 or del(9p) in transitional cell carcinoma of the bladder.
(b) Suggesting aetiological connections between histologically diverse tumours—for example, virtually all germ cell tumours of the testis, both teratomas and seminomas, have been found to show i(12p).
(c) Highlighting known histological subtypes—for example, the t(12;16) translocation specific to the myxoid form of liposarcoma.
(d) Suggesting a primary tumour site when a specific karyotype change is found in a metastatic tumour or in bone marrow.

When considering chromosome changes in solid cancers, it is possible, as in leukaemias and lymphomas, to distinguish between those of primary aetiological importance and secondary changes appearing in the course of tumour progression. For a given tumour type the former are recognised either because they are seen as the sole karyotype abnormality or because they recur as a common link between more complex abnormal karyotypes which may include multiple secondary rearrangements.

Sequential cytogenetic changes have been recorded in a variety of solid tumours as they develop from relatively benign forms to show more malignant, invasive, or metastatic behaviour. For example, Nowell has summarised the current knowledge of cytogenetic evolution in melanocytic tumours and in colon cancer. In the former, karyotypically abnormal clones are more common as the disease progresses from common naevus, through dysplastic naevus, to invasive and metastatic melanoma, with 9p and 10q rearrangement in early stages and...
Examples of specific primary changes in solid tumours

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Chromosomal abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningioma</td>
<td>Monosomy 22/DEL(22q)</td>
</tr>
<tr>
<td>Mixed salivary gland tumour</td>
<td>t(3;8)(p21q12)</td>
</tr>
<tr>
<td>Lipoma</td>
<td>Translocations of 12q13-14</td>
</tr>
<tr>
<td>Myxoid liposarcoma</td>
<td>t(12;16)(q13q11)</td>
</tr>
<tr>
<td>Small cell lung carcinoma</td>
<td>del(3)(p14p23)</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
<td>t or del (16q)</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>i(12p)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>t or del (1p) DMs/HSRs</td>
</tr>
<tr>
<td>Ewing’s sarcoma and related tumours</td>
<td>t(11;22)(q24;q12)</td>
</tr>
<tr>
<td>Alveolar rhabdomyosarcoma</td>
<td>t(2;13)(q35-37q14)</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>t(X;18)(p11;q11)</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>i(6p)</td>
</tr>
<tr>
<td>Wilms’ tumour</td>
<td>Constitutional del(13q14)</td>
</tr>
<tr>
<td></td>
<td>Constitutional del(1p13)</td>
</tr>
</tbody>
</table>

del—deletion, q—chromosome long arm, p—short arm, t—translocation, i—isochromosome, DM—double minute chromosome, HSR—homogeneously staining region.

Genetics are of intense clinical interest, with neuroblastoma being perhaps the best characterised tumour in this respect. Cells from neuroblastomas frequently show deletions or rearrangements of material from the distal short arm of chromosome 1 (fig 1). Karyotypes showing hyperdiploidy with 50–77 chromosomes per cell and normal chromosomes 1 are strongly associated with stage I, II, or IV–S disease in patients under 1 year of age, and a favourable outlook, while hypo- or pseudodiploidy with 1p involvement is more common in children over 1 year, with very poor prognosis stage III or IV tumours.

Another highly significant clinical indicator in neuroblastoma is amplification of the oncogene N-myc, located at 2p23–24. In one study patients with one, three to ten, or more than 10 copies of this gene in their tumour cells showed progression free survival at 18 months of 70%, 30%, and 5%, respectively. Simultaneous use of flow cytometry to measure DNA ploidy index, and Southern blot hybridisation to measure N-myc copy number, has been proposed as a means to distinguish between prognostic subsets in neuroblastoma. However, in at least one study the presence or absence of a cytogenetically visible 1p rearrangement emerged as a more accurate predictor of tumour progression than N-myc amplification.

Gene amplification may be visible at a cytological level in the form of “double minute” chromosomes (DMs) and “homogeneously staining regions” (HSRs). These have been frequently reported in neuroblastoma and brain tumours. DMs are paired, acentric chromosome bodies 0.3–0.5 μm in size; HSRs are integrated into the chromosomes as blocks of amplified genes, with these regions failing to
show differential chromosome banding.

Neuroblastoma is one of the small round cell tumours of childhood, along with Ewing's sarcoma, peripheral neuroepithelioma, rhabdomyosarcoma and malignant soft tissue lymphoma. The histopathological distinction between these entities can be problematic, and this has prompted the search for diagnostically useful chromosome aberrations. It was also hoped that cytogenetics might help to elucidate the histogenesis of these tumours, especially Ewing's sarcoma, in which the cell of origin remains unclear. As well as the neuroblastoma chromosome changes already described, two particularly important translocations have emerged within the group of small round cell tumours.20 21 First, a translocation t(11;22)(q24;q12) has been reported in over 80% of Ewing's sarcomas (fig 2) and has also been found in neuroepithelioma and Askin's tumour, lending support to an embryonic neural crest origin for Ewing's sarcoma. Second, a t(2;13) (q35-7;ql4) has repeatedly been observed as a specific marker for alveolar rhabdomyosarcoma (fig 3).22 (Highly complex karyotype rearrangements have been recorded in osteosarcoma cells,23 but no specific chromosome banding changes have yet been identified.) The chromosomal characterisation of the small round cell tumours is therefore beginning to reveal links between disease types, as well as having a more immediate practical impact in diagnosis.24 25

Similar hopes that cytogenetics might help in the classification of brain tumours have not yet been fulfilled. The specific aberration associated with meningiomas—loss of part or all of chromosome 22—was first identified even before the development of chromosome banding techniques. More recent investigation of the gliomas (astrocytoma, medulloblastoma, glioblastoma multiforme, ependymoma, etc), has identified frequent gains of chromosome 7 and losses of 10, 22, and either X or Y, but only tentative proposals have so far emerged for specific associations between chromosome changes and histopathological subtypes.26 Results from two series of paediatric intracranial tumours suggest that the karyotype changes in these cases may differ from adult tumours, with deletions, unbalanced translocations, and isochromosome 17q predominating, rather than the numerical changes commonly seen in the adult tumours.27 28

The clinical or diagnostic interpretation of a chromosome result from a solid tumour can be problematic in several respects. First, when only normal karyotypes are detected in direct preparations or in tumour cultures, it is usually unclear whether these represent tumour cells with, presumably, submicroscopic genetic lesions, or stromal fibroblasts—that is, the malignant cells may be present but not in mitosis. Second, it is now known that several normal (non-neoplastic) human tissues show clonal chromosome changes—for example, gains of chromosome 7 and losses of sex chromosomes in both brain29 and kidney.30 The possibility of constitutional tissue specific mosaicism must therefore be borne in mind when interpreting aneuploidies in solid tumour karyotypes. Third, there are now many reports of chromosomal abnormalities in non-malignant tumours. In one series of 109 non-malignant solid tumours of various types, thyroid and parotid adenomas, meningiomas, acoustic neuromas etc, 37% showed clonal numerical or structural chromosome changes.31 This breakdown of the previously well established correspondence between acquired clonal chromosome change and malignancy seems to be confined to solid cancers; in haematological diseases the traditional dogma appears to remain valid.

Another intriguing cytogenetic pattern which has emerged, particularly from studies of skin tumours, has been the phenomenon of multiple independent clones showing unrelated chromosome changes. This situation has been described in, for example, basosquamous papilloma,32 basal cell carcinoma,33 and squamous cell carcinoma.34 Several groups have cited these observations in support of the "field carcinisation" model of polyclonal initiation of epithelial tumours; others urge caution in relating in vitro karyotype findings to the tumour in vivo.35

What has the identification of tumour rela-
Chromosome studies of solid tumours

Chromosome studies of solid tumours have revealed a variety of chromosomal abnormalities that are important in the understanding and treatment of these diseases. The actions of oncogenes in solid cancers, and the general rule of chromosome rearrangements found in leukaemias and lymphomas, seem mainly to bring about a potentiation of onco-
genome function by increasing gene expression or by modifying the gene product to a more active form.56,57 In solid tumours, the disease is mainly cytogenetic, and the predominant mechanisms are chromosomal translocations, which may be either the oncogene amplification, such as t(11;22) in acute myeloid leukaemia or t(15;17) in acute promyelocytic leukaemia. Chromosome aberrations may also be found in solid tumours, such as t(2;13) in Ewing's sarcoma or t(I 1;22) in small round-cell tumours.

The potential clinical contribution of solid tumour cytogenetics to diagnosis and defining prognostic subgroups has therefore been estab-
lished. The number of United Kingdom cytogenetics centres involved in this research is increasing, and new disease specific karyotype abnormalities continue to be reported, permitting the identification of sites of oncogenic importance within the genome and acting as a springboard for molecular studies. This is a rapidly evolving area in which active collaboration among pathologists, oncologists, and cytogeneticists is proving both clinically important and insights into the fundamental biology of solid cancers.

Thanks are due to Dr A J Malcolm for his comments on the manuscript.

11 Barrios L, Caballin MR, et al. Cytogenetic effects of radiotherapy. Breakpoint distribution in induced chromo-
14 Hayashi Y, Hanada R, Yamamoto K, Bebsho F. Chromo-
22 Wang-Wuu S, Soukup S, Ballard E, Gorobis B, Lampkin B. Chromosomal analysis of sixteen human rhabdomyo-
24 Brichard B, Ninane J, Gossaye S, Vareille-Dumoulain C, Rhodin J, Corrin G. A disseminated alveolar rhabdo-
isarcoma in a 9-year-old boy disclosed by chromoso-
27 Bigner SH, Mark J, Friedman HS, Biegel JA, Bigner DD. Structural chromosome abnormalities in human medul-


