Chromosome studies of solid tumours

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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 leukaemia¹—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.²⁻⁴

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.^{5,6}

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.⁷

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^{8,9} have indicated several areas in which solid tumour cytogenetics has already proved valuable:

- 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.
- 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).
- Highlighting known histological subtypes—for example, the t(12;16) translocation specific to the myxoid form of liposarcoma.
- 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.¹⁰ 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

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Examples of specific primary changes in solid tumours

Tumour	Chromosomal abnormality
Meningioma	Monosomy 22/del(22q)
Mixed salivary gland tumour	t(3;8)(p21;q12)
Lipoma	Translocations of 12q13-14
Myxoid liposarcoma	t(12;16)(q13;q11)
Small cell lung carcinoma	del(3)(p14p23)
Bladder carcinoma	i(5p)
Breast carcinoma	t or del (16q)
Testicular teratoma/seminoma	i(12p)
Neuroblastoma	t or del (1p) DMs/HSRs
Ewing's sarcoma and related tumours	t(11;22)(q24;q12)
Alveolar rhabdomyosarcoma	t(2;13)(q35-37;q14)
Synovial sarcoma	t(X;18)(p11;q11)
Retinoblastoma	i(6p)
Wilms's tumour	Constitutional del(13q14) Constitutional del(11p13)

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

alterations of chromosomes 1, 6, and 7 occurring at more advanced stages. For colon tumours, the progression from colonic polyps to advanced colon cancer is marked by four successive genetic lesions: mutation in a *ras* oncogene; partial deletion of 5q; deletions of chromosome 17; and loss of chromosome 18.

Difficulties can arise in the interpretation of complex chromosome changes in tumours which have been subjected before biopsy to therapeutic irradiation or drugs, because both agents can induce chromosome rearrangements.^{11 12} A recent report described serial cytogenetic studies of a paediatric astrocytoma which evolved to a glioblastoma multiforme.¹³ At diagnosis, trisomy 1q was the sole abnormality, but after chemotherapy and radiotherapy multiple reciprocal translocations, involving chromosome breakpoints implicated as “hot-spots” in radiation induced chromosome damage, were detected.

The prognostic implications of tumour cyto-

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.^{14 15}

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.¹⁶ 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.¹⁷ 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.¹⁸

Gene amplification may be visible at a cytological level in the form of “double minute” chromosomes (DMs) and “homogeneously staining regions” (HSRs).¹⁹ 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

Figure 1 Neuroblastoma. Metaphase cell showing, among other abnormalities, rearrangement of the short arm of chromosome 1 (1p+) typical of this tumour. Inset: normal homologue on the left, rearranged 1p on the right (G-banding by trypsin and Leishman's stain).

Figure 2 Ewing's sarcoma. G-banded metaphase cell and partial karyotype showing $t(11;22)(q24;q12)$ translocation.

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 $t(2;13)(q35-7;q14)$ 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 chan-

Figure 3 Alveolar rhabdomyosarcoma. Partial karyotype of tumour cell showing $t(2;13)(q36;q14)$.

ges 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 cancerisation" 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-

ted chromosomal abnormalities shown about the actions of oncogenes in solid cancers? As a general rule the chromosome rearrangements found in leukaemias and lymphomas seem mainly to bring about a potentiation of oncogene function either by increasing gene expression or by modifying the gene product to a more active form.^{36 37} In solid tumours, on the other hand, the predominant mechanisms seem to be either oncogene amplification, such as *c-neu* in breast carcinoma and *N-myc* in neuroblastoma, or the loss or inactivation of "anti-oncogenes".^{37 38} Also known as "tumour suppressor genes", the normal function of these genes is to inhibit cell growth and promote differentiation. Their loss or inactivation is therefore a critical event in tumour initiation and loss of heterozygosity—evident at either the cytogenetic or molecular level—has now been reported in a wide range of human solid cancers. This model for oncogenesis has been most fully explored for retinoblastoma and Wilms's tumour,^{39 40} in both of which inherited constitutional deletions may predispose to tumour development.

The possible role of oncogenes in a number of recurring chromosomal aberrations, such as the t(11;22) and t(2;13) rearrangements described above, remains to be shown.

The interaction between cytogenetics and molecular genetics continues to generate important technical advances. Perhaps the most relevant to the study of solid tumours are the new methods of fluorescent (non-isotopic) in situ hybridisation which permit both "chromosome painting" and "interphase cytogenetics". In the former whole chromosome DNA libraries are hybridised to metaphase spreads to elucidate structural rearrangements. The latter technique involves hybridising chromosome specific probes to centromeric repetitive DNA sequences, allowing the number of copies of a particular chromosome to be identified in poor quality metaphases or in interphase nuclei. Used in appropriate circumstances, both approaches greatly enhance the karyotype information which may be obtained from cytogenetic preparations,⁴¹ and recent reports have described the application of these methods to solid tumours. Selleri *et al.*,⁴² for example, have used a panel of cosmid clones from distal 11q to localise the breakpoints of the t(11;22) seen in Ewing's sarcoma and related tumours. Other workers have used panels of centromere specific satellite DNA probes to identify monosomies and trisomies in brain tumour interphase nuclei.⁴³ Techniques have also been developed for combined cell morphology, immunophenotyping, and chromosome in situ hybridisation,^{44 45} although these have yet to be applied to large series of solid tumours.

The potential clinical contribution of solid tumour cytogenetics to diagnosis and defining prognostic subgroups has therefore been established. 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 producing both clinically important information and insights into the fundamental biology of solid cancers.

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