INVITED REVIEW

Advances in cytogenetic analysis of solid tumours

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Information from cancer cytogenetics has been accumulating rapidly in recent years. In the past decade in particular, many new reports of chromosomal aberrations have been added to the literature. This burgeoning collection of data is regularly updated in the *Catalog of Chromosome Aberrations in Cancer*, the fifth edition of which was recently published (Mitelman 1994) and describes cytogenetic abnormalities from over 22,000 human neoplasms. This is a significant and healthy increase from the first edition of the *Catalog* in 1983, when only 3,800 reports were available. However, a careful breakdown of the data shows that the clear majority (over 60%) are generated from haematological malignancies (Heim & Mitelman 1995). The analysis of chromosome abnormalities in leukaemia has been of significant value in understanding both the clinical prognosis and biology of the disease. The introduction of techniques for banding chromosomes (Caspersson et al. 1970) permitted the identification of specific and recurring sites of chromosome change. These consistent changes have allowed the subdivision of patients by chromosome abnormality into defined clinical groups. The karyotypic subdivision has been shown to be a strong prognostic indicator in terms of both the duration of remission and the mean survival time in these patients (Le Beau & Rowley 1984). From a biological point of view, identification of specific chromosomal changes provided a starting point for more detailed molecular investigations of leukaemia, and a clear correlation has emerged between the sites of chromosomal abnormality and the location of human oncogenes (Nowell et al. 1984, Heim & Mitelman 1995).

In contrast to the picture for haematological malignancies, karyotypic information on solid tumours has consistently lagged behind. Information on the cytogenetic abnormalities found in all solid tumours represents only 27% of total reported cases (Mitelman 1994). Although this is a significant increase since 1983, when the reports numbered a mere 12%, it is entirely disproportionate to the relative contribution of carcinomas to deaths due to cancer. The reasons for this uneven data distribution are threefold and centre on variations between laboratories in methods used for tissue preparation and difficulties inherent in the tumour material itself. First, there is a wide variation between different groups in the methods used to prepare tissues for analysis. Both the cell culture method and the duration of time in culture for the cells used for analysis can be very variable. Direct harvesting of cells for analysis often fails to yield any information if the mitotic activity of the tumour is low. This may be exacerbated by necrotic tissue or accompanying infection in the sample. Provided it is possible to prepare chromosomes for analysis, these will usually be of poor quality when compared with those made from blood cultures. *In vitro* culture of the cells before analysis increases the chances of success, and the number of cases with simpler abnormalities is higher. However, it is not clear whether these cultured cells are truly representative of the original tumour material or just clonal outgrowths of small subpopulations of cells. In addition, there may be significant differences in the treatment given to patients before chromosome analysis. Some may receive, for example, doses of radiotherapy, which may complicate the karyotype with random chromosome changes or bias the results by selection of subpopulations of cells. Second, it is clear that the cytogenetics of solid tumours will inevitably be more difficult to unravel as the chromosomes have undergone many more rearrangements than those seen in leukaemia cells, making the karyotypes far more complex. It is therefore difficult to identify the primary changes in such material. In addition, many published studies have not used chromosome banding methods and often have reported only partial karyotypes. This does not allow the definitive identification of all chromosome abnormalities for each tumour. Third, in some tumour types a large proportion of data has been gathered not from primary tumours but from more advanced metastases. This may generate a false picture of chromosome abnormalities based on a small, highly evolved subpopulation of tumour cells present at these sites.

A good example of the problems encountered in the cytogenetics analysis of solid tumours is seen in breast cancer (for reviews, see Trent 1985, Bieche & Lidereau 1995). Here the tumour karyotypes are widely variable, ranging from the highly complex with many chromosome abnormalities and markers of unknown origin (Figure 1) through to apparently normal karyotypes. The abnormalities may be both rearrangements of

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Chromosome Research Vol 4 1996 479
material within or between chromosomes and/or numerical anomalies. In addition, breast tumours can often exhibit both double minutes (DMs) or homogeneously staining regions (HSRs) of unknown origin. Double minutes represent small extrachromosomal bodies of DNA whereas HSRs are usually long and poorly stained regions of DNA identified after G- or Q-banding. It has long been known that these aberrations correlate with amplified genes in these cells (Cowell 1982).

Despite the difficulties outlined above, cytogenetic information on solid tumours has clearly been rapidly increasing in the last decade. Although the field is more complex than that of haematological malignancies, the methods used to analyse leukaemias have given clear guidelines for those working on solid tumours. At present, analysis of each tumour can be performed using three distinct methods, each one adding further information to the picture. It has first been important to establish clear and unambiguous sites of consistent chromosome change. This has been achieved for the most part by using conventional cytogenetic analysis and more recently by whole-genome in situ hybridization methods such as comparative genomic hybridization (CGH). Second, the regions highlighted in these studies have then been investigated in more detail at the chromosome level. Methods for fluorescence in situ hybridization (FISH) using a variety of molecular probes have been used to refine the analysis of chromosomal anomalies. Finally, the regions of chromosomal change can be examined at the molecular level to investigate mutations in candidate oncogenes or tumour-suppressor genes. This area is too wide to be covered adequately here; for a recent review see Bieche & Lidereau (1995).

The identification of consistent regions of chromosomal abnormality in solid tumours has been greatly aided by the use of chromosome banding. One of the best examples of the use of modern cytogenetic analysis to identify chromosomal abnormalities in a solid tumour is seen in the case of retinoblastoma (Cowell & Hogg 1992). The first indication of where the retinoblastoma (Rb) gene mapped in the human genome came from cytogenetic studies of rare patients who combined retinoblastoma with other congenital abnormalities, particularly mental retardation. Some of these patients had constitutional deletions of one copy of chromosome 13 (Vogel 1979). Although the deletions varied in size, analysis of banded chromosomal preparations from these tumours identified a minimal region of deletion around chromosome band 13q14. Dosage studies with the closely linked marker esterase D enzyme, in patients with 13q14 deletions, confirmed that this band contained a gene important in retino-

**Figure 1.** A representative GTG-banded cell from a breast tumour. The karyotype is 46,XX, add(1)(q32), t(6;16)(q16;q13), –8, –10, –11, –17, +20, –21, +4mar. Two clonal abnormalities are present: an add(1)(q32) (small arrow) and a t(6;16)(q16;q13) (large arrows). Non-clonal abnormalities are also present in this cell and include –8, –10, –11, –17, +20, –21 and the four unidentified marker chromosomes (figure courtesy of Dr Erika Mitchell, Paterson Institute).