ELSEVIER

European Journal of Cancer 40 (2004) 1960–1967

European Journal of Cancer

www.ejconline.com

Understanding cancer at the chromosome level: 40 years of progress ☆

Silvana Debernardi, Debra Lillington, Bryan D. Young *

Medical Oncology Laboratory, Barts and the Royal London School of Medicine, Cancer Research UK, Charterhouse Sq, London EC1 6BQ, UK

Received 26 May 2004; accepted 2 June 2004

Abstract

The review by D.T. Hughes examined the role of cytogenetics in cancer research in 1964. Despite the technical limitations of the day, he highlighted a number of known abnormalities which were to turn out to be crucial in our understanding of cancer genetics over the subsequent 40 years. These included the Philadelphia translocation and the Burkitt's lymphoma-associated marker chromosomes. In addition, he mentioned that a deleted chromosome had been observed in an example of retinoblastoma and double-minute chromosomes in neuroblastoma. The study of these events led to the identification of the key genes involved (*BCR*, *ABL*, *C-MYC*, *RB1* and *N-MYC*) and served as models for substantial further work. We review some of the technical advances in the field of molecular cytogenetics and show how they can be applied to the events reviewed by Hughes.

Keywords: Cancer genetics; Cytogenetics; Chromosomes; Translocations

1. Introduction

In 1964, a review of the role of chromosomes in cancer was published by D.T. Hughes. This paper highlighted a remarkable number of key chromosomal abnormalities which subsequently were to lead to landmarks in our understanding of the genetic basis of cancer. The importance of chromosomal alterations in the aetiology of cancer was first proposed in 1914 by Boveri who advanced his theory based on microscopic observations of mitosis. However, it was not until 1952 when the hypotonic treatment of metaphase cells was introduced that chromosomes could be accurately analysed [1]. A key study by Tjio and Levan in 1956 [2] determined for the first time the true number of human chromosomes. With this knowledge, it became possible to examine tumour cells for chromosomal abnormalities. At that time, in the pre-banding era, the identification of chromosomes was based on size and centromere position and chromosomes fell into 7 similarly sized groups (A–G). It was therefore possible to examine the chromosome number relatively easily, but was much more difficult to determine structural abnormalities. In this period, considerable progress was being made in describing constitutional numerical abnormalities, such as trisomy 21 associated with Down's syndrome. Indeed, the link between trisomy 21 and the increased occurrence of leukaemia had already been established [3].

Dr. Hughes reviewed a series of studies which addressed the issue of the role of chromosome number in cancer. A study by Levan [4] examined 40 human cell lines, half of which were derived from normal tissues and half from cancers. It was noted that most human tumours had chromosomal complements in the diploid to triploid range. In another study, Hauschka and colleagues examined a large number of ascites tumours. They observed that a small proportion of tumours were diploid or pseudo-diploid and a majority were aneuploid [5]. In addition, mentioned by Hughes is the study of Makino and collegues who observed that many tumours from different sites had characteristic modal chromosomal numbers. He observed that the most frequent chromosome numbers were found at different ploidy levels in the tumours. For example, gastric carcinomas were mainly hypotriploid, whereas

☆ S.D. is supported by the Kay Kendall Leukaemia Fund.
* Corresponding author.
E-mail address: bryan.young@cancer.org.uk (B.D. Young).

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.06.007

rectal carcinomas were mainly in the hypo- to hyperdiploid range [6].

Hughes also pointed out that very few studies had been made at that time of early cancer or pre-malignant neoplasias. He pointed out that some information on this question had been obtained in studies of cervical lesions and on primary tumours of the bladder. It was observed that in the cervix the most striking difference in chromosome number was found between dysplasia, with a main range of 40–55 chromosomes, and carcinoma *in situ*, with a range of 40–100, so that considerable aneuploidy was found to occur sometime after the start of malignancy [7]. In another study, Lamb and colleagues found extensive changes in chromosome numbers that did coincide with the capacity to invade. Nine benign bladder tumours were predominantly in the diploid range, whereas the majority of malignant tumours had chromosome numbers in the range of 40 to more than 100. It was pointed out by Dr. Hughes that it may be possible to diagnose malignancy in terms of chromosome number in this situation because over two thirds of the cells in the invasive tumours had chromosome numbers in excess of the diploid range. Thus, at that time it was felt that the ploidy of a tumour could have an important bearing on the origin and degree of malignancy of tumours.

Despite this concentration on chromosome number, Dr. Hughes also recognised that diploid cells could have a malignant phenotype. He stated "chromosome number is an inadequate criterion of the normality of a chromosome set" and went on to say that chromosomes would have to be examined in detail before they could be supposed to be without any anomaly. It was already clear that certain structural abnormalities did exist in cancer and are highlighted in this publication. Dr. Hughes reviewed the state of knowledge concerning the Philadelphia chromosome. The Philadelphia chromosome had been previously identified in 1961 by Nowell and Hungerford [8] as a small marker chromosome associated with chronic myeloid leukaemia. The identification of this chromosome as the product of the t(9;22) translocation in 1973 was a landmark in our understanding of genetic basis of cancer [9]. Hughes also highlighted the fact that Burkitt's lymphoma was characterised by two marker chromosomes. We now know that these corresponded to the translocation t(8;14) which results in the juxtaposition of the *C-MYC* oncogene into the immunoglobulin heavy chain (IGH) locus [10]. The introduction of chromosome banding allowed a much more detailed examination of the tumour karyotype and led to the identification of many other recurrent structural chromosomal abnormalities particularly in leukaemias and lymphomas.

Another abnormality discussed by Dr. Hughes concerns double-minutes which are small chromosomal fragments in neuroblastoma. It is now known that these abnormalities correspond to amplifications of the *N-MYC* oncogene [11] and the amplification of this gene is an indicator of a poor prognosis in neuroblastoma. Another key observation discussed in this paper is the finding of a deleted acrocentric chromosome in retinoblastoma. In the publication by Lele et al. [12] this was identified as chromosome number 15 and it was suggested that recessive genes could be involved in the origin of this tumour. This was confirmed when the retinoblastoma gene, *RB1*, was identified as the cause of hereditary retinoblastoma [13]. The difficulties in identifying the chromosome of origin are reflected in the fact that this chromosome was identified as number 15 in the original publication.

2. Advances in molecular cytogenetics over the last 40 years

We can see from the review by Dr. Hughes that several of the key events in the history of cancer cytogenetics (t(9;22), t(8;14), *N-MYC* amplification and putative retinoblastoma tumour suppressor gene) had already been identified and were becoming the focus of research. There have been many technical advances over the last 40 years which have revolutionised cytogenetics and we review how modern techniques can be used to analyse some of the events highlighted by Dr. Hughes in 1964.

The use of cytogenetics and molecular cytogenetic analysis has become particularly important in the haematology field. Fluorescence *in situ* hybridisation (FISH) has been incorporated into most diagnostic laboratories to complement chromosome analysis and further improve its accuracy. In the era of risk-adapted and mutation-directed therapy, accurate assessment of the genetic status is of paramount importance. In many current studies, patients are stratified on the basis of their cytogenetic or molecular rearrangements since numerous disease/subtype-specific abnormalities have independent prognostic outcome [14]. Such is the specificity of certain chromosomal rearrangements that it can provide unequivocal diagnosis of the type of malignancy. This is true for both leukaemia and lymphoma, although the number of recurrent chromosomal changes identified in leukaemia exceeds those in lymphoma. Nevertheless, a number of highly specific lymphoma-associated changes have diagnostic and therapeutic value. In national treatment trials for acute myeloid leukaemia (AML) and acute lymphoid leukaemia (ALL), cytogenetic information is vital to treatment stratification and in other diseases, such as chronic lymphocytic leukaemia (CLL) and myeloma, the impact of chromosomal abnormalities are beginning to be recognised.

FISH is used as a rapid, sensitive test to complement G-banded analysis allowing the detection of cryptic or subtle changes. In addition, FISH can be used to screen

non-dividing cell populations, such as bone marrow smears, tumour imprints and paraffin-embedded tissue sections (PETS). A vast array of FISH probes is currently available, aimed at detecting fusion genes, numerical abnormalities, chromosomal imbalances, chromosomal rearrangements and complex events. FISH has been further developed to allow the global detection of tumour-associated gain and loss using tumour DNA as a FISH probe against normal metaphase chromosomes. This technique is known as comparative genomic hybridisation (CGH) and in turn has led to the very recent array-CGH technique. Array-CGH promises to provide much higher resolution of genomic imbalance compared with chromosome-based CGH. Currently, custom-designed arrays containing oncogenes and tumour suppressor genes are available commercially and arrays with DNA clones spaced at 1Mb intervals throughout the genome are becoming available. Gene expression profiling also offers exciting prospects and coupled with the molecular and cytogenetic information, accurate molecular cytogenetic analysis looks set to revolutionise patient management.

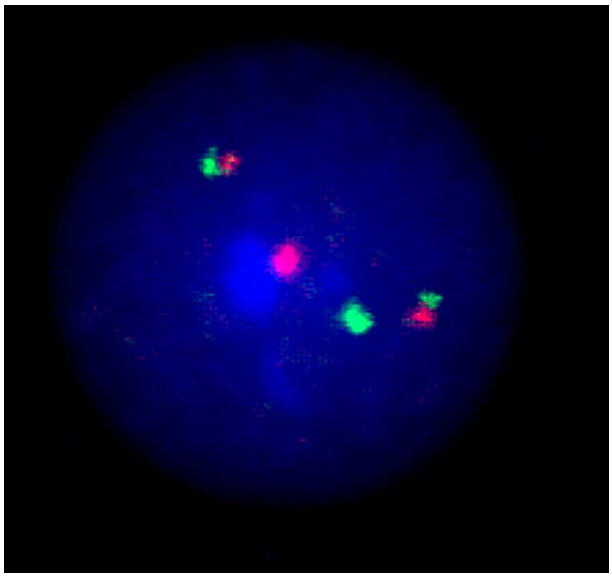

Fig. 1. *BCR-ABL* rearrangement in an interphase cell. The reciprocal t(9;22) generates two fusion signals using the Vysis dual fusion probes corresponding to the der(9) and der(22). A normal *ABL* (red) and *BCR* (green) are present.

3. FISH on metaphase chromosomes

In leukaemia and lymphoma, gene fusions are relatively frequent and well characterised at the molecular level. These novel disease-associated fusion events arise through chromosomal translocations, inversions or insertions and are usually visible by routine karyotype analysis, although subtle abnormalities do exist and some of the recurrent rearrangements can be cryptic. FISH probes mapping to the unique sequences involved in these fusions are readily available and detect their respective abnormalities by one of two methods. In the first strategy, probes mapping to the two genes involved are labelled in two distinct colours, e.g. the *BCR-ABL* fusion associated with the t(9;22)(q34;q11.2) is illustrated in Fig. 1. *BCR* is represented by the green fluorescence and *ABL* by the red signal. The t(9;22) translocation results in both *BCR-ABL* and *ABL-BCR* fusions and since the probe extends beyond the breakpoint for both genes, two fusion signals (red and green juxtaposed) are generated (dual fusion probes), one on the der(9), the other on the der(22). Usually, there will also be a normal 9 and a normal 22 (single red and green signal). However, to further complicate the analysis, deviations from this pattern may exist since some patients carry deletions around the breakpoint and some harbour cryptic insertions of part of one gene, thereby generating only one of the fusion sequences. The next generation of FISH probes look likely to utilise four fluorescent probes to enhance the sensitivity and specificity to simultaneously detect translocations and deletions around the breakpoint which may confer independent prognostic value. The second common type of FISH strategy is the "break apart" probe, specifically designed to detect abnormalities affecting one specific gene which rearranges with multiple partner loci e.g. *MLL* (11q23). Over 60 different *MLL* gene translocations have been cytogenetically reported and the FISH probe used most often for diagnosis consists of a probe mapping above the breakpoint labelled with one colour and a second probe mapping below the breakpoint in another colour. Translocations involving *MLL* therefore result in the separation of one set of probes and the displaced *MLL* signal will map to the partner chromosome. Single colour probes extending across the breakpoints can also be used which result in a split signal.

Unique sequence probes can also be applied to screen for copy number changes, particularly in cases with evidence of additional genetic material by karyotyping in the form of double-minute chromosomes (dm), homogeously staining regions (hsr) or additional pieces of chromosomes. Dm or hsr are manifestations of gene amplification and in certain malignant diseases, particularly solid tumours, are well recognised mechanisms for oncogene activation. FISH probes mapping to the genes commonly associated with amplification can very quickly confirm the presence of multiple copies of genes e.g *N-MYC* in neuroblastoma. Fig. 2 shows a bone marrow aspirate infiltrated by neuroblastoma and multiple copies of *N-MYC*. Alpha satellite probes are often used to determine chromosome number. Hyperdiploidy is a frequent phenomenon in ALL and is associated with a common pattern of gain, namely chromosomes 4, 6, 10, 14, 17, 18, 21 and X. Using a selected cocktail of alpha satellite probes mapping to these chromosomes,

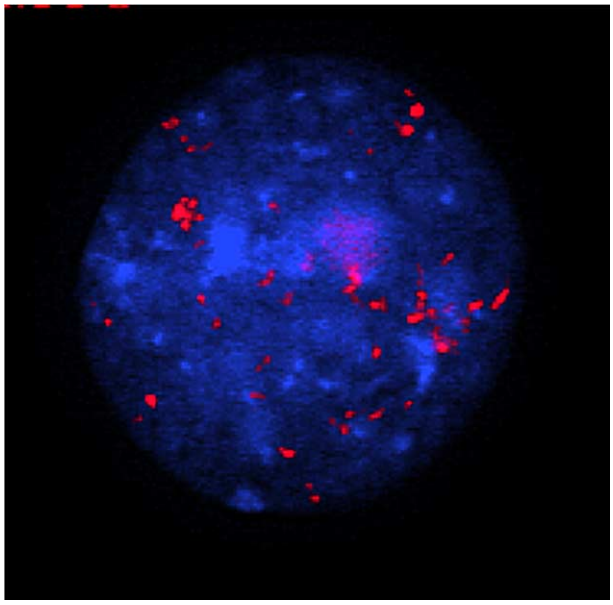

Fig. 2. Bone marrow aspirate from a patient with neuroblastoma. The interphase cell shows multiple copies of *N-MYC* (red).

hyperdiploidy can be detected in both metaphase and interphase cells. Metaphase cells derived from leukaemic blasts of patients with ALL can often have poor morphology and be difficult to fully characterise. In such situations, FISH with centromeric probes can be of particular value since it may help elucidate chromosomal gains and losses.

Whole chromosome painting probes (WCP), consisting of pools of DNA sequences mapping along the full length of a particular chromosome and labelled with a fluorochrome can be used individually or in combination with other WCP to characterise abnormalities whose origin is uncertain by G-banding. In simple karyotypes, requiring confirmation of a suspected rearrangement, two colour chromosome painting might be the most useful option. In more complex karyotypes, such as those associated with therapy-related leukaemia, a mixture of paints mapping to all 24 human chromosomes (24 colour karyotyping) is probably the most informative. M-FISH/spectral karyotyping (SKY) is not used routinely for diagnostic purposes, but has revealed cryptic rearrangements in several studies. M-FISH/SKY utilises a combinatorial labelling approach such that each individual chromosome paint is labelled with a unique combination of not more than 5 fluorochromes [15]. The 24 differentially labelled paints are then applied in a single hybridisation assay and visualisation achieved using one of two strategies. M-FISH uses a series of optical filters to collect the images from the different fluorochromes which are then merged into a composite image and a pseudo-colour assigned to each chromosome on the basis of its fluorochrome combination. A related technique known as SKY has also been developed. This uses an interferometer with Fourier transformation to determine the spectral characteristics of each pixel in the image and assigns a pseudo-colour [16]. These techniques have been particularly useful for the detection of cryptic abnormalities which would not be detected by conventional analysis [17]. We illustrate the use of M-FISH to analyse a case of myelodysplasia syndrome (MDS) detecting several chromosomal abnormalities (Fig. 3).

4. CGH and array-CGH analysis

CGH provides a global assessment of copy number changes, revealing regions of the chromosome which are either gained or lost in the tumour sample. A key feature of this technique is that dividing tumour cells are not

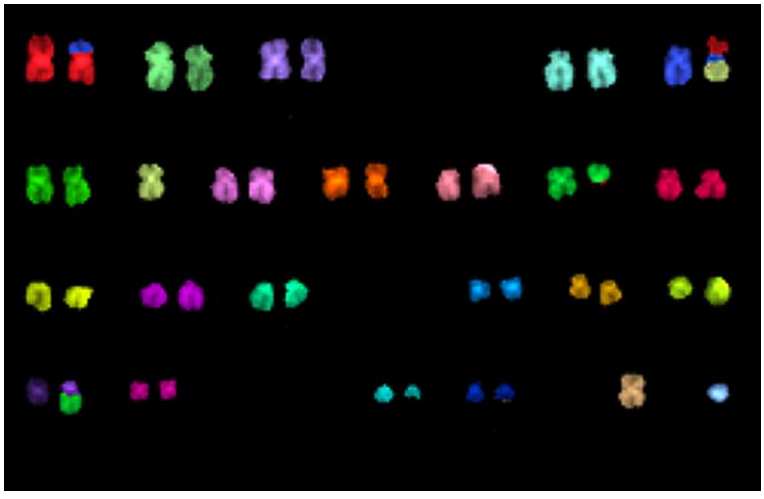

Fig. 3. M-FISH karyotype of a patient with sMDS. In addition to structural rearrangements of chromosomes 1, 5, 11 and 19, there is loss of chromosome 7.

required. DNA is extracted from the tumour, labelled (usually) with a green fluorochrome and compared with DNA from a normal reference labelled with a red fluorochrome. Labelled test and reference DNA are combined and hybridised to normal chromosomes and the resultant ratio of the two signals along the length of the chromosomes reflects the differences in copy number between the tumour and reference DNA samples. Regions of gain in the tumour DNA are represented by an increased green/red ratio, whereas deletions are indicated by a reduced ratio. We illustrate this with a CGH analysis of a case of retinoblastoma (Fig. 4) showing the gain of 1q and loss of 16q. CGH requires 50% abnormal cells to be present within the tumour sample for reliable detection of genomic imbalance and will not easily detect regions involving less than 10 Mb of DNA unless it involves high level amplification. Nevertheless, CGH is particularly applicable to the analysis of solid tumours since DNA can be readily extracted from them and the karyotype frequently involves loss or gain of whole or partial chromosomes.

The use of genomic DNA arrays as the hybridisation target essentially allows much higher resolution

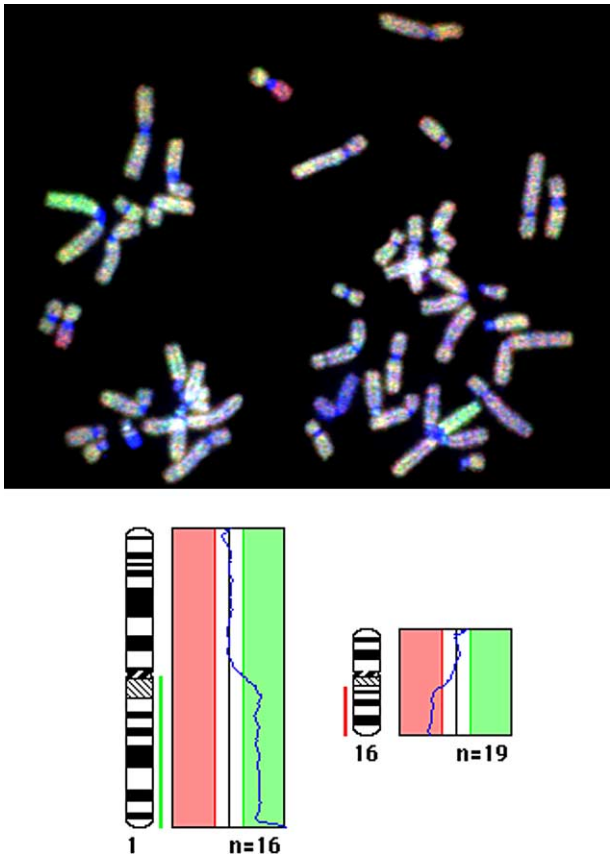

Fig. 4. CGH using a retinoblastoma tumour as the test DNA. Top image shows a metaphase following CGH, green fluorescence represents gain and red corresponds to loss of the region in the tumour. The CGH profile (bottom image) clearly demonstrates this corresponds to gain of 1q and loss of 16q.

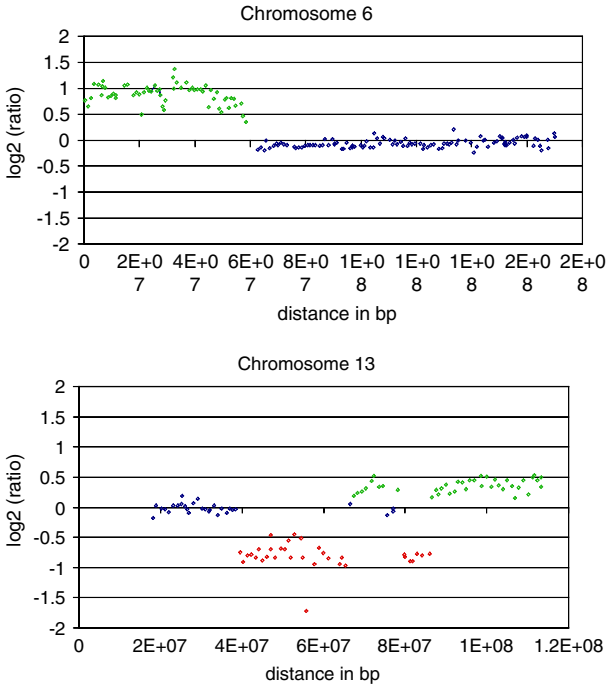

Fig. 5. 1 Mb CGH-array (Sanger Centre) of a retinoblastoma tumour, showing gain of BAC clones mapping to 6p (green) (top image), two regions of gain on 13q (green) and two regions of deletion on 13q (red) (bottom image).

for the detection of copy number changes. Array-CGH is based on the metaphase CGH methodology, but uses mapped sequences as the hybridisation target rather than metaphase chromosomes and hence the resolution is limited only by the density of the sequences spotted on the slides. Array-CGH was first introduced in 1997 [40] and since then a number of groups have set up their own facilities for spotting sequences onto slides. In addition, some companies have produced commercial chips ranging from specialist arrays containing 287 targets to 1Mb arrays containing around 3000 target clones. Array-CGH has the potential to provide a highly sensitive global assessment of gene copy number simultaneously screening hundreds or thousands of individual gene sequences, although its reproducibility and robustness needs to be thoroughly validated before it is used in the diagnostic setting. Fig. 5 clearly demonstrates the sensitivity of array-CGH, showing regions of simultaneous gain and loss along chromosome 13 in a case of retinoblastoma and giving a more precise definition than was achieved by metaphase CGH.

5. Gene expression profiling

The rapid advance of the Human Genome Project has led to the identification of most of the 30 000–35 000

genes that make up our genetic complement. The combination of this sequence information and advances in microarray technology has opened up the possibility of highly parallel genome-wide investigations of the cancer cell. Thus, it is now possible to investigate the transcriptional status of virtually every gene in a tissue sample leading to the concept of the expression profile or signature for a given tissue or tumour type. This approach is yielding highly detailed patterns that are characteristic of the cancer cell. Many aspects of cancer biology, including disease classification, chemosensitivity and prognosis can now be interpreteted in terms of these expression profiles [18]. As this technology improves and extends its coverage, we can expect even more detailed and informative maps of the cancer cell to emerge.

Since cancer itself involves the deregulation of gene expression, microarrays are becoming particularly valuable in the characterisation of such aberrant disruptions and have already demonstrated that previously unknown disease subtypes can be distinguished by their unique expression profiles. The expression profiles of many types of human cancer have been investigated, including colon [19–21], brain [22,23], breast [24–27], ovary [28–30], kidney [31] prostate [32–34] and gastric [35]. Many of these studies aim to define new classes with prognostic and diagnostic relevance and to increase our knowledge of the mechanisms underlying the biology of these diseases. The pathological diagnosis and classification of human neoplasia is currently based on well-defined morphological, cytochemical, immunophenotypic and clinical criteria. A molecular classification can take advantage of such prior knowledge, but would also have the potential to define new subgroups with greater prognostic and therapeutic significance and could offer many advantages over conventional classification methods.

The first classification of cancer based on the gene expression showed it was possible to distinguish between myeloid and lymphoid acute leukaemias, using arrays with approximately 6800 human genes [36]. Since then, the approach has been applied successfully to the classification of haematological malignancies and a large variety of solid tumours. Recently, ALLs with rearrangements to the *MLL* gene were shown to have expression patterns which could allow them to be distinguished from ALLs and AMLs without the *MLL* translocations [37]. Further microarray analysis of AML cases with a favourable outcome, AML M2 with t(8;21), AML M3 or M3v with t(15;17), and AML M4eo with inv(16), has shown a specific pattern of predictor genes associated with the three subclasses [38] In a subsequent microarray study, AML leukaemia samples were specifically chosen to represent the spectrum of known karyotypes common in AML and included examples with AML-FAB phenotypes from M1 to M5 [39]. Hierarchical clustering sorted the profiles into separate groups, each representing one of the major cytogenetic

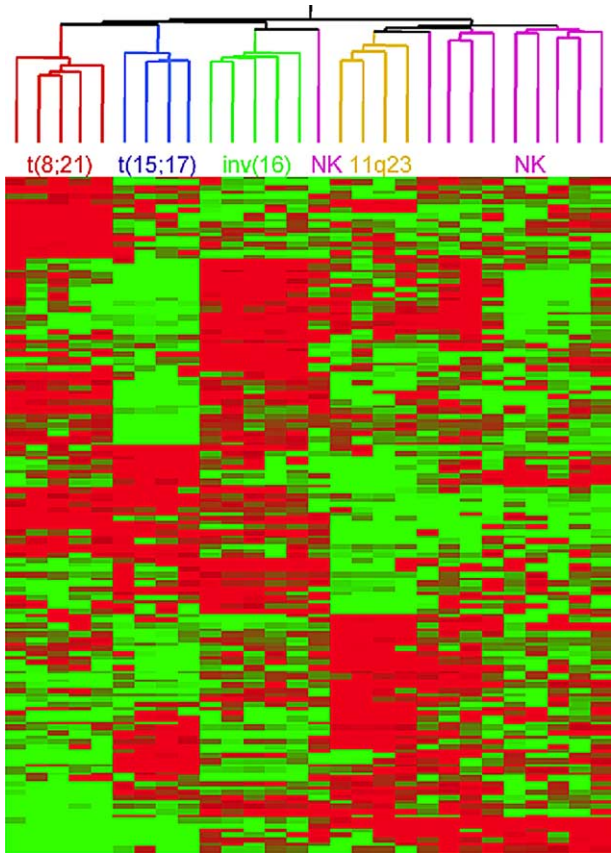

Fig. 6. Hierarchical cluster analysis of expression profiles of 28 cases of AML illustrates how samples with certain chromsomal abnormalities can be distinguished. This analysis is from Debernardi et al. [39] and is based on a list 145 genes which exhibited the greatest statistical power.

classes in AML, i.e., t(8;21), t(15;17), inv(16), 11q23, and normal karyotype as shown in Fig. 6. Statistical analysis identified genes whose expression was strongly correlated with these chromosomal classes. Importantly in this study, the normal karyotype AMLs were characterised by distinctive upregulation of certain members of the class I homeobox A and B gene families, implying a common underlying genetic lesion. These data reveal novel diagnostic and therapeutic targets and demonstrate the potential of microarray-based dissection of AML. The cluster analysis presented here illustrates the potential of expression profiling to distinguish the major subclasses. An important conclusion of expression profiling studies is that the major cytogenetic events in AML have associated expression signatures and this could form the basis of customised DNA arrays designed to classify leukaemia.

6. Concluding remarks

The combination of technical advances in molecular cytogenetics and the sequencing of the human genome

has revolutionised cancer genetics. It is possible to investigate any region of any chromosome in the minutest detail. Probes for any region are freely available and there is a continuum of investigation possible from the whole chromosome down to a single base. Genome-wide approaches are promising to further advance molecular cytogenetics. Patterns of gene expression can be used to detect the presence of chromosome translocations without having any dividing cells. CGH arrays can be used to detect copy number changes in a cancer cell. A major challenge for the future will be to integrate such approaches to yield a full picture of the cancer cell. If the rate of progress seen over the last 40 years continues, we can look forward to a much deeper understanding of the cancer process.

Acknowledgement

The authors thank Dr. N. Carter for Fig. 5.

References

1. Hsu TC. Mammalian chromosomes *in vitro* I. The karyotype of man. *J Hered* 1952, **43**, 167–172.
2. Tjio JH, Levan A. The chromosome number of man. *Hereditas* 1956, **42**, 1.
3. Stewart A, Webb J, Hewitt D. Aetiology of childhood malignancies. *Brit Med J* 1961, **1**, 452–460.
4. Levan A. Relation of chromosome status to the origin and progression of tumours. In: *Genetics and cancer*. London: Peter Owen Ltd; 1961.
5. Hauschka TS. Chromosome patterns in primary neoplasms. *Exp Cell Res* 1963, **9**, 86–98.
6. Makino S, Sasaki MS, Tonomura A. Cytological studies of tumours. XL. Chromosome studies of52 human tumours. *J Natl Cancer Inst* 1964, **32**, 741–777.
7. Spriggs AI, Boddington MM, Clarke CM. Carcinoma of the cervix uteri. *Lancet* 1962, **1**, 1383–1384.
8. Nowell PC, Hungerford DA. Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J Natl Cancer Inst* 1961, **27**, 1013–1035.
9. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973, **243**(5405), 290–293.
10. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci USA* 1982, **79**(24), 7824–7827.
11. Emanuel BS, Balaban G, Boyd JP, Grossman A, Negishi M, Parmiter A, *et al*. N-myc amplification in multiple homogeneously staining regions in two human neuroblastomas. *Proc Natl Acad Sci USA* 1985, **82**(11), 3736–3740.
12. Lele KP, Penrose LS, Stallard HB. Chromosome deletion in a case of retinoblastoma. *Am J Hum Genet* 1963, **27**, 171–173.
13. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, *et al*. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 1986, **323**(6089), 643–646.
14. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, *et al*. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML 10 trial. The medical research council adult and children's leukaemia working parties. *Blood* 1998, **92**(7), 2322–2333.
15. Speicher MR, Gwyn Ballard S, Ward DC. Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 1996, **12**(4), 368–375.
16. Schrock E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, *et al*. Multicolor spectral karyotyping of human chromosomes. *Science* 1996, **273**(5274), 494–497.
17. Veldman T, Vignon C, Schrock E, Rowley JD, Ried T. Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. *Nat Genet* 1997, **15**(4), 406–410.
18. Alizadeh AA, Ross DT, Perou CM, van de Rijn M. Towards a novel classification of human malignancies based on gene expression patterns. *J Pathol* 2001, **195**(1), 41–52.
19. Zou TT, Selaru FM, Xu Y, Shustova V, Yin J, Mori Y, *et al*. Application of cDNA microarrays to generate a molecular taxonomy capable of distinguishing between colon cancer and normal colon. *Oncogene* 2002, **21**(31), 4855–4862.
20. Lin YM, Furukawa Y, Tsunoda T, Yue CT, Yang KC, Nakamura Y. Molecular diagnosis of colorectal tumors by expression profiles of 50 genes expressed differentially in adenomas and carcinomas. *Oncogene* 2002, **21**(26), 4120–4128.
21. Notterman DA, Alon U, Sierk AJ, Levine AJ. Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. *Cancer Res* 2001, **61**(7), 3124–3130.
22. MacDonald TJ, Brown KM, LaFleur B, Peterson K, Lawlor C, Chen Y, *et al*. Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nat Genet* 2001, **29**(2), 143–152.
23. Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, *et al*. Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* 2002, **415**(6870), 436–442.
24. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, *et al*. Molecular portraits of human breast tumours. *Nature* 2000, **406**(6797), 747–752.
25. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, *et al*. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001, **98**(19), 10869–10874.
26. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, *et al*. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002, **415**(6871), 530–536.
27. Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, *et al*. Gene-expression profiles in hereditary breast cancer. *New Engl J Med* 2001, **344**(8), 539–548.
28. Jazaeri AA, Yee CJ, Sotiriou C, Brantley KR, Boyd J, Liu ET. Gene expression profiles of BRCA1-linked, BRCA2-linked, and sporadic ovarian cancers. *J Natl Cancer Inst* 2002, **94**(13), 990–1000.
29. Welsh JB, Zarrinkar PP, Sapinoso LM, Kern SG, Behling CA, Monk BJ, *et al*. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. *Proc Natl Acad Sci USA* 2001, **98**(3), 1176–1181.
30. Wang K, Gan L, Jeffery E, Gayle M, Gown AM, Skelly M, *et al*. Monitoring gene expression profile changes in ovarian carcinomas using cDNA microarray. *Gene* 1999, **229**(1–2), 101–108.
31. Takahashi M, Rhodes DR, Furge KA, Kanayama H, Kagawa S, Haab BB, *et al*. Gene expression profiling of clear cell renal cell carcinoma: gene identification and prognostic classification. *Proc Natl Acad Sci USA* 2001, **98**(17), 9754–9759.
32. LaTulippe E, Satagopan J, Smith A, Scher H, Scardino P, Reuter V, *et al*. Comprehensive gene expression analysis of prostate cancer

reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res* 2002, **62**(15), 4499–4506.
33. Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, *et al*. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 2001, **61**(16), 5974–5978.
34. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, *et al*. Delineation of prognostic biomarkers in prostate cancer. *Nature* 2001, **412**(6849), 822–826.
35. Hippo Y, Taniguchi H, Tsutsumi S, Machida N, Chong JM, Fukayama M, *et al*. Global gene expression analysis of gastric cancer by oligonucleotide microarrays. *Cancer Res* 2002, **62**(1), 233–240.
36. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, *et al*. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999, **286**(5439), 531–537.
37. Armstrong SA, Staunton JE, Silverman LB, Pieters R, den Boer ML, Minden MD, *et al*. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet* 2002, **30**(1), 41–47.
38. Schoch C, Kohlmann A, Schnittger S, Brors B, Dugas M, Mergenthaler S, *et al*. Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. *Proc Natl Acad Sci USA* 2002, **99**(15), 10008–10013.
39. Debernardi S, Lillington D, Chaplin T, Tomlinson S, Amess JA, Rohatiner AZ, *et al*. Prognostically important chromosomal events in acute myeloid leukaemia can be deduced from gene expression analysis. *Genes Chromosomes Cancer* 2002, **37**(2), 149–158.
40. Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, Cremer T, Lichter P. Matrix-based comparative genomie hybridisation biochips to screen for genomie imbalances. *Genes Chromosomes Cancer* 1997, **20**(4), 399–407.