REVIEW ARTICLE

Cytogenetics: Past, Present And Future

Thirumulu Ponnuraj Kannan, Zilfalil Bin Alwi

Human Genome Centre, Universiti Sains Malaysia Health Campus, Jln Raja Perempuan Zainab II, 16150 Kubang Kerian, Kelantan, Malaysia

Submitted: 11 Nov 2007
Accepted: 7 Apr 2009

Abstract

Fifty years have elapsed since the discovery of the number of human chromosomes in 1956. Newer techniques have been developed since then, ranging from the initial conventional banding techniques to the currently used molecular array comparative genomic hybridisation. With a combination of these conventional and molecular techniques, cytogenetics has become an indispensable tool for the diagnosis of various genetic disorders, paving the way for possible treatment and management. This paper traces the history and evolution of cytogenetics leading up to the current state of technology.

Keywords: *Cytogenetics, Chromosomes, medical sciences*

History of cytogenetics

It was the Swiss botanist Nageli who first described thread-like structures in the nuclei of plant cells in the 1840s, and what he called "transitory cytoblasts" are now known as chromosomes. Later, in 1888, Waldeyer coined the term "chromosome" after staining techniques had been developed to make them more discernible (chromos = Greek for colour; soma = Greek for body). Cytogenetics is the study of the structure and properties of chromosomes, their behaviour during somatic cell division during growth and development (mitosis), and germ cell division during reproduction (meiosis), as well as their influence on phenotype. Cytogenetics also includes the study of factors that cause chromosomal changes (1).

Initially, it was difficult to determine the diploid number of mammalian species because the chromosomes were crowded in metaphase. In the 1950s, several technical improvements, such as the addition of colchicines to arrest cells in metaphase and the use of hypotonic solution to obtain better chromosome spreads, were made (2,3,4). In 1956, the diploid number of chromosomes in man was established as 46 (5), and the peripheral leucocyte culture method of Moorehead et al. (6) was adopted by many cytogeneticists. It was then possible to describe correctly the normal human chromosome number and chromosome abnormalities. This enabled detection of numerical chromosome aberrations like trisomy 21 in Down syndrome (7), 45, X in Turner syndrome (8), 47, XXY in Klinefelter syndrome (9), trisomy 13 (10), trisomy 18 (11), and Philadelphia chromosome in a patient with chronic myeloid leukaemia (12). It was also reported that cells cultured from amniotic fluid could be used to determine the chromosome content of the foetus (13). The metaphase chromosomes were classified into seven groups based on the Denver classification (1960) (14), with revisions at the London Conference (1963) (15) and the Chicago Conference (1966) (16). Jau-hong Kao et al. (17) described a chromosome classification based on the band profile similarity along the approximate medial axis.

Advent of banding techniques

Caspersson et al. (18) discovered one of the first chromosome banding techniques (Q-banding), which involved staining chromosomes with a fluorochrome, such as quinacrine mustard or quinacrine dihydrochloride, and examining them with fluorescence microscopy. This technique, however, was less than optimal for routine studies, as the fluorescent staining quickly quenched. Hence, several other banding techniques were developed, for example, G-, R-, C- and NOR banding, each having its own specific properties and applications (19).

In due course, G-banding, produced by staining the chromosomes with Giemsa solution, became the most frequently used method (20). This gave better resolution than Q-banding, allowed permanent preparations, and did not necessitate the use of fluorescence microscopy. Pardue and Gall (21) first reported C-bands in 1970, when they discovered that the centromeric region of mouse chromosomes is rich in repetitive DNA sequences and stains darkly with Giemsa. C-bands localise in the heterochromatic regions of chromosomes. Many chromosomes have regions that differ among individuals but have no pathological importance. These polymorphic regions can be visualised optimally with C-band methods. C-banding is also useful to show chromosomes with multiple centromeres, to study the origin of diploid molar pregnancies and true hermaphroditism, and to distinguish between donor and recipient cells in bone marrow transplantation. Nucleolar organising region (NOR)-banding is a technique that stains NORs of chromosomes (22). These regions are located in the satellite stalks of acrocentric chromosomes and house genes for ribosomal RNA. Goodpasture et al. (23,24) developed a simple silver nitrate staining technique for NOR-banding that is useful in clinical practice to study certain chromosome polymorphisms, such as double satellites.

However, the resolution of chromosome studies remained relatively limited because the total number of bands produced on metaphase chromosomes was low, and it was difficult to detect rearrangements involving small portions of chromosomes due to excessive condensation. This situation was improved by the development of high-resolution banding by Yunis (25), which was achieved by synchronising the lymphocyte cultures and obtaining more cells in pro-metaphase or even prophase. High resolution cytogenetics provided greater precision in the delineation of chromosomal breakpoints and assignment of gene loci than earlier techniques could since analysis of late prophase sub-banding reveals more than twice the number of bands seen at metaphase (26). By applying this technique, several well-known clinical syndromes, like Prader-Willi and Angelman syndromes with deletions at the proximal long arm of chromosome 15, Smith-Magenis and Miller-Dieker syndromes with (different) deletions in the short arm of chromosome 17, and DiGeorge/Velo Cardio Facial (VCF) syndromes with deletions in the long arm of chromosome 22, could be linked to small chromosome aberrations, and the concept of the micro-deletion or contiguous gene syndrome was born (27).

The choice of banding technique

For routine analysis, however, the G-banding technique using trypsin and Giemsa became the most accepted worldwide (28). The banding pattern enabled the detection of various structural aberrations like translocations, inversions, deletions, and duplications in addition to the well-known numerical aberrations. This led to the cytogenetic investigation of healthy family members of known carriers and of couples suffering from repetitive spontaneous abortions (29).

Specialised techniques to visualise chromosomes

Sister Chromatid Exchange (SCE)

SCE enabled visualisation of interchanges between brightly and dully fluorescent segments of sister chromatids. This was made possible by incorporating BrdU (in place of thymidine) into replicating cells for 2 cell cycles. The biologic importance of SCEs is uncertain, but some mutagens and carcinogens increase their frequency (30). It has been noted there is an increase of SCE in patients with ankylosing spondylitis (31), in smokers (32), in women after exposure to biomass fuels (33), and in patients with carcinoma of the cervix uteri (34).

Fragile sites and chromosome breakage

Gaps that are consistently seen at the same chromosome locus are called fragile sites. Some fragile sites are associated with specific medical conditions such as Fragile X Syndrome, which is associated with a fragile site at Xq27.3 (35,36). All humans experience increased chromosome breakage when exposed to cytotoxic agents, but in certain autosomal recessive disorders, the inability to repair DNA is associated with certain kinds of chromosome damage (37). The detection of a fragile site in a patient often causes concerns due to its potential significance, and it must be followed-up properly with genetic counselling (38).

Molecular cytogenetics

Fluorescent in situ Hybridisation (FISH)

In 1986, Pinkel et al. (39,40) developed a method to visualise chromosomes using fluorescent-labelled probes called fluorescent in situ hybridisation (FISH). FISH technology permits the detection of specific nucleic acid sequences in morphologically preserved chromosomes, cells, and tissues. These techniques are useful in the work-up of patients with various congenital and malignant

neoplastic disorders, especially in conjunction with conventional chromosome studies. Using FISH, cytogeneticists can detect chromosomal abnormalities that involve small segments of DNA if their probe is situated, fortuitously or by design, in the affected chromosomal segment (41). FISH can be used to establish the order of DNA clones relative to bands, naturally occurring breakpoints, and other clones. Even more importantly, FISH permits karyotype analysis of nuclei in non-dividing cells. FISH has been used for the detection of t(2;5) (p23;q35) translocation in anaplastic large-cell lymphoma (42), for minimal residual disease in haematopoietic stem cell assays from peripheral blood stem cells of acute myeloid leukaemia (AML) patients with trisomy 8 (43), and for analysing chromosomal abnormalities of tumours in children (44).

Spectral Karyotyping (SKY) and Multicolour FISH (M-FISH)

FISH paved the way for a more powerful technology called spectral karyotyping (SKY) or multicolour FISH (M-FISH). M-FISH allows all of the 24 human chromosomes to be painted in different colours. By making use of various combinations and concentrations of fluorescent dyes, it is even possible to give every single chromosome a different colour (SKY), which can be of particular use when dealing with complex aberrations often associated with various types of solid tumours. SKY or M-FISH enables production of chromosome-specific 'paints'. Fluorochromes are combined to produce 24 colour combinations, one for each chromosome (45), resulting in multicolour analyses. SKY paints the entire chromosome in the same colour, whereas M-FISH uses various fluorescence dyes to represent different painting probes at the same time. This offers the simultaneous presentation of all 24 different human chromosomes with a single hybridisation. SKY and M-FISH have proven to be extremely useful in detecting translocations and other complex chromosomal aberrations. The main applications for M-FISH have been in solid tumours, which are often characterised by complex karyotypes, and in AML and acute lymphoblastic leukaemia (46).

Comparative Genomic Hybridisation (CGH)

FISH investigations have proven to be advantageous in many ways, but they are time consuming because preparations must be hybridised and then microscopically analysed. These problems led to the development of a variation of FISH called CGH (47). Later, a further improvement was developed an array based on comparative genomic hybridisation (48, 49). CGH does not require the preparation of metaphase chromosomes from cells. Instead of hybridising a labelled probe to human chromosomes on a slide, it is now possible to print thousands of different and well-characterised probes on a glass slide. The array-CGH is even more promising than the conventional CGH (50). Array-CGH is the equivalent of conducting thousands of FISH experiments at once, and it provides better quantification of copy number and more precise information on the breakpoints of segments that are lost or gained than does conventional CGH. It is faster and has a better resolution than available molecular cytogenetic tools (51).

Cancer cytogenetics

The involvement of chromosomal aberrations and the deviation from the normal copy number of a given chromosome (aneuploidy) in tumours have long been known. For example, balanced chromosomal translocations can have oncogenic effects through the production of fusion proteins. In the case of chronic myelogenous leukaemia (CML), 95% of the cases harbour a translocation between chromosomes 9 and 22, which results in the formation of what is commonly referred to as the Philadelphia chromosome. Glivec is the main treatment for CML, which is a biological treatment that targets a protein made by CML cells. Glivec is a type of growth blocker, called a tyrosine kinase inhibitor (TKI). Tyrosine kinases are a group of proteins that cells use to signal to each other to grow. This drug stops the messages from these proteins getting to cancer cells and thus interferes with their growth. Routine conventional cytogenetics is used to monitor the progress of this treatment. In most people, there is a major drop in the number of cells that carry the Philadelphia chromosome. As a consequence, the implementation of cytogenetic analyses, at least at diagnosis, is mandatory for analysing the outcomes of many clinical trials, and it can also be used to stratify patients for different types of therapy (52,53).

The current situation and the future

Currently, cytogeneticists are developing molecular approaches for deciphering the structure, function and evolution of chromosomes. Conventional cytogenetics using regular banded chromosomal analysis remains a simple and popular technique to get an overview of the human genome. Routine banded karyotype

analysis can now be combined with M-FISH and various other molecular techniques, leading to more precise detection of various syndromes in children. The combination of CGH (54) with multicolour FISH was seen from the beginning to be a powerful combination for 7characterising complex karyotypes (55,56,57,58). More recently, microarray-based formats using large insert genomic clones, cDNAs or oligonucleotides have replaced metaphase chromosomes as DNA targets (49), providing higher resolution and the ability to directly map the copy number changes to the genome sequence. In other words, chromosomal abnormalities exist as nature's guide to the molecular basis of many unexplained human disorders. Thus, techniques of cytogenetics are bound to continue to be indispensable tools for diagnosing genetic disorders and indicating possible treatment and management.

Correspondence

Dr. Kannan Thirumulu Ponnuraj
BVSc (Chennai), MVSc, PhD (Chennai)
Human Genome Centre/School of Dental Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan, Malaysia
Tel : +609 766 3684
Fax : +609 764 2026
E-mail: tpkannan@kb.usm.my

References

1. Hare WCD, Singh EL. Cytogenetics in Animal Reproduction. Slough: Commonwealth Agricultural Bureaux, UK. 1979.

2. Makino S, Nishimura E. Water pretreatment squash technic; a new and simple practical method for the chromosome study in animals. *Stain Technol*. 1952; **27**: 17.

3. Hsu TC, Pomerat CM. Mammalian chromosome in vitro: II. A method for spreading the chromosomes of cells in tissue cultures. *J Heredity*. 1953; **44**:23-29.

4. Ford CE, Hammerton JL. A colchicines hypotonic citrate squash sequence for mammalian chromosomes. *Stain Technol.*, 1956; **31**:247-252.

5. Tjio JH, Levan A. The chromosome number in man. *Hereditas*. 1956; **42**:16.

6. Moorehead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res*., 1960; **20**:613-616.

7. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongliens. *Comptes Rendus*., 1959; **248**:1721-1722.

8. Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet*. 1959; **1**: 711-713.

9. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature*. 1959;**183**:302-303.

10. Patau K, Smith DW, Therman E, Inhorn SL, Wagner HP. Multiple congenital anomaly caused by an extra autosome. *Lancet*. 1960;**1**:790-793.

11. Edwards JH, Harnden DG, Cameron AH, Crosse VM, Wolff OH. A new trisomic syndrome. *Lancet*. 1960;**1**: 787-790.

12. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science*. 1960;**132**:1497-1501.

13. Steele MW, Breg WR. Chromosome analysis of human amniotic-fluid cells. *Lancet*. 1966;**1**:383-385.

14. Denver Conference. The identification of individual chromosomes especially in man. *Am J Hum Genet*. 1960;**12**:384-389.

15. Hamerton JL, Klinger HP, Mutton DE, Lang EM. The London Conference on the normal human karyotype, 1963 August 2830; *Cytogenetics*.1963;**25**:264-268.

16. Chicago Conference: Standardization in Human Cytogenetics. Birth defects: Original Article Series, 11: 2, 1966. New York, The National Foundation.

17. Kao JH, Chuang JH, Wang T. Chromosome classification based on the band profile similarity along approximate medial axis. *Pattern Recogn*. 2008;**41**:77-89.

18. Caspersson T, Zech L, Johansson C. Differentia l binding of alkylating fluorochromes in human chromosomes. *Exp Cell Res*. 1970;**60**:315-319.

19. Rooney DE, Editor. Human cytogenetics: constitutional analysis. New York: Oxford University Press; 2001.

20. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973; **273**:290-293.

21. Pardue ML, Gall JG. Chromosomal localization of mouse satellite DNA. *Science*. 1970;**168**:1356-1358.

22. Matsui S, Sasaki M. Differential staining of nucleolus organisers in mammalian chromosomes. *Nature*. 1973;**246**:148-150.

23. Goodpasture C, Bloom SE, Hsu TC, Arrighi FE. Human nucleolus organizers: the satellites or the stalks? *Am J Hum Genet*. 1976;**28**:559-566.

24. Goodpasture C, Bloom SE. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma*. 1975; **53**:37-50.

25. Yunis JJ. High resolution of human chromosomes. *Science.*, 1976; **191**: 1268-1270.

26. Sawyer JR, Hozier JC. High resolution of mouse chromosomes: banding conservation between man and mouse. *Science*. 1986;**232**:1632-1639.

27. Schmickel RD. Contiguous gene syndromes: a component of recognizable syndromes. *J Pediatr*. 1986;**109**:231-241.

28. Seabright M. A rapid banding technique for human chromosomes. *Lancet*. 1971;**2**: 971-972.

29. Smeets DF. Historical perspective of human cytogenetics: from microscope to microarray. *Clin Biochem.,* 2004;**37**:439-446.

30. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature*. 1975;**258**:121-125.

31. Sönmez S, Sqenel K, Öztas S, Erdal A, Cerrahoglu L. Sister chromatid exchange analysis in the lymphocytes of patients with ankylosing spondylitis. *Ann Rheum Dis.*, 1997;**56**:275-277.

32. Lambert B, Bredberg A, McKenzie W, Sten M. Sister chromatid exchange in human populations: the effect of smoking, drug treatment, and occupational exposure. *Cytogenet Cell Genet*. 1982; **33**:62-67.

33. Sungu YS, Cinar Z, Akkurt I, Özdemir O, Seyfikli Z. Sister-chromatid Exchange Frequency in Women Who Exposed to Biomass in a Village of Central Anatolia. *Turk Resp J*. 2001;**2**:26-28.

34. Dhillon VS, Kler RS, Dhillon IK. Chromosome instability and sister chromatid exchange (SCE) studies in patients with carcinoma of cervix uteri. *Cancer Genet Cytogenet*. 1996;**86**: 54-57.

35. Lubs HA. A marker X chromosome. *Am J Hum Genet*. 1969;**21**:231-244.

36. Sutherland GR. Heritable fragile sites on human chromosomes, II: distribution, phenotypic effects, and cytogenetics. *Am J Hum Genet*. 1979; **31**:136-148.

37. Spurbeck JL, Adams SA, Stupca PJ, Dewald GW. Primer on Medical Genomics Part XI: Visualizing Human Chromosomes. *Mayo Clin Proc.*, 2004;**79**: 58-75.

38. Karadeniz NN, Tunca Y, Imirzalioglu N. New heritable fragile site at 15q13 in both members of a nonconsanguineous couple. *Am J Med Genet A*. 2003; **118(3)**:290-292.

39. Pinkel D, Gray JW, Trask B, van den Engh G, Fuscoe J, van Dekken H. Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. *Cold Spring Harb Symp Quant Biol*. 1986; **51**: 151-157.

40. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA*. 1986;**83**: 2934-2938.

41. Dewald GW. Interphase FISH studies of chronic myeloid leukemia. *Methods Mol Biol*. 2002;**204**:311-342.

42. Johnson WM, Leek J, Swinbank K, Angus B, Roberts P, Markham AF, et al. The use of fluorescent in situ hybridization for detection of the t(2;5)(p23;q35) translocation in anaplastic large-cell lymphoma. *Ann Oncol*. 1997;**8**: S65-S69.

43. Dodge W, Cruz J, Zamkoff K, Hurd D, Pettenati MJ. Use of Fluorescence In Situ Hybridization to Detect Minimal Residual Disease in Hematopoietic Stem Cell Assays from Peripheral Blood Stem Cells of 2 Patients with Trisomy 8 Acute Myeloid Leukemia. *Stem Cells Dev*. 2004; **13(1)**:23-26.

44. Raimondi SC. Fluorescence in situ hybridization: Molecular probes for diagnosis of pediatric neoplastic diseases. *Cancer Invest*. 2000;**18**:135-147.

45. Ried T, Landes G, Dackowski W, Klinger K, Ward DC. Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells. *Hum Mol Genet*. 1992;**1**:307-313.

46. Kearney L. Multiplex-FISH (M-FISH): technique, developments and applications. *Cytogenet Genome Res*. 2006;**114**:189-198.

47. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*. 1992;**258**:818-821.

48. Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Döhner H, et al. Matrix-based comparative genomic hybridization: biochips to screen for genomicimbalances. *Genes Chromosomes Cancer*. 1997; **20**:399-407.

49. Albertson D, Pinkel D. Genomic microarrays in human genetic disease and cancer. *Hum Mol Genet*. 2003;**12**: 145-152.

50. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nature Genet*. 1998;**20**:207-211.

51. Sanlaville D, Lapierre JM, Turleau C, Coquin A, Borck G, Colleaux L, et al. Molecular karyotyping in human constitutional cytogenetics. *Eur J Med Genet*. 2005;**48**: 214-231.

52. Hahna HP, Fletchera CDM. The role of cytogenetics and molecular genetics in soft tissue tumour diagnosis-a realistic appraisal. *Curr Diagn Pathol.*, 2005;**11**:361-370.

53. Ferrara F, Palmieri S, Leoni F. Clinically useful prognostic factors in acute myeloid leukemia, Crit Rev Oncol Hemat., 2008 Jun;66(3):18193. Epub 2007 Nov 8.

54. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*. 1992;**258**:818-821.

55. Tosi S, Giudici G, Rambaldi A, Scherer SW, Bray-Ward P, Dirscherl L, et al. Characterization of the human myeloid leukemia-derived cell line GF-D8 by multiplex fluorescence in situ hybridization, subtelomeric probes, and comparative genomic hybridization. *Genes Chrom Cancer*. 1999;**24**:213-221.

56. Speicher MR, Petersen S, Uhrig S, Jentsch I, Fauth C, Eils R, et al. Analysis of chromosomal alterations in nonsmall cell lung cancer by multiplex-FISH, comparative genomic hybridization, and multicolor bar coding. *Lab Invest*., 2000;**80**: 1031-1041.

57. Micci F, Teixeira MR, Bjerkehagen B, Heim S. Characterization of supernumerary rings and giant marker chromosomes in well-differentiated lipomatous tumors by a combination of G-banding, CGH, M-FISH, and chromosome- and locus-specific FISH. *Cytogenet Genome Res*. 2002;**97**:13-19.

58. Van Gele M, Leonard JH, Van Roy N, Van Limbergen H, Van Belle S, Cocquyt V, Salwen H, De Paepe A, Speleman F. Combined karyotyping, CGH and M-FISH analysis allows detailed characterization of unidentified chromosomal rearrangements in Merkel cell carcinoma. *Int J Cancer*. 2002;**101**:137-145.