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Advances in Cytogenetics

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Chromosomal abnormalities are a major cause of genetic disease. It accounts for a large proportion of reproductive wastage, congenital malformations, mental retardation and most importantly, in the pathogenesis of cancer. Chromosomal study, also conventionally known as cytogenetics is indicated to diagnose known chromosomal syndrome, multiple malformations, unexplained psychomotor retardation (with or without dysmorphism), abnormalities of sexual differentiation and development, infertility, monogenic disorder associated with dysmorphism, cancer, recurrent pregnancy loss, pregnancy at risk for aneuploidy (prenatal/preimplantation/preconception) and in coming days for neuropsychiatric disorders, neurodegenerative disorders, microdeletion/microduplication syndromes and so on. Chromosomal study is also indicated for comparative mammalian cytogenetics, chromosome evolution, localization of disease gene, detection of chromosome breakage and mutagenicity study.

Chromosomal study or cytogenetics conventionally means study of chromosomes by microscopy. However, in broader sense, it is a branch of genetics devoted to cellular constituents concerned in heredity i.e., chromosome, DNA, gene, episome, etc. Present form of conventional cytogenetics is evolved over years from accidental identification of correct human chromosome number as 46 through cell culture and microscopy [1]. Cytogenetic analysis by conventional chromosomal banding techniques, although highly precise and an important standard method, requires cell culture, skilled personnel and is labor intensive. Conventional cytogenetics is ineffective in analyzing complex karyotype, especially those of solid tissue cancer and often identification of marker chromosomes as well as telomeric rearrangements. Further, conventional cytogenetics may not reflect all the changes in cancers due to clonal selection in culture. Similarly, conventional cytogenetics is difficult to study chromosomes in single/few/condensed cells like blastomere (as in preimplantation diagnosis), polar body and gamete (oocyte and sperm). Conventional cytogenetics is possible only in live/dividing cells besides limited resolution (5-10 Mb, however can be reached to 2-3 Mb in pro-metaphase/long chromosome preparation). These drawbacks of conventional cytogenetics have led investigators to seek newer approaches for identifying chromosomal abnormalities.

Advances in human cytogenetics are largely due to innovations in molecular biology and instrumentation technology. Present form of molecular cytogenetics has matured into a multidisciplinary science that based heavily on molecular biology, bioinformatics and technological advances. The resolution has improved to the extent of single/few base pair/s and sensitivity by many folds. Most importantly, present cytogenetic advances have been freed from the dependence of cell culture. Present wave is now moving from microscope to DNA chips and/or sequencing for identification of submicroscopic chromosome alterations. These molecular techniques are now providing far more information at higher resolution than just chromosome number and morphology. These newer techniques can be applied throughout cell cycle, in non-dividing cells, dead cells and fixed cells [2]. This determines not only the presence of a particular DNA/RNA sequence, but also it provides information on mosaicism, parental origin of chromosomes as well as maternal contamination in prenatal diagnostic situation. High sensitivity, specificity, speed and the ability to provide information on single cell (e.g., interphase FISH) have made these newer

molecular approaches a powerful tool in modern cytogenetics. Newer molecular cytogenetic techniques (e.g., next generation sequencing) now can identify breakpoints as well as balanced chromosome structure alterations. Other important advantages over conventional cytogenetics include diagnosis of microdeletion (sub microscopic) and microduplication syndromes (copy number variations), identification of markers, etc. Present form of cytogenetics/molecular cytogenetics (should be named as cytogenomics) is now extracts far more information about the human genome than just chromosome number and morphology.

Molecular cytogenetic approaches used widely are conventional Fluorescence in situ Hybridization (FISH), spectral FISH karyotyping (SKY FISH), multiplex FISH karyotyping (M FISH), fiber FISH, comparative genomic hybridization (CGH), Primed in situ Labeling/Synthesis (PRINS), quantitative fluorescent PCR (QF-PCR), array CGH/SNP microarray, next generation sequencing (NGS)/massive parallel sequencing, etc. These techniques expanded the possibilities for precise genetic diagnosis, which are extremely important for clinical management of patients as well as research. This new wave is now on chromosome analysis from microscope to automated SNP microarray/NGS for identification of chromosomal abnormality beyond cytogenetic resolution. The microarray/NGS based molecular karyotyping has become the primary choice to direct analysis of all chromosomes/genes in one action without subjective nature, without restriction to cytogenetic experts and difficult cell culture, and above all for any indications.

FISH is the oldest molecular cytogenetic technique used to detect and localize presence or absence of specific DNA sequence on chromosomal/nuclear DNA. It uses fluorescent probes (a labeled nucleic acid sequence) that bind to only those parts of DNA that have high degree of sequence homology. Fluorescent molecules (FITC, TRITC, Cy 3, Cy 5, etc) are directly attached (enzymatic or chemical bonding methods) to the probe so that probe-target hybrid can be visualized under fluorescent microscope immediately after hybridization reaction. The probe can be cloned DNA sequence, or generated by PCR from flow-sorted chromosomes, micro-dissected chromosomes, whole genome or even oligonucleotide based combinations. Probes may be from alphoid sequence of centromeres (150-250 bp repeats), telomeric/subtelomeric sequence (5-6 bp repeats), or unique sequence (100-500 Kb), whole chromosome/one arm (p or q arm) of a chromosome, or even whole genomic DNA. Test and probe DNA is annealed following denaturation and visualized by fluorescence microscopy. Identification

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of targeted regions was made possible by direct observation based on DNA sequence location, rather than on morphology and banding patterns. It is a powerful tool for analyzing genes and chromosomes because of its high sensitivity and ability to provide information at single gene/cell level besides detecting cell-cell heterogeneity and mosaicism. Further, FISH has been modified to carry out spectral karyotyping/multiplex FISH to identify all 24 chromosomes in one experiment and comparative genomic hybridization (CGH) as well as array CGH (aCGH) to screen whole genome. The major application of FISH is in the field of cancer followed by prenatal diagnosis, preimplantation diagnosis, microdeletion-microduplication syndrome, etc. Detection of cancer-specific chromosome abnormality such as Philadelphia chromosome (bcr-abl fusion gene) assists in diagnosis/sub-classification of disease for selection of appropriate treatment besides more precise prognosis. It can also be used for monitoring of minimal residual disease, early relapse and engraftment of sex-mismatched allogeneic bone marrow transplant. Prenatal fetal chromosome analysis by conventional cytogenetics from amniotic fluid cells requires long time. Long delay in conventional method is un-acceptable to most parents and obstetricians in second half of pregnancy following ultrasound detected malformations or abnormal serum screening report because, in many countries, legal limit of pregnancy termination is 20-22 weeks. FISH is capable of providing the answer quickly (within 24 hours) in these situations, thus reduce parental anxiety, and guide obstetric management quickly. In PGD, typically one or two blastomeres are removed (biopsied) from 6-8 cell stage embryo and subjected to FISH for aneuploidy screening, translocation identification or sexing for X linked diseases. FISH is often extremely helpful in specific postnatal situations. FISH on interphase cells can be carried out within few hours in a situation like ambiguous genitalia at birth where immediate assignment of sex is required for not only social reason but also for appropriate management. It has similar value in acutely sick baby with congenital malformation syndrome suggestive of aneuploidy or microdeletion syndrome requiring urgent intensive care. FISH can be informative in all other situations where conventional cytogenetics is not possible. FISH is the main modality of chromosome analysis for meiotic chromosome segregation analysis, microdeletion syndrome diagnosis and testing on formalin fixed tissue.

CGH is a FISH technique that allows comprehensive analysis of chromosomal imbalance (relative copy number as gains or losses) in entire genome (molecular karyotyping at cytogenetic resolution) by single test without metaphase preparation and cell culture of the test samples. The strategy of CGH is based on co-hybridization of differentially labeled whole genomic test and control DNA in equal ratio on normal metaphase spread, like dual FISH. After standard washing, the slide is visualized using epifluorescence microscope. Image processing along with quantification of fluorescence intensities over the entire length of each karyotyped chromosome is done by computer software. Hybridization results into general staining of all chromosomes. If the test DNA contains additional copies of DNA material, hybridization will reveal higher signal intensity of test DNA (if labeled with green, then more green) at the corresponding target region of the hybridized chromosome. Similarly, deletion/monosomy will give rise to lower signal intensities. However, CGH cannot detect mosaicism of less than 50%, balanced translocation and polyploidy/tetraploidy. CGH although simple as well as cheap to perform but is labor intensive, time consuming and difficult to interpret (as karyotyping of DAPI banded chromosome is inaccurate), hence, getting replaced by array CGH.

Array CGH is carried out on the principle of CGH with some

modifications (co-hybridization is carried out on DNA spots/arrays (BAC/PAC/oligonucleotides) rather than metaphase chromosomes). Following hybridization of differentially labeled test and reference genomic DNA to the target sequences on the microarray, the slide is scanned to measure fluorescence intensities at each target on the array. The normalized fluorescent ratio for the test and reference DNA is then plotted against the position of the sequence along the chromosomes (as in CGH). Gains or losses across the genome are shown by values higher or lower than normal 1:1 ratio. The resolution depends on the size, number of targets and position of targets on the genome. This procedure is rapid (24-36 hours), provides whole genome view at a very high resolution, and does not depend on live cell, or cell culture. It detects majority of microscopic as well as sub-microscopic chromosomal changes from any DNA source in single experiment without prior knowledge of abnormalities. The only limitation is its inability to detect polyploidy or balanced chromosome abnormalities as with CGH. It is already recommended by several authorities as first line of test in evaluating multiple malformations, developmental/mental retardation, prenatal diagnosis, etc. Another important application of aCGH is in the field of cancer, which has enormous potential. SNP microarray is further refinement of aCGH to detect mosaicism, parental origin, zygosity, translocation (theoretically at present), etc besides better resolution (<100 kb).

NGS/massive parallel sequencing technology utilizes amplified or single molecule templates for sequencing massive parallel fashion. This increases throughput by several orders of magnitude. There are three main levels of sequencing analysis viz., targeted gene, exome and genome. It detect almost all principal types of genome alterations, including nucleotide substitutions, small insertions and deletions, copy number alterations, mosaicism, parental inheritance, chromosomal rearrangements including balanced translocation and microbial/foreign DNA integration. This method is becoming a serious challenge for conventional cytogenetics (balanced translocation detection advantage of conventional cytogenetics is also taken care by NGS) as this does not require cell culture, subjective nature of interpretation and provides more information quickly than conventional cytogenetics and hence NGS are now being widely adopted in clinical settings.

This century is observing a paradigm shift from cytogenetics to cytogenomics. NGS and SNP microarray based genome analysis are becoming acceptable tools for research and patient care. We are going to witness gradual fall of conventional cytogenetics usages in near future.

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