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Principle's and Role of Cyto-Genomic Hybridization in Biomarkers

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DESCRIPTION

CGH is a molecular cytogenetic method for analysing Copy Number Variations (CNVs) relative to ploidy level in the DNA of a test sample compared to a reference sample without the need for cell culture. The goal of this technique is to quickly and efficiently compare two genomic DNA samples derived from two sources that are often closely related, because differences in whole chromosome or sub chromosomal region gains or losses are suspected (a portion of a whole chromosome). This technique was originally developed to assess the differences between the chromosomal complements of solid tumour and normal tissue, and it has a higher resolution of 5-10 megabases when compared to more traditional cytogenetic analysis techniques such as giemsa banding and fluorescence *in situ* hybridization (FISH), which are limited by the resolution of the microscope used. This is accomplished by employing competitive fluorescence *in situ* hybridization. In short, this entails isolating DNA from the two sources to be compared, most commonly a test and a reference source, and labelling each DNA sample independently with fluorophores (fluorescent molecules) of various colours (usually red and green), denaturation of the DNA to make it single-stranded, and hybridization of the two resultant samples in a 1:1 ratio to a normal metaphase spread of chromosomes, to which the labelled DNA samples will bind at their locus of origin. The differentially coloured fluorescent signals are then compared along the length of each chromosome using fluorescence microscope and computer software to identify chromosomal differences between the two sources. A higher intensity of the test sample colour in a specific region of a chromosome indicates a gain of material from that region in the corresponding source sample, whereas a higher intensity of the reference sample colour indicates a loss of material from that region in the corresponding source sample. CGH can only detect unbalanced chromosome abnormalities. This is due to the fact that balanced chromosomal abnormalities such as reciprocal translocations, inversions, or ring chromosomes do not affect copy number, which is what CGH technologies detect. CGH, on the other hand, allows for the exploration of all 46 human chromosomes in a single test, as well as the discovery of deletions and duplications on a microscopic scale, which may lead to the identification of candidate genes to be further, investigated using other cytological techniques. The more specific form of array CGH (aCGH) has been developed through the use of DNA microarrays in conjunction with CGH techniques, allowing for a locus-by-locus measure of CNV with increased resolution as low as 100 kilobases. The aetiology of known and unknown conditions can now be discovered using this improved technique. The DNA on the slide is a reference sample, obtained from a karyotypically normal man or woman, though female DNA is preferred because it contains two X chromosomes, which contain far more genetic information than the male Y chromosome.

CONCLUSION

Colchicine is used to stop the cells from entering mitosis. The cells are then harvested and treated with hypotonic potassium chloride before being fixed in a 3:1 methanol/acetic acid solution. One drop of the cell suspension should then be dropped from a distance of about 30 cm onto an ethanol cleaned slide; ideally, this should be done at room temperature with humidity levels of 60%-70%. Slides should be evaluated using a phase contrast microscope, with minimal cytoplasm observed, and chromosomes that are 400-550 bands long with no separated chromatids and finally appear dark rather than shiny. The slides should then be air dried overnight at room temperature before being stored in groups of four at 20°C with either silica beads or nitrogen present to maintain dryness. Because hybridization can vary, different donors should be tested. Although commercially available slides may be used.

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CONFLICT OF INTEREST

The author's declared that they have no conflict of interest.

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