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Commentary

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Comparative Genomic Hybridization (CGH) in Cancer Research

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Abstract: Cancer is a multifactorial and multistep process. Conventional cytogenetics has become a major working field in cancer research. Chromosome rearrangements are recognized as critical in the pathogenesis of human cancer. Molecular biology and molecular cytogenetics technology have increased our ability to identify genetic alterations in cancer cells. Polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), oligonucleotide-primed in situ DNA synthesis (PRINS) are the most common molecular cytogenetic procedures in cancer research but they have limits with precise localization in their principles in cancer researching. However, comparative genomic hybridization (CGH) is a powerful molecular cytogenetic technique which allows the analysis of chromosomal gains and losses in tumor cells. In this technique, normal metaphase chromosomes are hybridized with differentially fluorescence-labeled genomic DNAs (tumor with green and normal with red), and analyzed with fluorescence microscopy. The chromosomal locations of amplifications and deletions in the DNA sequences between the normal and tumor DNAs that were not detected by conventional cytogenetic analysis are thus revealed. The CGH has also made retrospective studies with archival tumor samples possible. Conventional cytogenetic analysis of solid tumors is technically very difficult and requires a large number of viable cells. Therefore, complete genetic description which is considered to be an important diagnostic criteria is limited in tumors. The CGH and other new techniques such as multi-fluor and spectral karyotyping are required to analyze the whole genome of the tumor. The CGH may have widespread uses in the detection and identification of chromosomal gains and losses, the analysis of the clonal evolution of cancer, the implication of specific genes and regions in cancer progression, the classification of chromosomal aberrations in cancer genetics, and prenatal diagnosis clinical genetics

Key Words: Cancer, comparative genomic hybridization(CGH), molecular cytogenetics, genomic instability

Introduction

The genetic basis of neoplasia is well established, and chromosomal alterations are recognized as critical in the pathogenesis of human cancer. Cellular oncogenes, tumor suppressor genes, DNA mismatch repair genes, and genes associated with cell aging or apoptosis can be affected by translocations, inversions, deletions, and aberrant regions of gene amplification (1-8). Molecular biology and cytogenetics technology have increased our ability to detect genomic instabilities in cancer cells, to determine the mechanism(s) responsible for their occurrence, and to assess their role in the pathogenesis of neoplasia (9-12).

Polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH) are the most powerful molecular cytogenetic techniques for detecting genetic alterations in cancer cells. Nonisotopic in situ hybridization technique (NISH) and especially oligonucleotide-primed in situ DNA synthesis (PRINS), are alternative procedures to the conventional in situ hybridization (13-21).

Chromosome banding has been used to recognize chromosomal abnormalities. However, chromosome banding resolution has limits, as many cryptic changes involving small chromosome segments and markers cannot be resolved. FISH is the most efficient and reproducible approach for precise localization of single sequences within metaphase chromosomes. Among the most recent additions to the FISH repertoire are comparative genomic hybridization (CGH), for detecting changes (losses and gains) in DNA copy throughout the tumor genome (22-24), and two new methods namely

combinatorial multi-fluor FISH and multicolor spectral karyotyping (25, 26). Therefore, in this review, we shall explain the basic principles of CGH for our scientists who work in the field of cancer research.

Comparative Genomic Hybridization (CGH)

The CGH technique is a fluorescence in situ hybridization technique that allows the detection and mapping of chromosome imbalances in a tumor genome relative to a normal genome, using total genomic DNA as a probe (22-24). Delineation of genetic changes in solid tumors has proved difficult because of the complexity of the karyotype and difficulties in chromosome preparation. Molecular genetics and molecular cytogenetics have provided an insight into the organization of specific regions. However, CGH makes the analysis of whole genomes possible. CGH is an analytic method based on FISH and digital fluorescence ratio measurements that enables one to compare cytogenetically the entire genome of malignant and normal cells, as well as to map gains and losses of DNA in tumor cells. Recently, CGH has been used to analyze the genomic alterations in several malignant tumors (27-37). CGH has also made retrospective studies with small amounts of the archival tumor samples possuble (38-40). Amplifications and deletions detected by CGH analysis might reveal any oncogene(s) or tumor suppressor gene(s) playing an important role in tumorigenesis.

Digital Image Analysis

The hybridization is analyzed by using a digital image analysis system based on a microscope equipped with a cooled CCD camera and a filter system. Excitation of each fluorochrome is accomplished by using these filters in a computer-controlled filter wheel. Three fluorochromes (DAPI, SpectrumGreen, and SpectrumRed) images are properly registered and processed with a workstation using software for pseudocolor display. Three color images are used to visualize the color changes along the metaphase chromosomes. A quantitative analysis of green and red flourescence intensities is performed by the above mentioned software. Local background fluorescence is determined for each chromosome and subtracted from the green and red images before analysis. All profiles are normalized that the overall green-to-red ratio for the entire metaphase is set at 1.0 (41-43).

Interpretation of CGH Images

Ten metaphases are analyzed for the chromosomal locations of DNA sequence gains and losses. These regions are determined by using green-to-red fluorescence intensity ratio profiles. The definition increase and loss of DNA-sequence copy number in tumors is based on a comparison of normal DNAs labeled with two different colors according to the described protocols (42, 43). The decision limits of the green-to-red ratios are determined for the decrease in the DNA copy number < 0.75, and for the increase in the DNA copy number >1.25, respectively (these ranges can change according to the software and laboratory conditions). Processing and evaluation of CGH analysis has been described in detail previously (41-43).

Conclusion

Conventional cytogenetic analysis of solid tumors is technically very difficult and requires a large number of viable cells. Therefore, complete genetic description, which is considered to be an important diagnostic criteria, is limited in tumors. CGH and other new techniques; multi-fluor and spectral karyotyping are required to analyze the whole genome of tumor samples.

The CGH technique will have widespread use in (1) the detection of consistent chromosomal gains and losses in tumors, (2) the implication of specific genes and regions of the genome in cancer progression, (3) the analysis of clonal evolution of cancer, (4) the dissection of genetic changes in experimantal models of tumor progression, (5) the diagnostic evaluation and refined classification of chromosomal aberrations in cancer genetics, and (6) prenatal diagnosis and clinical genetics (44). For these applications, the CGH strategies and methodologies must be optimized with new research programs.

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