CHROMOSOME STUDY FOR LEUKEMIA: A REVIEW ON LABORATORY TECHNIQUE

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ABSTRACT

Identification of chromosome abnormalities and precise localization of breakpoints involved in specific chromosomal abnormalities has prompted the recognition, molecular characterization and isolation of genes possibly responsible for the condition studied, which could potentially form the basis for new approaches to therapy. The cytogenetic and molecular cytogenetic techniques used for chromosome study of leukemia are reviewed in this article. Conventional cytogenetic (karyotype) and molecular cytogenetic techniques (fluorescent in situ hybridization) are the main methods used in chromosome study of leukemia. We conclude that chromosomal analysis by conventional cytogenetic in combination with fluorescent in situ hybridization is important for the management of patients with leukemia.

Key words: Leukemia, karyotype, fluorescence in situ hybridization

INTRODUCTION

Leukemias are malignancies that affect the blood-forming stem cells found in bone marrow. Myeloid leukemias are cancers that arise from myeloid stem cells, which normally mature into red blood cells, white blood cells, and platelet-producing cells. Lymphoblastic leukemias are cancers that arise from lymphocyte stem cells, which normally mature into white blood cells, also known as leukocytes. Leukemias are a heterogeneous group of cancers in terms of both biological and clinical features. Acute types refer to cancers arising in immature stem cells, while chronic types refer to cancers arising in mature stem cells. The incidence of leukemia in the world is about 1 per 100,000 per year1. Identification of chromosome abnormalities and precise localization of breakpoints involved in specific chromosomal abnormalities has prompted the recognition, molecular characterization and isolation of genes possibly responsible for the condition studied, which could potentially form the basis for new approaches to therapy2. This review focuses on the cytogenetic and molecular cytogenetic techniques used for chromosome study of leukemia.
CHROMOSOME ANALYSIS: CYTOGENETIC METHODS

Cytogenetic analysis is essential in the diagnosis and prognosis of leukemia. Acquired chromosomal abnormalities, structural or numerical, are detected in malignant bone marrow cells in more than 75% of patients with hematologic malignancies, with an increasing incidence due to the application of complementary detection methods provided by molecular cytogenetics.

Conventional cytogenetic analysis is a routine procedure allowing for the detection of chromosomal aneuploidies and large structural abnormalities at a single cell level. An analysis of chromosome patterns in malignancy must be based on a study of the karyotype of the tumor tissue itself. In the case of leukemia, the specimen is usually a bone marrow aspirate that is processed immediately or is cultured for a short time. Cells in metaphase from a 24-hour culture of peripheral blood will have a karyotype similar to that of cells obtained from the bone marrow. The chromosome analysis may be performed by means of one of several pretreatments prior to staining with Giemsa.

Conventional chromosome analysis requires metaphase preparations which are stained with various banding techniques. Chromosomal aberrations found on karyotypic analysis may include numerical abnormalities (e.g., loss or gain of a chromosome) and structural aberrations (e.g., translocations, amplifications or deletions). The findings are described according to the international system for human cytogenetic nomenclature (ISCN).

High resolution chromosome analysis, introduced in 1976, involves synchronization of dividing cells in prophase or prometaphase, resulting in longer chromosomes with multiple bands. At this level of resolution (over 600 bands per chromosome), structural abnormalities of 3-5Mb of DNA can be detected, while alterations smaller than 3Mb and translocations involving telomeric regions are extremely difficult to identify. Significant proportion (15%-20%) of bone marrow karyotypes in leukemia patients are reported as normal by conventional cytogenetic analysis and, despite improvements, the detection of abnormalities rate has not increased. To overcome these limitations and to identify submicroscopic alterations, fluorescent in situ hybridization (FISH) was developed. In acute lymphoblastic leukemia (ALL), at least two cultures should be performed, one for 24 hr and one for 48 hr. If sufficient material is available, direct preparation and a 72-hr culture may be considered. Before harvesting, 0.5- to 2-hr Colcemid exposure is recommended. In acute myelogenous leukemia (AML), at least two cultures should be performed, one for 24 hr and one for 48 hr. If sufficient material is available, a second 24- and 72-hr culture may be considered. In AML with French-American-British (FAB) subtypes M3/M3v, it is mandatory to perform a 48-hr culture since the aberrant clone might be only detected in this culture and not after 24-hr cultivation. Before harvesting, 0.5- to 2-hr (24 hr optional) Colcemid exposure is recommended.
Figure 1. Karyotype of patient with chronic myeloid leukemia (CML) revealed three aberrations: t(9;22)(q34;q11), t(3;21)(q26;q22) and inversion of chromosome 2 (adapted from reference 11).

FLUORESCENT IN SITU HYBRIDIZATION (FISH)

Fluorescent in situ hybridization (FISH) is a very robust molecular cytogenetic technique and yet equally suitable for the detection of gene translocation, gene deletion/amplification and chromosomal aneuploidy. FISH has wide applications in many branches of medicine including oncology. The FISH technique supplements conventional cytogenetics and in some cases provides additional information, which is not detected by karyotyping (Table I). A large number of cells can be studied by FISH, since interphase nuclei can also be analyzed. This helps in the detection of minimal residual disease, assessment of the rate of cytogenetic remission and detection of disease recurrence (Figure 2). A large variety of probes cloned in cosmids or PI bacteriophage vectors, yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), and PI-derived artificial chromosomes (PACs), are available for demonstrating specific chromosomal aberrations (Figure 3). Oligonucleotide probes can be chemically synthesized.
Table 1. Comparison between conventional cytogenetic and molecular cytogenetic techniques.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Procedure type</th>
<th>diagnostic property (sensitivity, specificity)</th>
<th>Cost</th>
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<tbody>
<tr>
<td>Conventional cytogenetic</td>
<td>Slow</td>
<td>Low</td>
<td>High costly</td>
</tr>
<tr>
<td>Molecular cytogenetic</td>
<td>Fast</td>
<td>High</td>
<td>Less costly</td>
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1. DNA unmasking

2. Probe and target denaturation

3. Probe-target DNA hybridization

4. Detection

5. Image analysis on fluorescent microscope

Figure 2. The basic steps of FISH. (adapted from reference 16).
Peptide nucleic acid (PNA) probes and padlock probes are used for quantitative and high specificity in situ hybridization (ISH), respectively. Currently, enzymatic incorporation with modified nucleotides (nick translation) is commonly used to label the probes with reporter molecules like biotin or digoxigenin, fluorochromes like fluorescein isothiocyanate (FITC) (green), tetramethylrhodamine (TMR) (red), amino-methylcoumarin-acetic acid (AMCA) (blue) or Cyanin 5 (Cy5) (far red), or enzymes like alkaline phosphatase or horseradish peroxidase. When fluorochromes are used for the detection of the hybridized probe, the ISH technique is usually referred to as FISH. The high sensitivity and high signal resolution, the possibility to analyze multiple different sequences on the cellular DNA and to quantify signal intensity, makes FISH a powerful technique. DNA sequences of 1 kb or more, but also whole chromosomes can be visualized with FISH. Fluorescence microscopy with dual or triple band pass filter sets, confocal laser scanning microscopy, digital cameras and imaging systems contribute to the visualization of chromosome abnormalities at the single cell level. Enzyme labeled probes are visualized by precipitation reactions and are mainly used when ISH is applied to tissue sections.

Figure 3. Examples of different types of fluorescence in situ hybridization (FISH) probes. (adapted from reference 23).
MULTICOLOR-FISH (M-FISH AND SKY)

Multicolor, even 24-color FISH (M-FISH) or spectral karyotyping (SKY) is based on the individual painting of each pair of chromosomes in metaphase cells. Probes carrying two or more fluorochromes at different ratios are applied. With image analysis systems, all chromosomes in a single metaphase can be studied. These techniques have led to the detection of cryptic chromosome abnormalities and to the identification of complex chromosome rearrangements.

An interesting approach to multi-color FISH has been introduced: the combined binary ratio labeling (COBRA) technique which is based on labeling of the probes using combinations of three fluorochromes, each combination in either the presence or absence of a fourth label.

In the absence of metaphases, comparative genomic hybridization (CGH) can give information on genetic gains and losses in tumor cells. In CGH, normal DNA and tumor DNA are labeled with two different fluorochromes. Then, equal amounts of DNA are mixed. Following competitive hybridization onto metaphase preparations of cytogenetically normal cells, the intensity of the two fluorochromes on each chromosome is measured by digital image analysis. If there are no changes in tumor DNA, the ratio between the two fluorochromes will be one. In case of amplification or deletion, the ratio will shift. Gains, losses and high level amplifications can be detected when clonal aberrations are present in at least 50% of the cells.

In fiber FISH, naked DNA fibers immobilized to microscope slides are used. The technique is especially useful for assessing gene rearrangements.

In leukemia using FISH it is possible to identify the breakpoint cluster region / Abelson murine leukemia viral oncogene homolog (BCR/ABL) fusion gene in chronic myeloid leukemia (CML) and the promyelocytic leukemia/ retinoic acid receptor alpha (PML/RARA) fusion gene in acute promyelocytic leukemia (APML). These fusion genes are the molecular consequences of the translocation t(9;22) characterized by the Philadelphia chromosome in CML and the translocation t(15;17) in APML (Figure 4). These fused transcripts are endowed with tumorigenic properties.
Figure 4. CML patient with masked Philadelphia chromosome. FISH analysis revealed the fusion between \textit{BCR} and \textit{ABL} genes on chromosome 22. The probe BCR/ABL dual color, dual fusion- red signals seen on chromosome 9q34, green- on chromosome 22q11.2 (adapted from reference11).

**GENOMIC ARRAYS**

Genomic microarrays were developed for CGH applications, based on the same principles as traditional CGH, except that cloned DNA segments were substituted for metaphase chromosomes as targets for the hybridization. Targets for array CGH can also be polymerase chain reaction PCR-generated sequences, cDNA clones or oligonucleotides \textsuperscript{36} - \textsuperscript{39}. Ratios between labelled genomes are compared with computer imaging and software analysis. Arrays have been developed for the analysis of whole chromosomes, portions of chromosomes, site-specific regions and the entire genome. Array CGH has been applied to a number of malignancies including lymphomas \textsuperscript{40}. 
CONCLUSION

Chromosomal analysis by conventional cytogenetic in combination with FISH is important for the management of patients with leukemia.

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