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Review

Impact of Cytogenetic and Molecular Cytogenetic Studies on Hematologic Malignancies

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Abstract. *Conventional cytogenetic analysis of chromosome abnormalities in hematologic malignancies is hampered by the low mitotic index and poor quality of metaphases. A range of techniques based on fluorescence in situ hybridization (FISH) has greatly enhanced the identification of non-random translocations and deletions, pinpointing regions which contain genes involved in leukemogenesis. One of the main advantages of FISH is its ability to use non-dividing interphase cells as DNA targets, enabling the screening of large numbers of cells and providing access to a variety of cells with different hematopoetic activity. Furthermore, multicolor FISH (SKY, M-FISH and CGH microarrays) combines the screening potential of cytogenetics with the accuracy of molecular genetics, allowing the visualization of the entire human genome in 24 different colors.*

Cytogenetic analysis is essential in the diagnosis and prognosis of hematologic malignancies. Acquired chromosomal abnormalities, structural or numerical, are detected in malignant bone marrow cells in more than 75% of patients with heamatologic malignancies, with an increasing incidence due to the application of complementary detection methods provided by molecular cytogenetics (1).

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 (2). Consequently, cytogenetic analysis is considered mandatory for validating the outcome of many clinical trials.

Specific chromosomal abnormalities have been included in the World Health Organization (WHO) classification of hematologic malignancies and, together with morphology, immunophenotype and clinical features, are used to define distinct disease entities (2-5). Cytogenetic anomalies identified in patients with hematologic malignancies are among the most important independent prognostic factors and are currently used to plan for different types of therapy (6-8).

This review focuses on the cytogenetic and molecular cytogenetic techniques used for the diagnosis and study of hematologic malignancies, with some insight into their future roles.

Cytogenetic analysis

Conventional cytogenetic analysis is a routine procedure allowing for the detection of chromosomal aneuploidies and large structural abnormalities at a single cell level.

The accuracy of cytogenetic analysis has been significantly improved over the last 30 years due to technical advances regarding culture methodology and banding techniques (10,11). High resolution chromosome analysis, introduced in 1976 by Yunis, involves synchronization of dividing cells in prophase or prometaphase, resulting in longer chromosomes with multiple bands (11). 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 (12). Furthermore, high resolution chromosome analysis is labor-intensive and has the limitation of the inconsistency of band resolution. The possible presense of multiple abnormal clones, the poor quality of metaphases and the low mitotic index associated with the disease have been widely recognized as the major problems associated with

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applying conventional cytogenetic analysis to hematologic malignancies. As a result, a 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* hybridisation (FISH) was developed.

Fluorescent *in situ* hybridization (FISH)

Human DNA, which is propagated in a variety of vectors (plasmids, cosmids, bacterial artificial chromosomes or yeast artificial chromosomes), is labelled with fluorochromes and can be used as probes to hybridize to specific regions on chromosomes. The hybrid formed is visualized under a fluorescent microscope (13).

One of the greatest advances of the FISH technique is the ability to use non-dividing cells as targets (interphase FISH) (14), allowing for the identification of both numerical and structural chromosome abnormalities in a large number of nuclei. This has considerable advantage for some hemopoietic malignancies, where the proliferative activity is low, or when dividing cells do not represent the neoplastic clone. Thus, interphase FISH seems to be more sensitive for the detection of some chromosomal aberrations such as t(8;21), inv(16), +8q, +11q, +21q and +22q (15). In the cytogenetic analysis of B-CLL with interphase FISH, a much higher incidence of trisomy 12 is found in comparison to conventional cytogenetic analysis (16, 17). Furtheremore, FISH analysis has been used to demonstrate a high frequency of *RB1* and *p53* deletions in B-cell malignancies (18-21). Interphase FISH has also been useful in identifying the critical region of deletion on 11q13, associated with B-cell lymphoid malignancy and, subsequently, the mutations of the ATM gene in T-prolymphocytic leukemia (PLL) (22).

Interphase FISH is the method of choice for the detection of residual disease in patients with hematologic malignancies or after allogeneic bone marrow transplantation (23-25).

When combined with immunological staining for cell surface antigens, FISH is a powerful diagnostic tool for the detection of lineage involvement in chronic myeloid leukemia (CML), myelodysplastic syndromes (MDS) and other myeloproliferative syndromes (26-30). This technique has allowed for the demonstration of leukemia arising in donor cells after sex-mismatched allogeneic bone marrow transplant (31).

The sensitivity of FISH analysis, the ease with which large numbers of cells can be scored and the availability of a wide range of quality controlled probes are the main reasons for the widespread implementation of the technique. It is important, however, that each probe is evaluated in every laboratory on a series of normal controls for each tissue investigated (32).

Types of probes. Chromosome-specific centromeric probes are the most commonly used in the molecular cytogenetic analysis of hemetologic malignancies, targeting repeated alpha or beta satellite sequences present in the heterochromatin of the centromeres (32). They are available for all human chromosomes, thus, allowing for the detection of numerical chromosome abnormalities both in metaphase and interphase cells. This type of analysis is especially useful in cases where the morphology of the chromosomes is poor and banding indinstinct.

Whole chromosome painting probes (paints) are complex mixtures of sequences from the entire length of a specific chromosome deriving from chromosome-specific libraries, PCR amplification, flow-sorted chromosome fractions, or microdissected DNA specific for each chromosome (32-36). Chromosome paints for all chromosomes, as well as arm-specific and region-specific paints, are available. They are used to clarify complex translocations, but they can not detect intrachromosomal structural anomalies or alterations involving the centromeric and telomeric regions.

Locus-specific probes target unique sequences in the genome. The various types currently used in clinical cytogenetic laboratories aim to screen for specific chromosomal translocations, inversions, microdeletions and oncogenes associated with specific desease entities (37-40). Their use has simplified the process of identifying complex or masked known translocations (BCR/ABL, AML) and has particular application in interphase analysis. DNA probes for the fusion genes involved in most specific chromosomal translocations and inversions in leukemia are now commercially available. Differential labelling and detection of these probes with various fluorochromes enables a direct visualization of the fusion gene.

Telomeric and subtelomeric probes have also been produced from the repetitive ends of the chromosomes proximal to the telomere (41). They contain unique sequences specific for the chromosome end and are currently used to detect cryptic translocations in patients with apparently normal karyotypes.

Multicolor whole-chromosome painting (M-FISH and SKY)

For FISH analysis, prior knowledge of the chromosomal abnormality is needed in order to select the appropriate probe. Therefore, research was previously aimed at the development of a genome-wide screen for chromosome abnormalities. This was achieved in 1996, when two groups independently developed a method for labelling and

detecting all 22 autosomes and the sex chromosomes, visualized in different colors (42, 43). Multicolor whole-chromosome painting (M-FISH) and SKY techniques combine the global screening power of cytogenetic analysis with the accuracy of molecular analysis, allowing for the identification of highly rearranged chromosomes, such as those present in complex karyotypes. The limitation, however, of this technology is that it relies on the resolution of metaphases and whole chromosome paint probes. Therefore, for practical reasons, reference back to the conventional cytogenetic karyotype is recommended, along with a combination with FISH approaches, to fully identify complex chromosome aberrations.

Comparative genomic hybridization (CGH)

CGH has the advatange over multicolor FISH of overcoming the technical difficulty of preparing chromosomes from leukemia cells, because neither dividing cells nor prior knowledge of the chromosome constitution are required. Compared to FISH analysis, CGH allows the survey of the entire genome in one experiment.

The technique is based on a two-color FISH of differently-labelled tumor (green) and reference DNA (red), mixed in equal volumes and hybridized onto normal metaphases (44). Differences in copy number between the reference and tumor DNA are reflected by a variation of the red to green fluorescence ratio along the length of the chromosome. The major limitation of CGH, however, is the need for metaphases to be used as targets. For deletions, the resolution of CGH has been estimated at >10 Mb (45).

Over the past 12 years, CGH has been widely used to identify new regions of amplification and deletion in various tumors (46), but its application in hematologic malignancies is limited due to the method's inability to detect balanced rearrangements, and to the requirement for >50% cells with the clonal abnormality. However, CLL and some lymphomas have clearly benefited from the application of CGH (47-51).

CGH arrays

Recently, 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 (52-54). Targets for array CGH can also be PCR-generated sequences (54, 55), cDNA clones or oligonucleotides. 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 (56).

Conclusion

In the relatively short time since its introduction, FISH has had an impact on cytogenetic analysis due to the speed, sensitivity anad flexibility.

With the completion of the Human Genome project, it is reasonable to propose that whole genome arrays will be developed that could be used to screen for regions known to be involved in hematologic malignancies. The new methods involving the detection of genes using CGH arrays may provide a useful diagnostic tool in the future. However, cytogenetic analysis in combination with FISH is still important for the management of patients with hematologic malignancies.

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