Chapter 4

MOLECULAR CYTOGENETIC STUDIES FOR HEMATOLOGICAL MALIGNANCIES

Gordon W. Dewald, Stephanie R. Brockman, Sarah F. Paternoster
Cytogenetics Laboratory, Mayo Clinic, Rochester, MN

1. INTRODUCTION

“FISH” is an acronym that is applied to genetic technology which uses fluorescent-labeled DNA probes. This acronym is derived from the ability to visualize fluorescent-labeled probes at the place of in situ hybridization with complementary DNA within a nucleus. Experts working with FISH often refer to their field as molecular cytogenetics because their work crosses the fields of molecular genetics (DNA probes) and cytogenetics (evaluation of chromosomes).1

FISH is widely used today in clinical practice to help diagnose and select appropriate treatments for patients with hematological malignancies.2 This method permits analysis of proliferating (metaphase cells) and non-proliferating (interphase nuclei) cells, and is useful to establish the percentage of neoplastic cells before and after therapy (minimal residual disease).3 Thus, FISH is helpful to assess the effectiveness of treatment and to monitor the durability of remission. In research, FISH studies are used to investigate the origin and progression of hematological malignancies, and to establish which hematopoetic compartments are involved in neoplastic processes.4

This chapter is intended for clinicians and hematopathologists who wish to use FISH in the workup and management of their patients with hematological malignancies. Information is provided about appropriate specimen collection and transportation, laboratory procedures and interpretation of results. A review of different FISH strategies to detect chromosome anomalies is presented to appreciate the strengths and
limitations of this method. FISH studies are summarized for chronic myeloid leukemia (CML) and other myeloproliferative disorders (MPD), myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), B-cell chronic lymphocytic leukemia (B-CLL), multiple myeloma, and lymphoma. Some algorithms for conventional cytogenetics and FISH are suggested to help accomplish appropriate application of FISH studies in clinical practice.

2. CHROMOSOMAL BASIS OF MALIGNANCY

2.1 Chromosome Anomalies

The results of FISH can be used to detect neoplastic clones with either numeric or structural anomalies of chromosomes. The term polyploid refers to chromosome complements that are multiples of 23; the haploid number of chromosomes for humans. Diploid refers to 46 chromosomes, triploid to 69 chromosomes, and tetraploid to 92 chromosomes. In neoplastic disorders, most polyploid clones are associated with advanced stages of disease and are derived from fusion of neoplastic cells or endoreduplication. Aneuploid refers to chromosome complements that involve irregular multiples of the haploid chromosome number. Thus, any cell with trisomy 8 is characterized by 47 chromosomes and includes three number 8 chromosomes. A cell that is monosomy 7 contains 45 chromosomes and is lacking a chromosome 7. Aneuploid anomalies usually occur as a consequence of mitotic malfunction, such as chromosome nondisjunction.

The results of FISH can be used to discriminate among various anomalies of chromosome structure including translocations, deletions, inversions, duplications, or isochromosomes. Reciprocal translocations involve the interchange of parts of different chromosomes and are the most common type of translocation in hematological malignancies. Deletions involve loss of part of a chromosome and are either terminal or interstitial. Inversions produce a reversal in the direction of an interstitial part of a chromosome and are either pericentric or paracentric. Pericentric inversions involve both the short and long arms of any chromosome while paracentric inversions occur on only one of the arms of any chromosome. Duplications produce two or more copies of a particular DNA segment on the same chromosome. Amplification results in hundreds of copies of a gene or chromosomal segment, which can either occur within a chromosome as a homogenous staining region or as separate acentric double minutes within the nucleus. Isochromosomes produce a mirror-image band pattern with respect to the