The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2020

Volume 9, Pages 18-22

ICRES 2020: International Conference on Research in Education and Science

The Use of Molecular Biology Methods in Evaluating Hematologic Diseases

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Abstract: Leukemia is a malignant disease originating from lymphopoietic or hematopoietic stem cells of bone marrow. Leukemias are classified as acute or chronic, based on the spread and development characteristics of the tumor. Leukemias exhibit phenotypic and genotypic heterogeneity according to their classification. Therefore, hematology is one of the sciences that most frequently use molecular biology tests. Although the classification and risk assessment are mainly based on cytogenetic analysis, molecular tests play a complementary role. The most commonly used tests include conventional karyotyping, fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR)-based single nucleotide polymorphism analyses (RFLP, ARMS etc.), comparative genomic hybridization (CGH), and sequence analysis methods. Molecular biology tests have become essential for the final diagnosis of malignant diseases, as well as determining the prognosis and even selection of treatment methods. Although cytogenetic and molecular indicators play a key role in determining the risk status of patients, there are also other prognostic indicators in long-term remission. The treatment response can also be evaluated by carrying out morphological, cytogenetic and molecular biology tests (MBT) on bone marrow samples collected at various times throughout the treatment period. In practice, MBTs can be used in the diagnosis and follow-up of benign hematologic disorders (hemoglobinopathies etc.) as well as malignant diseases. MBTs are also frequently used in the diagnosis of congenital or acquired hemolytic anemias, hemophilia, thrombophilia and platelet disorders. Therefore, it is possible to say that the genetic parameters obtained using different MBTs are of utmost importance in the diagnosis, treatment and follow-up period of prevalent malignant or benign hematologic diseases.

Keywords: Hematology, Leukemia, MBT, PCR

Introduction

Hematology is a science that focuses on blood cells, blood-forming organs and the diseases of these organs. Millions of erythrocytes, leukocytes and platelets are produced in the human body each day in order to replace blood cells that are lost within the normal cell cycle (1). Hemostatic mechanisms somehow increase leukocyte production as a response to stress triggers such as bleeding and infection, wherein these cells return to normal levels when stress disappears. Hematopoiesis is the formation of blood cells and maintains the balance in a highly organized manner (2). In hematopoiesis, the fate and differentiation path of a cell is controlled by growth factors or growth factor receptors, specific transcription factors, and the microenvironment (3).

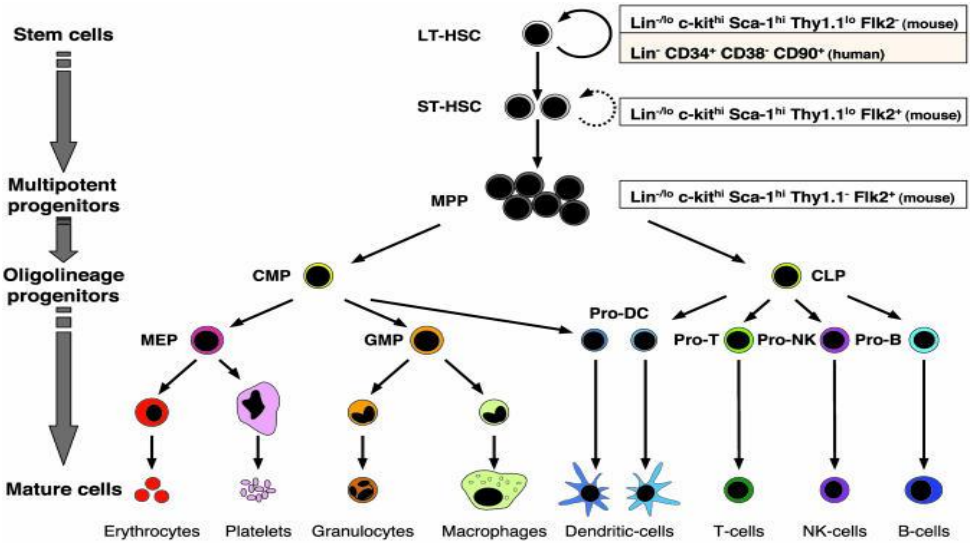

Figure 1. Hematopoiesis (4)

If the hematopoiesis process works as it should, hematopoietic stem cells differentiate into peripheral blood cells (mature lymphocytes, granulocytes, monocytes, erythrocytes and megakaryocytes/platelets etc.) as shown in Figure 1 (5,6). In leukemia however, the cell proliferation rate increases during the stages of bone marrow development cells in one cell line, wherein neoplastic clones proliferate within the bone marrow and start to replace other bone marrow cells. The increase in the number of cell lines is sufficient to replace peripheral blood cells (7). Leukemias constitute a large group of malignant hematologic diseases that are characterized by neoplastic cells covering the bone marrow and other tissues and accumulate in peripheral blood as a result of clonal proliferation, or discontinuation of a specific stage of normal myeloid or lymphoid hematopoiesis. The etiology of leukemia is not entirely known and leads to death if left untreated (8,9).

Leukemias are divided into two groups, i.e. acute and chronic, depending on maturity and survival characteristics (10). Immature hematopoietic or lymphoid progenitor cells are dominant in acute leukemias. There is accumulation of leukocyte progenitors and scarcity of mature cells. Leukemias are divided into two groups, i.e. Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL), according to the structural, cytochemical, immunologic and cytogenetic characteristics of the proliferating cells (11,12). Chronic lymphoid leukemia (CLL) is a type of cancer that develops as a result of the accumulation of small lymphocytes of mature appearance from the morphological aspect. CLL develops as a result of the accumulation of monoclonal cell surface antigens, in other words B lymphocytes, in the blood, bone marrow, lymphoid tissue and spleen (13). Since leukemia is a complex group of diseases that exhibit both phenotypic and genotypic heterogeneity, genetic evaluation is very important in determining the risk level and management of patients, wherein the majority of leukemia patients present with recurring chromosomal abnormalities. Although cytogenetic analyses are essentially used for the classification and risk assessment of leukemias, molecular biology tests play a complementary role (14).

Therefore, this review will evaluate the important molecular biology tests which are currently used and can be used in the future, that are widely accepted in the evaluation of hematologic diseases.

Biological methods used in the diagnosis and follow-up of leukemias

Studies concerning molecular genetics in leukemias started with the identification of recurring chromosomal abnormalities in this disease. Studying the genes with altered/impaired functions as a result of translocation helped to determine the main control pathways of hematopoietic cells and understand how they caused leukemogenesis when impaired. As a result of translocations, either 2 opposite chromosomes may become juxtaposed, thus causing the creation of a new oncogenic fusion gene with the combination of the relevant genes. Alternatively, reciprocal translocation may enhance the function of a gene on one side, causing it to acquire oncogenic characteristics, or result in the gene losing its existing suppressive properties, thereby rendering it unable to prevent the cells from leukemogenesis (15,16).

Cytogenetic studies are primarily necessary in order to investigate the structural or numeric chromosomal abnormalities of leukemic cells. It is accepted that conventional karyotyping is still the first method of choice for this purpose. Fluorescent In Situ Hybridization (FISH) technique, which is a molecular cytogenetic method, and the molecular biology method (MBT) provide results when chromosome analyses fail to suffice (17,18). On the other hand, sub-microscopic changes at DNA level, such as point mutations, can only be detected by molecular methods. Genetic changes shown by these methods play an important role in the diagnosis, classification, determination of subtypes and prognosis of hematologic neoplasia, as well as treatment selection and minimal residual disease (MRD) monitorization. With this purpose, the entire genome is studied to detect mutations using next generation DNA sequencing technologies (19,20). In order to correctly identify these genetic abnormalities that have high clinical significance, it is necessary to first provide the suitable material and then select the suitable method. Therefore, a comparison of the cytogenetic and molecular biology methods used in the diagnosis and treatment of leukemias is provided in Table 1 (21).

Table 1. Comparison of the cytogenetic and molecular biology methods used in leukemias

Properties	**Karyotyping**	**FISH**	**PCR**	**CGH**	**SNP**
Does it ensure the study of the entire genome?	YES	NO	NO	YES	YES
Can it show translocation?	YES	YES	YES	NO	NO
Can it show point mutation?	NO	NO	YES	NO	NO
Can it show deletion?	YES	YES	YES (qPCR)[2]	YES	YES
Can it show amplification?	YES	YES	YES (qPCR)[2]	YES	YES
Can it show rare mutations?	NO	YES	YES	NO	NO

As seen in the Table 1, karyotyping is still the gold standard and other MBTs should be applied as complementary to the Karyocytogenetic Methods (KM), when KMs do not provide results or when the karyotype is normal.

Use of molecular biology methods in benign hematologic disorders

Hematology is a science that investigates not only malignant diseases but also various disorders known to be benign. Erythrocyte disorders can be classified as anemias, thalassemia and sickle cell disease, spherocytosis and G6PD deficiency, acquired hemolytic anemias, erythrocytosis and other congenital hemolytic anemias. Benign leukocyte disorders consist of granulocyte function disorders, granulocytopenia, lymphopenia, lymphocyte function disorders, and leukocytosis. Platelet disorders include acquired platelet function disorders, hereditary platelet disorders, other thrombocytopeniae, and ITP (immune thrombocytopenic purpura). An important group of these benign diseases, i.e. hemoglobinopathies and globin gene disorders, is a large group that encompasses various diseases (sickle cell anemia and thalassemia etc.) (22). Hemoglobinopathies are considered as recessive monogenic (single gene) diseases that are prevalent both globally and in Turkey, with an increasing global burden each year. Nearly 7% of the world's population carries a globin gene mutation. The most common type is beta thalassemia, which is mainly seen in Mediterranean countries, followed by Southeast Asia, India, Africa, Central America and Middle East countries. The incidence of hemoglobinopathies is 2.1% in Turkey, ranging between 0.7% and 13% depending on the region (23). Hemoglobinopathies are systemic diseases. This brings new genetic approaches to the diagnosis and treatment of hemoglobinopathies, although they are monogenic diseases. In order to diagnose and treat hemoglobinopathies completely, it is required to prepare a global map detailing the distribution of the relevant globin gene mutations; reveal the relationship between molecular genetic infrastructure and mutation types, prenatal diagnosis and preimplantation genetic diagnosis, mutation and phenotype; and to be familiar with the pharmacogenetic, immunogenetic, nutrigenetic and gene therapy approaches for globin metabolism and drug use (24,25). Despite the technological advances in detecting mutations, hematologic and molecular biology techniques should be used together in order to reach the correct diagnosis in screenings for hemoglobinopathies.

Therefore, it is necessary to interpret the hematology results and confirm the genotypes using methods such as DNA analysis or mass spectrometry in order to make the correct diagnosis in a majority of carrier screening tests. These methods would be beneficial when hematology or biochemistry results are unclear. In addition, recent studies involving fetal DNA in maternal plasma and state of the art precise technologies, such as digital

PCR and next generation sequencing, provide the routine prenatal diagnosis of globin gene disorders in a manner noninvasive to the fetus (26,27).

Conclusion

New revolutionary discoveries in molecular biology enhance the quality of life and prolong the lifespan of leukemia patients, in addition to providing benefits in various fields. In addition to the advances in molecular biology, close cooperation between the laboratory and clinical findings also play an important role. This review aimed to convey the current molecular biology methods that are frequently used in the diagnosis, screening and follow-up of both malignant and benign important hematologic diseases. It is apparent that technological advances in genetic diagnostics achieved using molecular biology methods, would bring molecular diagnostics to a better position as a fast and inexpensive whole that encompasses the genome sequence of patients.

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