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POTENTIAL OF CLASSICAL AND MOLECULAR CYTOGENETICS IN THE DIAGNOSIS OF LEUKEMIA: ASSESSMENT OF THEIR CLINICAL AND THERAPEUTIC IMPLICATIONS

Hajar Azami Idrissi[1,2], Laila bouguenouch[1,2], Badr El Mekhzen[1,2], Said Tghanint[1,2], Ihssane El Otmani[1], Karim Ouldim[1,2], Mohamed El Azami El Idrissi[2]

1: Unit of Medical Genetics and Oncogenetics CHU Hassan II, Fez

2: Faculty of Medicine and Pharmacy, Fez.

Corresponding author: Hajar Azami Idrissi, PhD student.

ABSTRACT

Cytogenetic analysis plays an essential and significant role in the biological evaluation of malignant hemopathies in combination with cytology, immunophenotyping and molecular biology. It has the advantage of recognizing unique and non-specific cytogenetic defects that have diagnostic and prognostic significance, allowing the prediction of treatment response, period of remission and overall survival. In addition, cytogenetics constitutes the basis of the discovery of genes involved in leukemogenesis phenomena, and molecular biology is logically integrated into its suite for their understanding. Its applications in clinical practice are increasing, whether for the refined detection of certain translocations or for the assessment of residual disease.

Keywords: Malignant hemopathies, cytogenetic studies, chromosomal anomalies, molecular biology.

I. INTRODUCTION

Searching for chromosomal abnormalities in hematological malignancies is an essential element in the management of these diseases. Abnormalities are detected by conventional and molecular cytogenetic techniques (karyotype, FISH (Fluorescent in situ hybridization) performed on bone marrow samples or peripheral blood in leukemias, or lymph nodes in lymphomas. Karyotype modifications are acquired modifications (limited to malignant cells), clonal (a clone is defined by at least two metaphases having the same supernumerary chromosome or the same structural abnormality, or three metaphases lacking an identical chromosome), primary or secondary, whether numerical and/or structural, they are not always random, they are indeed non-random, and some are specific to a pathological entity [1]. The discovery of chromosomal abnormalities in hematological malignancies has two major benefits: clinical interest in patient management on one hand, and scientific interest on the other. The first cytogenetic abnormality associated with hematological malignancies is the Philadelphia (Ph) chromosome associated with chronic myeloid leukemia (CML). To date, the majority of cytogenetic abnormalities are discovered in leukemias and lymphomas [2].

II. HEMATOPOIESIS

Malignant hemopathies may be caused by hematopoietic homeostasis dysfunction [3]. Hematopoiesis is the process by which the body produces and renews all the figurative elements of blood. This process takes place in the bone marrow in successive stages. All blood cells are produced from the same undifferentiated cell, the hematopoietic stem cell. This cell has two essential properties which are self-renewal and differentiation ability. In response to an exogenous signal, it is able to differentiate into red blood cells (or red blood cells), white blood cells (myeloid or lymphoid line) or platelets (or thrombocyte).

Hematopoiesis requires an adapted medullary microenvironment and the intervention of growth factors such as IL-7 (interleukin 7), EPO (erythropoietin), TPO (thrombopoietin) or GM-CSF (granulocyte-monocyte colony stimulating factor). Ultimately, hematopoietic cells differentiate and determine their lineage under the influence of transcription factors such as PAX-5 (Paired-box gene 5), PU-1 or GATA-1. The hematopoiesis disruption can therefore be the cause of a hematopoietic malignancy. This

dysfunction may result from inappropriate expression or structural alterations of certain genes caused by point mutations or structural abnormalities such as chromosomal translocations [4].

III. CLASSIFICATION OF HEMATOLOGIC MALIGNANCIES

Malignant hemopathy is defined as a group of pathologies involving leukemia and lymphoma. They are classified according to their degree of severity and the stage of maturation. Leukemia is classified according to the cell type affected, it's frequently divided into lymphoblastic and myeloblastic [5].

There are two major forms of myeloid lineage pathologies. Chronic forms include myeloproliferative syndromes (MPS) with quantitative abnormalities and myelodysplastic syndromes (MDS) with qualitative abnormalities (excessive apoptosis). Acute forms are characterized by myeloid proliferation with blocked maturation. Impairment of the lymphoid line may give rise to leukemia or lymphoma.

Lymphomas are lymphoid hemopathies characterized by lymph node or extra-ganglionic infiltration, by malignant and monoclonal lymphoid cells from either B lineage (70% of cases) or T lineage. Lymphoma cell morphology and proliferation architecture define the histological type of lymphoma (example: Burkitt's lymphoma, Hodgkin's lymphoma) [6].

Acute leukemia differs from chronic leukemia by their spontaneous evolutionary speed. Moreover, Acute leukemias are characterized by a blockage of the maturation of medullary cells. This immaturity reflects an abnormality of differentiation.

IV. CHROMOSOMAL ABNORMALITIES

Chromosomal abnormalities are found in more than 95% of chronic myeloid leukemia cases, 70 to 80% of acute lymphoid leukemia and 50% in acute myeloid leukemia.

The three key mechanisms of malignant transformation in hematological malignancies are irregular gene expression caused either by mutation, fusion of two genes and the absence of genes (tumor suppressor genes) regulating the mutagenic process. These acquired genetic abnormalities were generally identified from chromosomal abnormalities detectable under a microscope. Malignant cells containing chromosomal abnormalities stem from the same cell clone [3]. These chromosomal abnormalities may be numerical (loss/gain of a chromosome) or structural (breaks followed by rearrangements, amplifications or deletions). The modal number of a tumor is the number of chromosomes found in the largest number of abnormal metaphases; it therefore corresponds to the majority clone in the tumor.

Structural abnormalities include chromosomal translocations, which are defined by the transfer of a segment of DNA from one chromosome to another. They can be reciprocal, involving two chromosomes (exchange of chromosomal material) and balanced, i.e. without loss of DNA fragment or unbalanced with loss or duplication of material.

Translocations lead to a rearrangement in the organization of genes. In some cases, the expression of these genes can be modified. Indeed, some translocations give rise to new fusion genes transcribed into a chimeric mRNA, itself translated into a new hybrid protein equipped with oncogenic properties (qualitative anomaly). In a transcription active region, translocation of a gene may lead either to over-expression of this gene or to ectopic expression in a type of cell where it is typically absent (quantitative anomaly). These genes are usually genes that regulate cell survival and/or development, giving rise to a proliferative advantage. [3]. Thus, oncogenes or proto-oncogenes have been classically identified as genes that lead to the emergence of a malignant phenotype when their expression is deregulated or when their structure is altered.

Currently, more than 100 different oncogenes have been discovered and described in human hematologic malignancies. These genes code for proteins with a wide range of functions. A large number of them encode transcription factors such as PAX5, transcription co-activators or co-repressors such as ETV6, but also tyrosine kinases (KIT, PDGFRβ, ...), proteins involved in cell cycle control (cyclin D1...) or finally anti-apoptotic proteins, such as the BCL2 molecule [7].

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V. INTEREST IN INVESTIGATING CHROMOSOMAL ABNORMALITIES IN HEMATOLOGICAL MALIGNANCIES.

In the classification of hematologic disorders, the key anomalies found represent a significant criterion. The first unique anomaly discovered in a malignant process was the Philadelphia chromosome (or Ph1) in chronic myeloid leukemia (CML) (1960). In 1973, Rowley revealed that Ph1 emerged from t(9;22)(q34;q11) translocation, which led to a rearrangement between the bcr gene and the abelson kinase (bcr-abl). The diagnosis of CML is mainly based on Karyotype observation of a Ph1 chromosome associated with hyperleukocytosis. Cytogenetics is an important approach for the detection of these pathologies, but when it comes to cryptic anomalies, molecular biology approaches are also essential, which is the case in 5% of CML for bcr-abl translocation or in the search for residual transcripts by RT-PCR. [8].

For certain hematologic diseases, the existence of the Philadelphia chromosome (Ph1) has significant diagnostic and prognostic effects. In more than 90 percent of cases, the anomaly is typical of chronic myeloid leukemia (CML), it also constitutes a major anomaly in 30 percent of acute lymphoblastic leukemia adult cases (ALL) and in 2-10 percent of children with ALL. [9]. This rearrangement is sometimes observed in rare acute myeloblastic leukemia cases (AML) [10]. Following the Philadelphia translocation t (9;22) (q34;q11), The 3' sequences of the proto-oncogene abl (Abelson) in 9q34 are bound to the 5' sequences of the bcr (Breakpoint Cluster Region) gene in 22q11, giving rise to bcr-abl "fusion" gene. In CML, breakpoints were found at the 22q11.21 and 9q34.1 sub-bands by Prakash and Yunis (1984). Although the position of the breakpoint at chromosome 9 is quite variable, almost all breaks are located within a 200 kb region covering exons 1b to 1a. There are two regions of breakpoints in the bcr, m-bcr and M-bcr gene. In CML, most translocations involve the M-bcr (Major Breakpoint Cluster Region), which lies approximately between exons 12 and 16. In LAL, the breaks are mainly clustered in the Minor Breakpoint Cluster Region (m-bcr), which lies between exons 1 and 2, although the resulting translocation is cytogenetically identical to that seen in CML [11]. The other breakpoints connect different sets of exons from the bcr gene to a common subset of exons in the abl gene, resulting in 2 alternative chimera oncogenes, p210 (bcr-abl) and p185 (bcr-abl)). These two fusion proteins have a substantially enhanced tyrosine kinase activity due to the activation of tyrosine kinase activity of the abl gene after direct binding of the sequences within the first exon of the bcr gene. This kinase activity is thought to be necessary for the oncogenicity of the chimeric oncogene.

In ALL, rearrangement is associated with an extremely unfavorable progression, with an event-free survival of up to 15% at 5 years in adult and pediatric patients treated with chemotherapy alone [12]. There are no reports of long-term survivors [13]. Allogeneic bone marrow transplantation is the only curative treatment for these patients. Pediatric patients are treated on the basis of a high-risk protocol and adults are referred for immediate bone marrow transplantation. Philadelphia chromosome-positive acute myeloid leukemia (AML) is characterized by resistance to conventional standard chemotherapy and a bad prognosis. Accurate and rapid identification of this chromosomal abnormality is therefore vital. In a small number of cases of ALL, the translocation does not result in the formation of a cytogenetically visible Philadelphia chromosome. In these cases, FISH is essential to reveal the fusion gene [14].

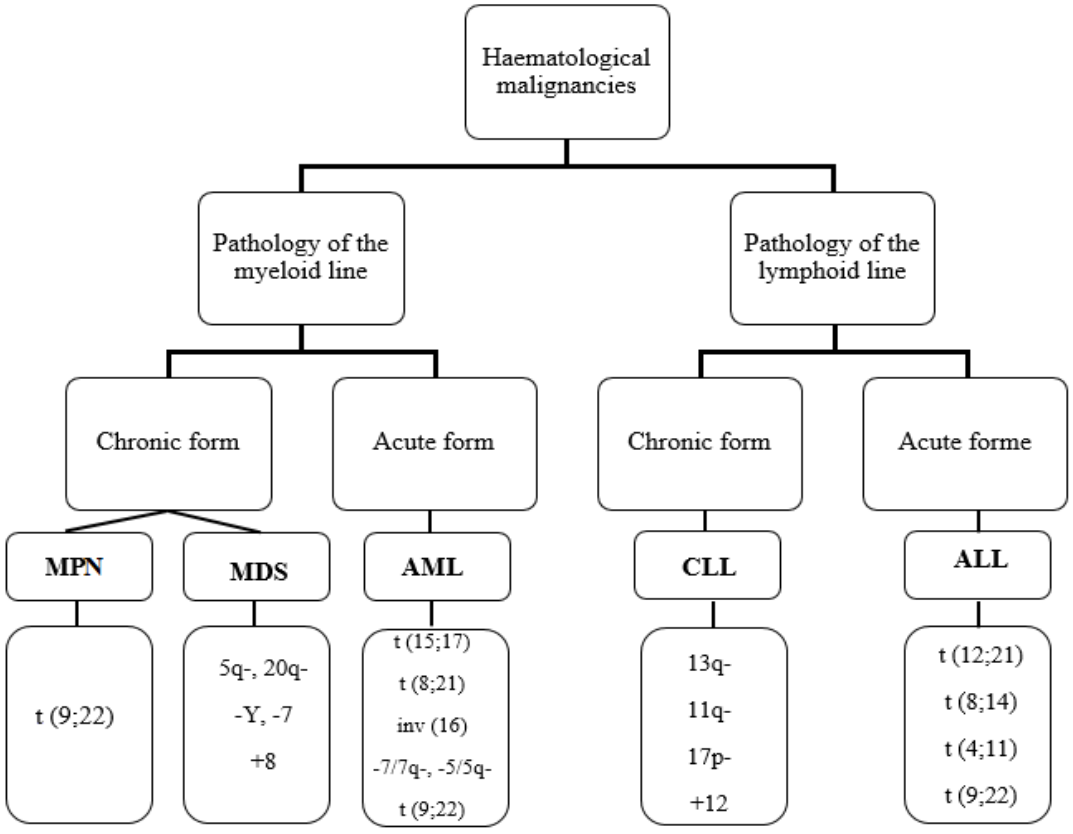
Cytogenetics often provides a prognostic parameter and an element of treatment choice. In pediatric ALL subjects, forms associated with Ph chromosome, t-translocation (4;11) or t-translocation (1;19) have a high potential for relapse and a low hope of curability, justifying the use of an allograft of bone marrow as soon as a first complete remission is achieved. In AML, the forms associated with the inversion of the chromosome 16 or translocation t(8;21), have on the contrary, a high potential for curability with chemotherapy alone. The identification of the genes involved in these translocations may lead to more targeted therapy. For example, in acute promyelocytic leukemia (APL), the t(15;17) (q22;q11) translocation is now recognized as being specifically associated with this pathology. It juxtaposes a gene called pml (promyelocytic leukemia) normally located at 15q23 with the gene coding for the retinoic acid receptor (RARalpha) in 17q21. The hybrid protein PML-RARα resulting from this fusion prevents the

action of endogenous retinoids on the differentiation of the promyelocytic leukemia line which remains blocked at an early stage. The use of retinoic acid in the treatment of this condition allows the specific targeting of cells carrying the translocation [15].

Cytogenetics also facilitates the assessment of disease progression (Residual Disease or complete remission) and the evaluation of treatment efficacy. Additional defects in CML may occur during the evolution of the disorder, suggesting a clonal progression (ph, trisomy 8, 17 isochromosome, y chromosome loss) and poor prognosis. Conversely, the resumption of hematopoiesis Ph negative under interferon is a prognostic improvement. Cytogenetics can also be complemented by molecular biology techniques such as RT-PCR. Indeed, after therapy, the search for residual disease can be apprehended by RT-PCR if the fusion transcript arising from the translocation is already known [16]. Characterization of new chromosome translocations has often brought to light genes that are critical for understanding oncogenesis. (myc, bcl2, bcl1, pml, mll, abl, pdgfrβ....). The primarily translocated genes are two families: genes that encode tyrosine kinases and genes that encode transcription factors. Finally, there are also microRNAs, a class of small non-coding RNAs that could obviously play a significant role in oncology.

VI. CONCLUSION

Cytogenetic exploration in hematological tumor pathology is based on karyotype and fluorescent in situ hybridization (FISH), which are essential to make the diagnosis, determine the phase of the disease and also evaluate its prognosis in order to choose the appropriate treatment for the patient. Chromosomal abnormalities in hemopathies are numerous and complex. Understanding their pathophysiological mechanisms enabled the development of targeted therapies for chronic myeloid leukemia. Indeed, CML, one of the most common hematological malignancies, has undergone significant therapeutic developments through both classical and molecular cytogenetic techniques and molecular biology techniques.



MPN: myeloproliferative neoplasm, **MDS:** myelodysplastic syndromes, **AML:** acute myeloid leukemia, **CLL:** chronic lymphoblastic leukemia, **ALL:** acute lymphoblastic leukemia, **t:** translocation, **inv:** inversion

Conflicts d´intérêt :

The authors do not declare any conflict of interest.

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