OVERVIEW

MOLECULAR TECHNIQUES IN MONITORING MINIMAL RESIDUAL DISEASE IN LEUKEMIA

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Summary

Leukemia is the most common childhood cancer in both Vietnam and another country around the world. Although the rate of successful treatment has increased due to the improvements of therapy and supportive care, the rate of relapse is still high. The main reason is the persistence of cancer cells after treatment. Detecting minimal residual disease is considered as "gold standard" in evaluating treatment efficiency, selecting alternative therapies, and predicting relapse in leukemic management. In the past, cytological techniques were used for MRD detection, but now the molecular techniques has gradually replaced due to their high sensitivity and specificity. The most common techniques are PCR-based assays and next generation sequencing.

Key words: *Leukemia, cytological techniques, MRD*

1. INTRODUCTION

Leukemia, which refers to cancers of the bone marrow and blood, is the most common childhood cancer. It accounts for about 31% of all cancers in children in which acute leukemia constitutes 97 % of all childhood leukemia. The most common types are acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) which account for 75 % and 25 %, respectively [11].

About 3000 children in US and 5000 children in Europe are diagnosed with ALL each year. There are about 500 newly AML diagnosed cases in US per year. In Vietnam, leukemia is also the cancer with highest incident in children [11].

Although treatment in leukemia has been gradually intensified during the last 30-40 years, leading to a significant improvement of the outcome, there is still a remarkable high rate of relapse, about 20 - 25%. Since minimal residual disease (MRD) has played an important role in leukemic management due to its value in evaluating treatment success, following, prognosis, early detection and predicting relapse in leukemia patients [4] [6], methods which can detect MRD earlier with high sensitivity and specificity are preferred. Here, we introduce some molecular techniques used widely in detection MRD in leukemia.

2. MINIMAL RESIDUAL DISEASE

2.1. The concept of minimal residual disease

Minimal residual disease is defined as the smallest number of cancer cells that persist in a patient during or after treatment, even though clinical and microscopic examinations confirmed complete remission (CR) and the patient show no signs and symptoms of disease [6][9].

2.2. Value of MRD in leukemic management

MRD provides an important feedback about conventional treatment success and help in selecting therapeutic alternatives. Also, MRD is useful in following patient, and in predicting relapse. Moreover, MRD studies have demonstrated prognostic value when measured before or after allogeneic haematopoietic cell transplantation. Because this minimal number of cancer cells is the main cause leading to relapse or recurrence sooner or later, early detection of MRD is very important in leukemic management. The earlier MRD can be detected, the better long-term outcome the patient can obtain [4][6][10].

3. MOLECULAR TECHNIQUES USED IN DETECTION MRD

In the past, CR was confirmed merely based on clinical examinations (clinical CR) and

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microscopic examinations (haematological CR), then the cytological techniques (karyotype, FISH, CGH) (cytological CR). However, the sensitivity of these methods is obviously not sufficient for MRD detection required today because of the availability of high sensitive molecular techniques such as PCR-based assays, quantitative PCR, next generation sequencing [6].

Table 1. Levels of detection of MRD in leukemia [11]

Method	Lowest Levels of Detection*
Morphology	5 per 100
Conventional cytogenetics	2 per 100
Karyotyping by flow cytometry	1 per 100
Southern Blot for receptor gene rearrangement	1 per 100
FISH	1 per 1000
Double immunological marker analysis (leukemia-associated phenotype)	1 per 100 000
Polymerase chain reaction (PCR)	1 per 1 000 000

*: Number of leukemic cells per number of normal bone marrow cells

3.1. MRD detection in Acute Lymphoblastic Leukemia

3.1.1. Characteristics of Immunoglobulins (Ig) and T-cell receptor (TcR)

Immunoglobulins and T-cell receptor are antigen receptors of B-cell and T-cell, respectively, which were encoded from Ig and TcR genes [3]. Ig and TcR rearrangements are genetic events that happen very early in differentiation process of pluripotent hematopoietic stem cells into B-lineage and T-lineage. Immunoglobulins include heavy chains and light chains. Heavy immunoglobulin (IgH) rearrangement is the first genetic event that can be detectable and happens in all differentiated B-lineage cells. Conversely, not all differentiated B-cell lineage experience light immunoglobulin rearrangement [2][3].

Somatic rearrangement of IgH and TcR genes occurs by joining the germline variable (V), diversity (D), and joining (J) gene segments. By this combination, each lymphocyte gets a specific combination of V-D-J segment that encodes for the variable domain of Ig or TcR molecule. The uniqueness of each rearrangement further depends on random insertion and deletion of nucleotides at junction sites of V, D, J gene segments (result in V-(N)-D-(N)-J), making the junctional region of Ig and TcR genes as "fingerprint-like" sequence. This combined sequence constitutes a specific signature of each lymphoid cell clone, normal or malignant. Therefore, each of malignant lymphoid disease will represent an expansion of a clonal population with a specific IgH/ TcR rearrangement [3][8][12]. Because of these reasons, analysis of IgH and TcR rearrangement is among the best choices for monitoring MRD.

3.1.2. Common molecular genetic abnormalities in Acute Lymphoblastic Leukemia

Seventy five percent of childhood ALL cases have evidence of chromosomal translocation [11]. Cytological techniques with low resolution (karyotype, FISH) require the translocation must be large enough to be detected. Otherwise, it can be only seen at molecular level which in most cases, the translocation results in a fusion gene that can be analyzed by molecular techniques.

Some common fusion genes in ALL include BCR-ABL fusion gene t(9,22); TEL-AML1 (ETV6-RUNX1) fusion gene t(12,21); E2A-PBX1 fusion gene t(1,19) or MLL gene rearrangement [6][11].

3.1.3. Molecular techniques used in detecting MRD in Acute Lymphoblastic Leukemia

3.1.3.1. PCR and realtime PCR

Based on the characterization of IgH/TcR rearrangement and the present of common translocation in ALL patients, conventional PCR and realtime PCR used to amplify and quantify sequences including IgH/TcR cancer-specific rearrangements or cancer patient-specific translocations are considered as standard methods in monitoring MRD.

Samples are usually derived from bone marrow of leukemic patients for DNA or RNA extraction at diagnosis, then during follow-up. The reason is that MRD expression in bone marrow is higher than in peripheral blood, at least in AML and B-lineage ALL [4][7][9].

For detecting MRD by analyzing IgH/ TcR rearrangement, the various IgH/TcR rearrangements must be identified in each patient at diagnosis. The sequence information

enables the design of junctional region-specific oligonucleotides which can be used as primers in PCR to specifically amplify the rearrangement of malignant clone or as probes to distinguish the PCR product derived from leukemic cells with the one derived from normal lymphoid cells. The leukemic-specific sequence identified at diagnosis then can be used as a target to access MRD in follow-up samples [4].

Similarly, detecting MRD by analyzing translocation can be done only if a translocation exists in the patient. PCR amplification of chromosomal translocation can be used in approximately 37% of cases, usually using reverse transcriptase (RT) PCR with RNA sample, whereas PCR amplification of IgH/TcR rearrangement can be used in about 80% of patients [8]. That's why detecting MRD by analyzing IgH/TcR rearrangement is preferred.

MRD detection using PCR has major advantages because of its high sensitivity, accuracy, saving time, reproducibility, need of small DNA amount and irreplaceable use in retrospective studies. However, it also has noticeable disadvantages such as risk of contamination, degradation of RNA, the need of designing individual specific primers or probes [4].

3.1.3.2. Next generation sequencing

Next generation sequencing refers to non-Sanger-based high throughput DNA sequencing technologies. Millions or billions of DNA strands can be sequenced in parallel, yielding substantially more throughput and minimizing the need for fragment-cloning methods that are often used in Sanger sequencing genome. With the development of this new method, it has become possible to search not only for known mutations, translocations, but also for all clonal gene mutations and rearrangements present in diagnostic samples, and to track their evolution during therapy [5][6].

Regard to IgH/TcR rearrangement identification, consensus primers are employed to universally amplify rearranged IgH and TcR-gene segments in a sample and relies on high-throughput sequencing and specifically designed algorithms to identify clonal gene rearrangement in diagnostic samples and quantify these rearrangement in follow-up MRD samples [5].

However, this method is costly, requires high-skill technicians, modern laboratory. Therefore, this method is mainly used for research, not in clinical practice of MRD monitoring.

3.2. MRD detection in Acute Myeloid Leukemia

The fact that AML cells lack specific antigen receptors, like IgH or TcR, causes limitation of MRD detection based on gene rearrangement in AML patients [4]. About 55% of AML patients have cytogenetic abnormalities [1]. PCR amplification of fusion-gene transcripts or gene mutations is mainly used in detecting MRD in AML patient, particularly in patient receiving chemotherapy. The genomic breakpoints of the most common known leukemic fusion genes are spread over large distances within each gene locus [9][10]. Therefore, amplification of fusion gene at DNA level is limited while amplification of fusion-gene transcripts at RNA level is often chosen.

Some common known fusion gene in AML include PML-RARa t(15;17), AML1-RUNX1T1 (AML-ETO) t(8;21), orinv(16)(p13q22)/t(16;16)(p13;q22)/CBFBMYH11 rearrangements [6][11]. Common mutations used in detecting MRD in AML patient include FLT3 internal tandem duplication (FLT3/ITD) or NPM1 mutations [4][9][10].

The achievement of detecting MRD by PCR-based assays in AML is observed in 50% of the patients with suitable molecular targets [6].

Table 2. Most common genes and translocations used for detection of MRD [6]

Disease	Targets
Acute Lymphoblastic Leukemia	Patient-specific IgH or TcR genes
	BCR-ABL t(9;22)
	ETV6-RUNX1 (TEL-AML1) t(12;21)
Acute Myeloid Leukemia	PML-RARa t(15;17)
	AML1-RUNX1T1 (AML-ETO) t(8;21)
	FLT3/ITD
	NPM1

3.3. MRD detection in post-transplant period in leukemia patients

For leukemia patients who received allogeneic haematopoietic stem cell transplant (HSCT), besides the possibly applied methods mentioned above, another method that can be used for detecting MRD is microsatellite analysis by quantitative fluorescent PCR (QF-PCR) using short tandem repeats (STRs). The purpose is to evaluate the chimerism status of patient by quantifying the ratio of donor and recipient cells in the post-transplant period. This information helps to evaluate the treatment efficiency of HSCT and predict the risk of relapse. This methodology guarantees high sensitivity of 10^{-4} to 10^{-5}. A state of 100% hematopoiesis from donor origin is called "complete chimerism", while the coexistence of donor and recipient hematopoiesis is called "mixed chimerism". The term "increasing mixed chimerism" means increasing of recipient cells and vice versa for the term "decreasing mixed chimerism" [1][10].

4. CONCLUSION

MRD detection has an irreplaceable important role in leukemia management because of its value in evaluating treatment efficiency, selecting alternative therapies, follow-up, and predicting relapse as mentioned above. With the availability of molecular techniques with high sensitivity and specificity, the range of application of cytological methods in MRD detection has narrowed. Each method has its own advantages and disadvantages.

MRD detection by PCR-based assays can access nearly 90% of leukemia patients. Therefore, monitoring MRD by PCR-based assays has become a conventional method in clinical practice in regard of leukemia management in developing countries.

In Vietnam, doctors usually conclude complete remission based on clinical CR and haematological CR to evaluate the response to the treatment. These examinations are clearly indispensable but not sufficient, because even though the patient obtained haematological CR, he or she may still have a large number of cancer cells that can be detectable by cytological techniques, or by higher sensitive molecular techniques. If we can apply PCR-based assays, at least simply conventional PCR, in MRD detection, surely the outcome of leukemia patients will be improved.

ABBREVIATION

ALL: acute lymphoblastic leukemia
AML: acute myeloid leukemia
CR: complete remission
D: diversity
HSCT: haematopoietic stem cell transplant
IgH: immunoglobulin heavy chain
J: joining
MRD: minimal residual disease
N: nucleotides
TcR: T-cell receptor
V: variable

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