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A Hypothesis for Diagnosis of Chronic Myeloid Leukemia based on miR-15a

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Abstract:

Chronic myeloid leukemia (CML) is a type of leukemia characterized by myeloid proliferation. Morgana is a chaperone protein, whose levels are lower due to the overexpression of miR-15a in CP phase. By quantitatively detecting miR-15a and Morgana, we could verify the correlation of them to CP-CML, thus as biomarkers of CP-CML diagnosis.

Keywords:-chronic myeloid leukemia (CML); miR-15a; Morgana; diagnosis.

1. Introduction

Chronic myeloid leukemia (CML) first described in 1845 by Rudolf Virchow and John Bennett respectively is a type of blood cancer characterized by myeloid proliferation [1,2]. The additional number of CML every year is 2 per one hundred thousand, accounting for roughly 15 percent of the total number of adult leukemia [3]. The cancer can occur in all age groups.

The mechanisms underlying the development of CML are not yet fully understood. Despite this, it's widely recognized that most CML cases happened due to chromosome variation. In most cases, there is a balanced genetic translocation between No.22 and No.9 chromosome. After translocation, the changed No.9 chromosome getting shorter is well known as Philadelphia (Ph) chromosome [4]. The consequence is that a new fusion gene called BCR::ABL1 forms. Then, the oncogene expressed a problematic tyrosine kinase, BCR::ABL1 oncoprotein, which will cause proliferation and division out of control by affecting downstream pathway[5]. The Ph chromosome is highly associated with CML. However, only nine in ten patients have a typical chromosome translocation, while 5% have variant or complex translocation. Approximately 2%-5% of patients are Ph-negative, in spite of presenting with a morphologic picture of CML [3]. Therefore, CML is now identified by the BCR::ABL1 fusion originating from t(9;22)(q34;q11), according to the WHO Classification of Haematolymphoid Tumours[6]. But some (2%-5%) have different variants of transcripts which may end up with a false-negative by routine probes [3]. For patients with atypical chromosome or gene mutations, they are at risk of misdiagnosis.

People with chronic myeloid leukemia may go through 3 illness stages: chronic phase (CP), accelerated phase (AP) and blast phase (BP) [3]. 90%~95% patients present in CML-CP, where around a half patients are asymptomatic or have mild symptoms, or symptoms overlapping with other diseases [3]. Here we focus on patients with these symptoms owing to the lack of timely diagnosis. For most cases, the current diagnostic process is quite complete. In general, initial evaluation consists of a medical history and physical examination including spleen size by palpation, full blood count (FBC) with differential, blood chemistry studies [7]. To confirm the diagnosis of CML, bone marrow aspiration and biopsy for morphologic (to observe cell morphology) and cytogenetic (to seek Ph chromosome) evaluation are indicated for all sufferers in whom CML is suspected [3]. In addition, it's recommended to have a quantitative reverse transcription polymerase chain reaction (RT-qPCR) test to evaluate the amount of BCR::ABL transcripts. If someone is Ph chromosome positive or BCR::ABL1 gene positive, he or she is diagnosed with CML. On the contrary, if somebody is both Ph chromosome and BCR::ABL1 gene negative, doctors will evaluate for diseases other than CML[7].

In the remaining cases, regular molecular or cytogenetic studies do not adequately characterize the disease from which patients suffer. Our idea is to detect (potential) biomarkers to assist in the diagnosis of the leukemia. Ogasawarac et al. reported a simple but effective screening method for the diagnosis of CML [8]. By their method, an absolute basophil count more than 4.3 × 108 per liter is a mark of CML. Inspired by their work, here we come

up with a hypothesis based on miR-15a and Morgana. Recently, research showed that low Morgana expression level is relevant to whether the patient is in CP-CML phase or not; Morgana is a chaperone protein, whose levels are lower due to the overexpression of miR-15a in CP phase [9]. In this work, how to quantitatively detect miR-15a or Mogana by using biosensor and other methods is further elaborated on.

2. Hypothesis

As mentioned before, CML cannot be diagnosed directly at present. The diagnosis of CML is still based on some strongly correlated markers (such as Ph chromosome and BCR::ABL1 mRNA transcript detection, etc.), and the final confirmation of CML in high probability patients is achieved by observing various indicators after pre-treatment with TKI drugs. Recent studies have shown that overexpression of miR-15a [a kind of miRNA (small non-coding RNAs interact with their target mRNAs to modulate gene expression at the post-transcriptional stage [10].)] leads to a decrease in the expression level of Morgana protein in CP-ph+ CML patients [9]. Therefore, we suppose that the quantitative detection of miR-15a and Morgana protein can be used as a strongly correlated marker for the diagnosis of CML, thereby improving the diagnosis rate of CP-CML patients and shortening the treatment time for CML patients due to delayed diagnosis.

3. Quantitative Detection

3.1 Quantitative detection of miR-15a

There are already many established methods for quantitatively detecting specific miRNAs, some of the most common and mature methods, which can detect miR-15a in live cells, are as follows:

3.1.1 RT-qPCR.

RT-qPCR is a combination of Quantitative real-time PCR and Reverse transcription-PCR technology based on amplification. The specific procedure of RT-qPCR is represented in Fig.1.

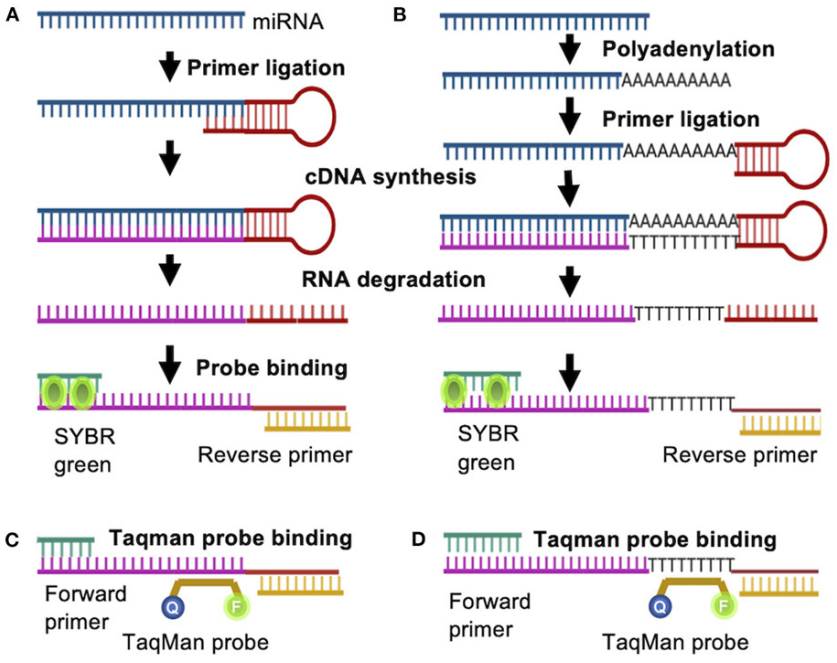

Fig. 1 Diagram of miRNA detection by RT-qPCR [10].

In A, a hairpin DNA (Single strand of DNA that forms a stem-ring "hairpin" configuration, hpNDA) is directly added to do primer ligation, to synthesize a cDNA (Complementary DNA) with the hairpin DNA. Through RNA degradation, a single strand cDNA plus hpDNA is formed, and after probe binding, usually using SYBR green, a double stranded DNA is formed, and the whole process is finished. SYBR green shows low fluorescence in solution, but when it binds to double-stranded DNA, it produces strong fluorescence, making it a great marker for double-stranded DNA concentrations. In scenario B, adding adenine nucleotides to the 3′-end (Polyadenylation) allows hpDNA to indirectly facilitate primer ligation. To address potential inaccuracies in SYBR green readings due to contamination from double stranded DNA or primer dimers, TaqMan probes are employed to monitor amplification rates as depicted in scenarios C and D. Normally, the Quenched group Q in TaqMan will reduce

the fluorescence of F group, when Taq DNA Polymerase works and touches TaqMan, the F group will separate from Q group to make the fluorescence detectable, and that is how TaqMan works. Currently, RT-qPCR is still the favored method for validating data from microarrays and next-generation sequencing. However, the variability in primer design, data analysis methods, and normalization procedures often leads to poor reproducibility, underscoring the importance of using different detection techniques [10].

3.1.2 Next-generation sequencing (NGS).

The application of Next-generation sequencing (NGS) develops rapidly in the last couple of years in sequencing miRNA.

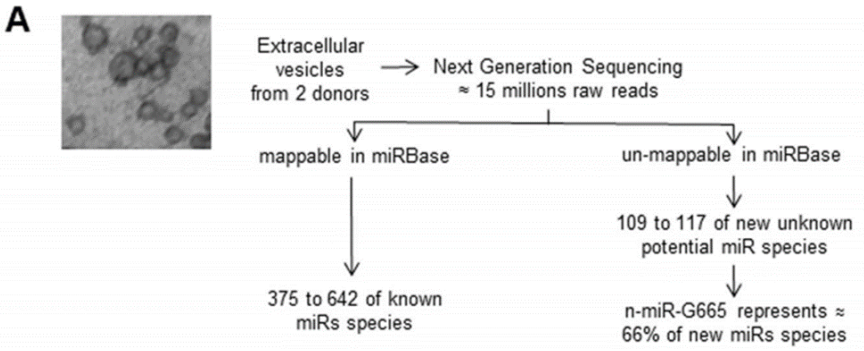

Fig. 2 Diagram of a novel approach for identifying new miRNA variants using NGS [11].

First, the target miRNA is extracted, broken down into many small fragments, got labeled, and then loaded on the sequencer to quickly read the sequence of each fragment to obtain sequencing data (see Fig.2). After that, compare the data with the miRNA library generation to confirm whether it is the target miRNA. Next-generation sequencing (NGS) provides unmatched, comprehensive data collection, sequence coverage, and depth, making it the sole discovery-based method capable of identifying new miRNAs. However, the widespread use of NGS may be constrained by its high cost and the need for complex computational analysis [10].

3.1.3 Bead-Array Based Profiling

MiRNAs are usually different on a single nucleotide. The issue of cross-hybridization among closely related miRNAs has driven the creation of bead-array-based profiling techniques.

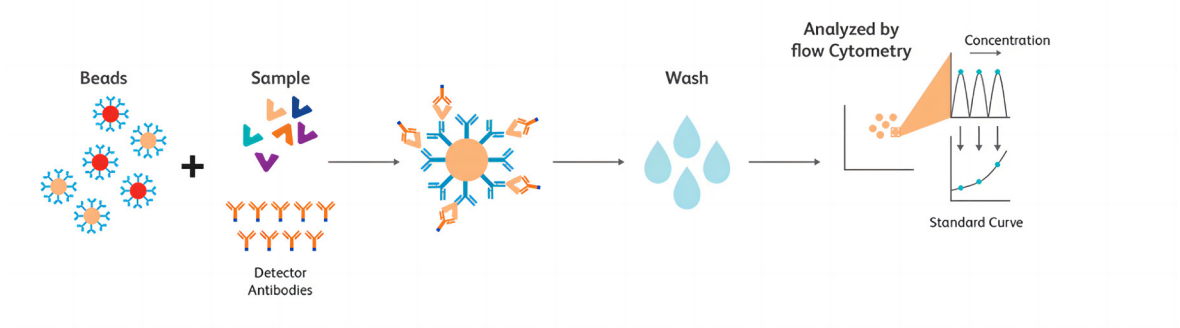

Fig. 3 Schematic diagram of bead-array based profiling [12].

During reverse transcription amplification, a biotin tag is incorporated. Beads are then ligated with oligonucleotide probes that are complementary to the miRNAs. The biotinylated cDNAs are mixed with capture probes, followed by washing away unbound probes and subsequent analysis using flow cytometry (guiding individual cells through a light source and measuring the scattering and emission of light energy produced at various wavelengths) to identify and quantify miRNA based on bead color and intensity. Compared to microarrays, low cost is the advantage of bead-array based profiling, and so is fewer steps and medium throughput. Requiring specific equipment to do flow cytometry is its limitation (see Fig.3).

3.2 Quantitative detection of Morgana protein

As is known to all, directly quantitatively detect a unique kind of protein outside the lab is tough. Common way is to detect its coding gene mRNA (i.e. CHORDC1 mRNA) to make the question easy. Methods are almost same with

detecting miRNA, such as RT-qPCR, and the following is an exception.

3.2.1 Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a modern variation of in situ hybridization that substitutes isotope labeling with fluorescent labeling.

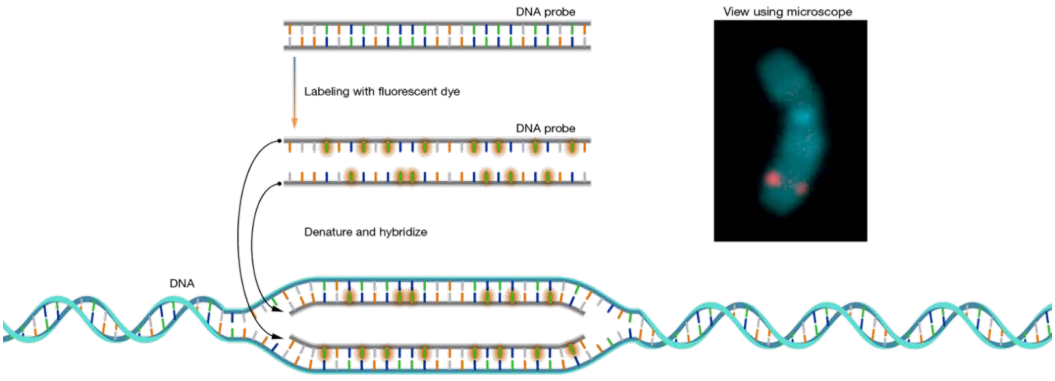

Fig. 4 schematic diagram of FISH [13].

The first step is to generate double-stranded cDNAs from mRNA through Reverse Transcription PCR. These cDNAs are then treated with a "probe," which is a diminutive fragment of purified DNA labeled with a fluorescent dye. The fluorescent probe then identifies, binds to probe's matching sequence, which is in the cDNAs. By way of fluorescence detection system under microscope and other techniques (about quantitative detection), the fluorescence gets detected and counted. Nonuse of radiolabel means safety and economy, meanwhile, high probe stability, specificity, accurate localization and quickly obtaining results makes FISH an excellent method. Nevertheless, not 100% hybridization affects its efficiency and quantitatively accuracy (see Fig.4).

3.2.2 Direct detection of Morgana protein

There are many kinds of quantitative detect methods of protein can be used, but only with peptide and amino acid sequence determination and specific structure determination. For example, Western blot is used in the reference to quantitatively detect Morgana and track it[9]; Isotope-coded protein label (ICPL) reagent technology allows labeling almost whole peptides exist in the specimens, but is unworkable without the peptide sequence of target protein; MS is a strong, mature and powerful way to accurately quantitatively detect Morgana, especially combined with other methods such as plurally MS, HPLC, ESI, MALDI and so on; Relative quantification, including chemical labeling, metabolic labeling, enzymatic labeling and LFQ(label-free quantification) based on spectral counting and intensity figures up the abundance ratios by contrasting signals between proteins and peptides from diverse specimens. Besides, absolute quantification provides precise measurements of mass, copy numbers, or mole numbers of proteins, whether labeled or unlabeled. An overview of quantitative detection of protein is as follow: *Quantitative mass spectrometry-based proteomics: an overview* [14].

4. Conclusion

With the data of CP-CML patients and normal people obtained by the quantitative detection methods mentioned above, we could verify whether miR-15a and Morgana is correlated with CP-CML or not. If the consequence supports our hypothesis, we could do further work to determine the cut-off level of miR-15a and Morgana/CHORCD1 mRNA to make it possible to be markers for diagnosing CP-CML. Assuming that current CP-CML diagnosis is 99% accurate with additions of 100,000 per year, our work may increase the percent to 99.5%, so we could help 500 CML patients per year to confirm early and save their lives, and that's the significance of what we're doing, we think. In addition, research also shows that when CP-CML patients turn to BP stage [9], the expression of miR-15a decreases, resulting in increased expression of targets: Morgana protein, which could become a biomarker of CP stage to BP stage. However, the lack of sample data of BP patients means that this hypothesis needs to be verified by stronger evidence.

5. Acknowledgement

Rui Huang, and Yishi Yan contributed equally to this work and should be considered co-first authors.

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