

The Evolution of Genetics Techniques for Leukemia Diagnosis

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Leukemia is characterized by abnormal proliferation of hematopoietic cells as the result of underlying genetic lesions. There are four main types of leukemia-Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL) - as well as a number of less common types [1]. Genetic studies into the cause of leukemia are considered to be crucially important, with regards to prognostication, therapeutic choice. Since the first specific chromosomal abnormality was identified in chronic myeloid leukemia in 1960, the so-called Philadelphia chromosome, genetic changes underlying leukemia have been continuously discovered and new methods are being established aiming to improve treatment and to decrease treatment side effects such as classic cytogenetics, Polymerase Chain Reaction (PCR), Fluorescence In Situ Hybridization (FISH), Array Comparative Genomic Hybridization (array-CGH), Sanger sequencing (first generation sequencing) and more recently Next Generation Sequencing (NGS). These evolutionary techniques have been successfully employed to help refine diagnosis, establish prognosis and monitoring leukemia follow up [2].

Cytogenetic analysis (G-banding) has been routinely used as part of clinical practice helping to determine disease aggressiveness, response to treatment, and prognosis [3]. The role of cytogenetics in determining the biologic basis of leukemia is now widely recognized. Metaphase analysis of leukemia samples is useful to detect changes such as inversions, translocation deletions, duplications and aneuploidies, which are common in AML, ALL and CML. However, the relative resolution of banding analysis, the lack of sensitivity (5%), and the requirement for preparation of metaphase chromosomes using fresh material, as well as for experienced personnel for the analysis significantly limited the usage of this technique. Banding techniques also face difficulties with highly rearranged chromosomes. The introduction of FISH in the late 1980s was heralded as a further evolution in cytogenetic analysis. By using entire chromosomes or short DNA fragments as probes, FISH can be applied to detect genetic abnormalities such as characteristic gene fusions, aneuploidy, loss of a chromosomal region or a whole chromosome or to monitor the progression of an aberration serving as a technique that can help both in the diagnosis of a genetic disease and in suggesting prognosis [4]. Due to the high sensitivity and specificity, FISH has gained general acceptance as a clinical laboratory tool. The extension of FISH to fibre-FISH, Q-FISH, flow-FISH and multiple colour FISH also further extend the usage of this technique in research or in the clinical laboratory. Although FISH improves the sensitivity level when comparing with banding technique it is still considered as labour-intensive. In addition, around 40% to 50% of patients with AML lack karyotypic abnormalities at diagnosis but could bear cryptic copy number alterations which cannot be detected by FISH.

Array-CGH allows genome-wide high-resolution analysis of copy number alterations that are not detectable by banding technique and FISH. In addition, high-resolution genomic microarrays enable simultaneous detection of cryptic copy-number aberrations. Array-CGH has greatly increased genomic resolution over classical cytogenetics techniques and has become a powerful tool in the analysis of unbalanced chromosomal rearrangements. Array-CGH is supplied

in different formats ranging from low resolution 44k to high resolution 1M, which offer the flexibility of lowering the cost with low resolution when necessary.

In leukemia, mutations can be single base changes found at multiple sites across the majority of a gene (e.g., CEBPA gene) or very specific mutations at a single codon (e.g., JAK2V617F), or involve base changes, such as deletions (e.g., TP53 gene), insertions (e.g., NPM1 gene) and duplications (e.g., FLT3 gene). In most cases, Sanger sequencing will be considered as the first choice for the detection of these mutations as this approach has been particularly successful in delivering the diagnosis result. However, in a clinical molecular diagnostic laboratory, with the continuously increasing candidate genes, the Sanger sequencing technique can hardly deliver the ever-increasing numbers of relevant mutation tests on each patient at an affordable cost. Multigene screening with Sanger sequencing is also facing the challenge of the need for relatively large quantities of DNA to assess one gene at a time (e.g., Factor 8) as well as fair low sensitivity (20-30%).

With regards to leukemia patients follow up, minimal residual disease (MRD) detection is now becoming routinely implemented in several treatment protocols, as an important tool for clinical decision-making, if not at least serving as a monitoring marker for the treatment. For example, according to the European Leukemia Net (ELN), early molecular response at 3 months in Philadelphia chromosome-positive CML patients receiving the tyrosine kinase inhibitor Imatinib is associated with significantly higher rates of overall survival [5]. In this case the PCR technique serves as the main technique for MRD detection. PCR/Real-time PCR can generally achieve a sensitivity of at least 10⁻³, but preferably 10⁻⁴-10⁻⁵ should be reached for MRD detection in leukemia. MRD-PCR targets can be analysed at the DNA as well as at the RNA/cDNA level. The sensitivity of PCR is also its major disadvantage since very small amounts of contaminating DNA (from a different sample) can also be amplified. Also, PCR only targets one gene, thus with an ever increasing number of target genes, PCR techniques can hardly cope.

Each method described above can only address part of current demands, as no single method can replace the others. However, the emergence of new NGS widely used in research and clinical application is changing current technical approaches. The massively parallel sequencing ability of NGS technologies has made high throughput

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and multiplexed sequencing of specific panels of genes or whole exomes/genomes feasible. The widespread use of NGS is not only inevitable, but will also be beneficial, as it now offers the opportunity to sequence a human genome for a cost of just 1000 USD comparing with the first human genome project which cost 3 billion USD [6]. Dramatic advances in NGS technologies have also provided a novel opportunity to understand the molecular genetics of leukemia through the comprehensive detection of multiple genes in a single run. For example, in 2014 a multiplex panel containing 54 cancer-related genes was developed using NGS methods, including genes relevant in AML such as NRAS, KRAS, FLT3, NPM1, DNMT3A, IDH1/2, JAK2, KIT and EZH2 [7]. However every coin has two sides, the development of NGS strategies as diagnostic tools also leads to some novel interpretational problems and ethical issues. Also from our point of view software analysis has a long way to go for routine diagnosis as not all clinical laboratories will have in-house bio-informaticians available to handle the data analysis. The ethical issues raised by NGS are not completely new compared to those related to previous work in genetics and genomics. However, NGS presents some unique features, such as the amount and quality of data. Furthermore, with regards to the patient the potential information overload, the complexity of information, the limited ability of individuals to sufficiently understand or even just to remember the information given, the biased understanding and false hopes can potentially only bring disturbance to the patient's normal life without any obvious benefits at least not currently. These matters require serious consideration before presenting the information to the patients.

In summary, all the genetics techniques are essential in clinical diagnosis. Each one of them presenting its own unique advantages and disadvantages, they can co-exist in the laboratory to serve the purpose of leukemia diagnosis and research for many years to come.

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