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A Review on the Relevance of Molecular Karyotyping in AML

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Abstract: Acute myeloid leukemia (AML) is an aggressive disorder characterized via the overproduction of immature myeloid cells that accumulate in blood and bone marrow. Integration of genetic findings and clinicopathological facts is critical in establishing the diagnosis, prognosis and identifying the therapeutic approach in the administration of AML patients. In current years, the AML classification has developed from morphology to cytogenetics/molecular genetics-based findings, which is indispensable in the detection of chromosomal abnormalities and has provided the framework for the analysis and riskstratification in AML. Moreover, with advances in molecular karyotyping such as comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays, a number of obstacles of traditional diagnostic techniques have been overcome. Hence, this review focuses on the insights into molecular karyotyping using CGH and SNP arrays which allow the identification of replica wide variety versions (CNVs) at a higher resolution and facilitate the detection of replica impartial loss of heterozygosity (CN-LOH) otherwise undetectable by using traditional cytogenetics. Technical hindrances of these strategies (e.g. regions of losses, gains, or "undulating waves") are also mentioned in the context of AML.

Keywords: Copy Number Variants, Genomic Analysis, MicroarrayAcute Myeloid Leukemia, Comparative Genomic Hybridization

1. Introduction

Leukemia is a group of hematological malignancies characterized with the aid of unusual proliferation of hematopoietic cells which result in impaired maturation and excessive accumulation of immature cells in bone marrow and/or blood [1]. This leads to bone marrow failure with anemia, neutropenia, thrombocytopenia or pancytopenia and improved hazard of organ infiltration through the malignant cells. Leukemia itself can also be widely labeled to acute and persistent leukemia. Out of 4573 cases of leukemia mentioned from 2007-2011 in the Malaysian population, 54.2% of the instances were AML [2], making up the majority of leukemia instances inside the region. The records of AML within Malaysia may also be in contrast to AML cases in the United States (US), whereby in 2015 alone, 20830 new cases were diagnosed with a recorded mortality charge of over ten thousand [3]. Although remedies such as allogeneic hematopoietic stem mobile transplantation (HSCT) [4] and induction remedy with cytarabine [5] do exist to improve common survival (OS) of patients, untreated sufferers over the age of 60 are pronounced to solely have an common survival between 5 to 10 months [6],

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further emphasizing the significance of a strong diagnostic method to better stratify the disease. Thus, this overview focuses on the deoxyribonucleic acid (DNA) microarraybased molecular cytogenetic approaches, advances, and boundaries in the context of AML with the addition of how these can also be top-quality to traditional techniques to diagnose this disease.

2. Overview on Current AML Diagnostic Approaches

Over the years, the important diagnosis of AML lies on the morphologic assessment of leukemic myeloblast in the peripheral blood or bone marrow. Diagnosis of AML is installed in the presence of blast exceeding 20% in theblood or marrow with the exception for instances with t (15; 17), t (8; 21), t (16; 16) or inv (16) and erythroleukemia. In some section of the world, lineage assignments depend on cytochemistry such as myeloperoxidase (MPO) or Sudan Black B (SBB), non-specific esterase (NSE) and periodic acid Schiff (PAS) [7, 8]. Morphological assessment, however, has limitations as it requires know-how to distinguish the cells, particularly in instances the place ambiguities exist in differentiating leukemic cells from reactive cells, whereby other ancillary testing is required to verify the lineage of the leukemic cells. Aside from morphologic assessment, glide cytometry immunophenotyping (IP) is an fundamental device in the prognosis of AML as it enables the characterization of leukemic cells via assessment of mobilephone surface, cytoplasmic and nuclear antigen expression patterns [9–11]. Characteristic expressions of myeloid lineage markers such as CD13, CD33 and CD117 enable the big difference of AML from different types of leukemia. Asynchronous antigen expression, also regarded as aberrant antigen expressed in leukemic cells serves as an auxiliary tool in the diagnosis and prognosis of AML. Aberrant expressions of CD56 in AML and CD2 in APL have been implicated as terrible prognosis. In particular, CD56 expression in AML with t (8;21) corresponds to a shortened DFS and OS, such as in patients who have undergone transplantation [12]. In addition, waft cytometry immunophenotyping (IP) also aids in the monitoring of minimal residual ailment (MRD) with greater sensitivity as in contrast to morphological examination of bone marrow aspirates, even though at a 1-log much less touchy as in contrast to polymerase chain response (PCR) primarily based methods [13]. Moreover, MRD with the aid of float cytometry IP is viable for cases the place molecular techniques cannot be utilized. However, there are drawbacks in these method mainly phenotypic shifts in the course of and/or after therapy [14]. Secondly, there is no clear consensus on the list of markers for AML research and so the resolution of markers is commonly based on the individual laboratory's discretion and experience. However, with larger adoption of multicolor go with the flow cytometry, this downside will probably be mitigated in the future. Cytogenetics remains an critical device in prognostication in AML and performs a key function in the medical management of AML patients. Specific cytogenetic aberrations are associated with particular abnormalities that are subsequently correlated with response to therapy and patient's survival. However, efforts to elucidate submicroscopic genetic transformations are thwarted via limited resolution, negative chromosome morphology apart from other sampling and technical issues [15]. Techniques such as FISH are beneficial in identification of structural and numerical aberrations as it ambitions non-dividing cells (interphase FISH). This technique, however, is extra of a focused approach and requires prior expertise of anomalies of interest; thus, it is unsuitable as a screening tool in the diagnosis of leukemia. Based on the traditional cytogenetics

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method, the World Health Organization (WHO) said a classification of AML in 2016, whereby the sickness was once subdivided into 25 entities with extra subclasses: provisional entities the place mutations happening in AML with regular karyotype particularly NPM1 and CEBPA; AML associated with Down syndrome, acute panmyelosis with myelofibrosis, granulocytic sarcoma, and blastic plasmacytoid dendritic mobilephone neoplasm as shown in Table 1 [16]. Having been proven to possess distinct cure responses, this classification helps to improve prognosis of AML through molecular trying out and further stratify predicted patient outcomes. Dohner et al. for instance, similarly associated molecular signatures that some AML sub-classes have into favorable, intermediate I, intermediate II, and damaging genetic groups [7].

Table 1. WHO classification of AML and related neoplasms (2016) [16].

AML with recurrent genetic abnormalities
AML with t (8;21)(q22;q22.1);RUNX1-RUNX1T1
AML with inv (16)(p13.1q22) or t (16;16)(p13.1;q22);CBFB-MYH11
APL with PML-RARA
AML with t (9;11)(p21.3;q23.3);MLLT3-KMT2A
AML with t (6;9)(p23;q34.1);DEK-NUP214
AML with inv (3)(q21.3q26.2) or t (3;3)(q21.3;q26.2); GATA2, COM
AML (megakaryoblastic) with t (1;22)(p13.3;q13.3);RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1
AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

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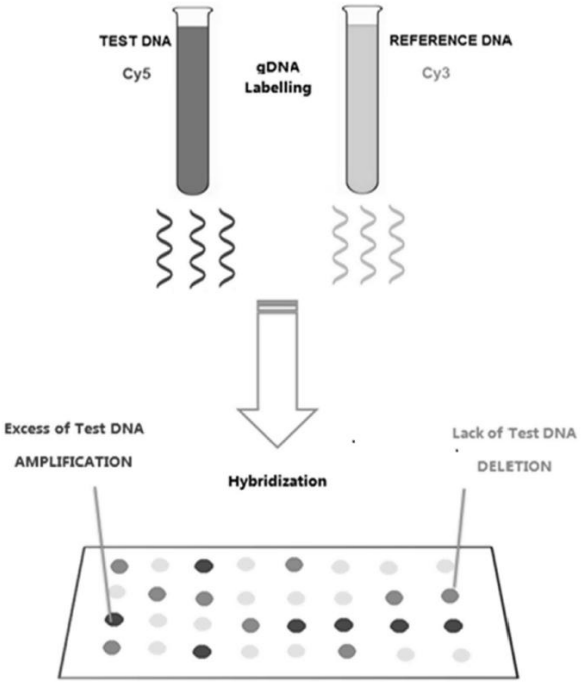

Figure 1. CGH gDNA labeling and hybridization. Test DNA is labeled with Cy5 and reference DNA is labeled with Cy3. Fluorescence signals are measured after hybridization. Excess and lack of test DNA is detected as amplification and deletion respectively.

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3. CGH + SNP in a Single Array

Recently, SNPs have been delivered to array CGH which enable elucidation of CNVs and CN-LOH. This gives insights into areas of obtained homozygosity that may additionally harbor tumorigenic changes that maybe predispose to AML. Combined arrays have shortened pattern processing time as the protocol of CGH and SNP are mixed into a single scan which leads to a much less laborious workflow. Data analysis turns into feasible as dual records of CGH and SNP is presented concurrently. Although SNP probe insurance on the mixed platform tends to be lower than the regular platform, other studies have highlighted that the overall performance of this blended array is tremendous in aiding investigation of CN-LOH, mosaic aneuploidy and CNVs [21].

4. Mosaicism in AML

In mosaic samples, there will be share of regular cells and tumor cells ensuing in a fractional of total CN in nondiploid regions. Previous research have theorized the presence of mosaicism via assessing log2 ratios or allele precise reproduction range and predicate the variations as doable warning signs of clonal heterogeneity [22-24]. In a study on clonal evolution in hematology malignancies by combining comparative genomic hybridization and single nucleotide polymorphism array on sixteen patients, guide top reassignment evaluation enabled the detection of heterogeneity in 10 patients [24]. In this study, the researchers recognized the clonal heterogeneity by means of referring to clonal fraction, discrepancy between CGH and SNP data, distribution of log2 ratios and peaks in the distribution plot where a CN was now not assigned as shown in Figure 2.

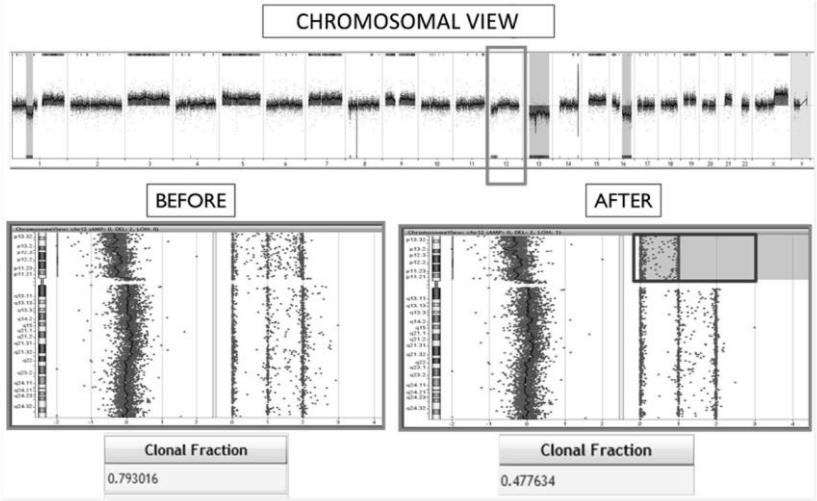

Figure 2. Clonal heterogeneity indicators. A) View of chromosomes generated by way of Cytogenomics software version 2.9.2.4 which indicates a deletion in the p arm of chromosome 12. Picture B) and C) exhibit the CGH and SNP data of the chromosome 12. Different log2 ratios point out the presence of sub-clones in the sample. Clonal fraction based on log2 ratios were estimated to be 79.3% (A) and 47.8% (B) respectively.

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CGH records shows a deletion of 12p however SNP information was normal for chromosome 12. This indicates a presence of sub-clone in the sample. Manual peak reassignment used to be carried out and the SNP records showed similar findings with CGH information submit reassignment (C). In our learn about on AML genome, clonal heterogeneity used to be through referring to clonal fraction discrepancy in our custom-made blended array CGH+SNP records through guide peak reassignment analysis. Low amplitude of array CGH was once determined except confirmation of SNP probes which indicated presence of minor clone. Figure three shows data earlier than and after guide peak reassignment in an AML patient.

5. Conclusion

AML is a heterogeneous hematological disorder which growth swiftly and requires instant treatment. It requires incorporation of a variety of scientific and laboratory findings in a timely manner to treat the sufferers effectively. Conventional cytogenetics has been extraordinarily precious in prognostication of AML over the last three decades. However, there are boundaries in this technique the place it is laborious in nature, requires technical understanding and thwarted by means of incapacity to uncover many doable sub-microscopic genomic ameliorations due to decision limitations. With the use of combined array CGH+SNP platform, a complete strategy for elucidation of clinically applicable CNVs and CN-LOH have been possible in a single assay. Delineation of tumor related genomic aberrations from inherited genomic variants was once feasible with the use of matched germline DNA for every of the case and minimized false discoveries in our case series. Chromosom alabnormalities with breakpoints coordinates should be greater accurately compared to traditional cytogenetics. Array CGH+SNP had a number of wonderful advantages in phrases of resolution and detection of cryptic CNVs aside from regions of CN-LOH which had been undetectable by way of conventional cytogenetics.

Besides that, in instances with no analyzable metaphase using traditional cytogenetics, array CGH+SNP were beneficial in presenting chromosomal aberrations as no cellphone culturing is required as the beginning cloth was once DNA. These findings can then be established with other techniques such as FISH, quantitative PCR (qPCR), multiplex ligationdependent probe amplification (MLPA), or a different microarray platform. In terms of analysis, the laboratory desires to determine the CNVs interval sizes, pathogenic aberrations as wells as the diagnostic, prognostic and therapeutic magnitude of the abnormalities detected. Further studies on clinically uncertain, received and probably pathogenic somatic aberrations be carried out prior to reporting of these abnormalities to the clinicians without speculating the pathogenicity of the CNVs. Although there are rapid developments in the other molecular techniques, array CGH and SNP holds fantastic practicable to be built-in in clinical administration of AML sufferers in phrases supplying diagnosis, prognostication and stratification of patients in accordance to their genomic abnormalities. Ultimately this will pave the way to improvement of stronger treatment modalities as this technique presents quicker detection of chromosomal aberrations with higher accuracy in mapping the breakpoints in somatic aberrations detected in AML.

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