Recent Diagnostic Techniques and Their Implications towards Acute Myeloid and Chronic Neutrophilic Leukemia

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Abstract

Leukemia is characterized by the uncontrolled proliferation of abnormal white blood cells within bone marrow, leading to immaturities within blood cells. In acute myeloid leukemia and chronic neutrophilic leukemia, genetic mutations induced by either foreign or natural processes lead to the development of leukemia. Although leukemia is a diagnosable disease, the diverse subtypes and a complex, multi-step diagnosis makes identifying expedient, accurate diagnosis methods a top priority. With specific methods being attributed as the only means of diagnosing leukemia, and the importance of proper subtype classification in patient prognosis, the lack of research regarding novel diagnostic approaches remains a concern due to the important role leukemia classification plays in the prognosis of the patient. Currently, three diagnosis methods can be regarded as prevalent to the modern diagnosis of leukemia: epigenetics, molecular genetics, and cytogenetics. Each of these current methods are capable of identifying specific subtypes of leukemia. Furthermore, developing more detailed diagnosis criteria will provide better prognostic stratification. This review will cover various diagnosis methods regarding acute myeloid leukemia and chronic neutrophilic leukemia. Further inquiry in the area of diagnostics could reveal novel patterns within the diagnosis of Leukemia, furthering the ability to treat patients more specifically which can increase the prognosis of the patient.

Introduction

Leukemia is a group of malignancies in the hematological, or blood, cells of the body, typically originating in the bone marrow. Over countless decades since its discovery, leukemia has remained a large concern in the field of public medicine. Due to the complexity and diversity of its many subtypes, leukemia requires an accurate patient diagnosis with targeted treatment plans. The modern diagnostic approaches integrate newer technology such as cytogenetics, molecular genetics, and epigenetics and has allowed for a comprehensive identification of subtypes. These methods have, overall, contributed to the improvement of the overall prognosis of patients with leukemia due to their contributions in creating new treatment methods that can be applied to patients. Given their critical role in treatment approach and patient outcome, modern techniques play crucial roles in the diagnostics of leukemia and it is a necessity to understand as well as to improve upon possible limitations of these modern techniques. Research to further analyze and explore novel diagnostic tools and approaches serves to improve patient diagnosis and ultimately survival.

While modern diagnostic specialties and their respective tools have contributed to significant advances within the world of medicine, a more holistic, comprehensive approach integrating each discipline is currently lacking. An integrated approach affords researchers and clinicians a more thorough analysis of genetic, epigenetic, and proteomic biomarkers, opening new possibilities for disease classification and patient stratification. The valuable insight provided by such integration would better inform diagnosis and treatment plans, ultimately improving patient care and prognosis.

In this review, we will highlight both the individual disciplines and their interdisciplinary use. Through an understanding of the benefits, complexities, and potential drawbacks of an integrated diagnosis system, we envision an improved era of precision medicine, whereby patients receive a more accurate diagnosis, targeted treatment plans, and ultimately leading to

increased survival with fewer treatment side effects.

2. Cytogenetics

Cytogenetics is the study of chromosomal structures and abnormalities. While decades old, it still plays a pivotal role in the diagnostic approach to leukemia. Many discoveries made through cytogenetics, such as the aberrations t(9;22) and t(15;17), have been instrumental. These breakthroughs have allowed further subclassification of leukemia into distinct molecular subtypes. (Takahashi et al., 2011)

2.1 The Principles of Cytogenetics

Cytogenetics analysis, also known as karyotyping, requires meticulous examination of chromosomal aberrations that underlie the subtypes of leukemia. The technique analyzes the collection of metaphase chromosomes in dividing cells. The chromosomes are stained, arranged, and visualized. These data are analyzed to visualize to identify any structural changes, particularly deletions, duplications, and rearrangements. Notably, the constant recurring discoveries of chromosomal abnormalities found through karyotyping has been pivotal in classifying the different subtypes of leukemia. Additionally, greater than 3 aberrations detected by karyotyping identifies a risk of leukemia. (Mrózek, 2008)

To improve its accuracy in diagnosis and risk stratification, cytogenetics has integrated more modern technologies such as fluorescence in situ hybridization (FISH). The FISH process involves hybridizing fluorescently-labeled DNA probes onto target sequences of DNA. It is effective at identifying subtle chromosomal abnormalities that can be missed by the traditional karyotyping method and has been used to identify the t(15; 17) translocation or the RARA genes. (Shakoori, 2017)

More advanced cytogenetic methods such as array comparative genomic hybridization (aCGH) directly compare cytogenetic analysis to reference DNA samples for diagnosis. These comparisons highlight differences in hybridization intensities, which identifies copy number changes in regions of DNA. Copy number changes are phenomenons where the genome sequence is constantly repeated in a different way. This method will not be discussed in this review paper, as this method has been extensively reviewed by (Ahn et al., 2015).

2.2 Chromosomal Aberrations Across the Subtypes of Leukemia

Using cytogenetics, chromosomal aberrations can be identified in patient samples to inform disease subtypes. The two subtypes of leukemia addressed in this paper are acute myeloid leukemia (AML) and chronic neutrophilic leukemia (CNL).

The AML subtype has been one of many to be identified by cytogenetics. Specific chromosomal alterations, such as translocation of t(8;21)(q22;q22), t(15;17), t(8;21)inv(16), t(16;16) have been identified in more than 50% of patients with AML. Notably, the t(8;21)(q22;q22) translocation has prognostic implications, as this alteration is associated with improved survival (Reikvam et al., 2011).

Chromosomal translocations, however, are not the only identifiable factors revealed by cytogenetics. Fluorescence-based polymer-chain reaction (PCR) has also allowed for the identification of genetic alterations in AML, such as NPM1 (33%), FLT3-ITD (18%), and CEBPA (19%); each percentage indicating the likelihood of being found within AML. (Chauhan et al., 2013) The percentage of patients who possess these identifiable genes are much lower than alterations discovered by karyotyping, however the application of fluorescence-based PCR

alongside karyotyping has increased prognostic stratification of patients. Furthermore, FISH has been used to identify the AML-M2 mutation which occurs within the t(8;21)(q22;q22) translocation (Suto et al., 2015).

CNL is also identifiable through cytogenetics. The (8;22)(q11;q11) translocation is a key biomarker found in the majority of CNL patients. Furthermore, the ability to identify the formation of fusion genes such as PCM1-JAK2 has made FISH the primary method for diagnosing CNL. Presence of these fusion genes indicate a poor prognosis.

2.3 Prognostic Significance of Cytogenetic Findings

With this paper's focus on diagnosis, it is also worth noting the importance of these diagnostic tests on patient prognosis and treatment options. This section will focus on the influence of cytogenetic analysis on prognosis for AML and CNL patients.

In AML, patients with a translocation of RUNX1-RUNX1T1 t(8;21) are given a favorable risk. In contrast to such favorable prognostics, karyotypes with multiple chromosomal abnormalities are associated with poor outcomes and typically require patients to undergo risky and aggressive treatment regimes. In general, the usage of cytogenetics alone is ineffective in establishing a reliable prognostic outcome and requires assistance from other methods that allow for a more refined view (Reikvam et al., 2011). Certain molecular markers such as NPM1 with standard cytogenetics provide a favorable prognosis. However, FLT3-ITD with the absence of NPM1 provides a less than favorable prognosis for the patient. (Juliusson et al., 2020)

2.4 Challenges and Future Prospects of Cytogenetics

Cytogenetics techniques have made significant contributions to identifying the genomic landscape of leukemia. However, many challenges, lay ahead. The dynamic nature of cancer and the field studying it demands constant innovation to address the growing challenges. However, these limitations and challenges also provide opportunity to capitalize on prospects for the future through continual improvement.

While cytogenetic analysis provides remarkable insights into diagnosing the different subtypes of leukemia, it faces challenges identifying more complex features on the genomic landscape of leukemia. Some key genetic and chromosomal alterations are missed, revealing the need for more sensitive detection methods and innovations. For example, a traditional method such as karyotyping is accurate but possesses limitations as it can only be as fast as the growing cells. If it was necessary to analyze slow growing or underdeveloped leukemia cells, karyotyping would not be an efficient approach for diagnosis.

Cytogenetics also poses a high possibility for error in data analysis as there is simply too much information to analyze effectively within a timely manner. Differences in lab practices may also pose challenges to the accuracy of cytogenetic analysis. The most crucial measure to overcome this is standardization of data, ensuring that all data is reliable. However this is not always a possibility due to extensive amounts of data that is constantly collected.

Despite these current challenges, the future of Leukemia diagnosis regarding cytogenetics is bright. With next generation technology, such as FISH and fluorescence PCR, paving the way to a new generation of cytogenetics, continued improvement in capabilities cytogenetics remains promising.More recent techniques such as single nucleotide polymorphism (SNP) analysis allows for genome wide analysis. This technique allows greater sensitivity for more subtle genomic abnormalities. The integration of cytogenetic data with new

generation methods proves effective, providing a much more holistic understanding of leukemia. Furthermore, technological advancements would also assist in the diagnostic capabilities of cytogenetics as well as the ability to compare data to other methods.

2.5 Ethical Considerations of Cytogenetics and Leukemia Diagnostics

The field of cytogenetics has substantially improved our understanding of leukemia and opened up the opportunity for accurate disease prognosis. It is, however, important to acknowledge the ethical considerations surrounding the context of cytogenetic analysis and its implications for patients, researchers, and clinicians.

For ethical purposes, a patient must be well informed about the purpose, outcomes, and limitations that follow the usage of cytogenetic procedures. Acquiring informed patient consent prior to any procedure(s) ensures patient understanding regarding research access to their genetic information. If targetable alterations are identified, available treatment options will be discussed with the patient, and they will be able to make an informed decision based on the information provided. Furthermore, when tumor samples are being utilized for research purposes beyond clinical care, patient material is de-identified to maintain confidentiality. The availability and use of patient material is paramount to continued advancements in the field. Cytogenetic data from these sources informs possible hereditary, or familial, as well as epidemiological implications of disease. For example, cytogenetic findings could identify the increased familial risk of leukemia amongst family members. Provided patient consent and ethical disclosure is strictly adhered to, this may result in preventative measures which increase survivorship.

2.6 Conclusion: Challenges and Future Prospects for Cytogenetics

Cytogenetic analysis through identifiable aberrations such as t(8;21)(q22;q22) and t(8;21)(q11;q11), allow for diagnosis to be made along with accurate prognosis. Certain translocations in tandem with identified aberrations may lead to (un)favorable prognosis and modifications to treatment plans. The success rate of accurate diagnosis and prognosis with Cytogenetics was studied to be 70-75% within adults, while for children it increased upwards to 85%. Such accuracy provides valuable insight onto the majority of patients and highlights the significance of cytogenetics in the field of leukemia diagnostics (Mrózek et al., 2009).

Even with reliable results, cytogenetics must still be used with caution. More intricate translocations and aberrations cannot be identified by cytogenetics, which presents a risk to relying on cytogenetic data alone for diagnosis. For this reason, it is recommended that cytogenetic approaches be coupled with other methods to allow for a more comprehensive assessment of genomic alterations, translocations, and aberrations.

3.0 Molecular Genetics

Molecular genetics is a branch of genetics that delves into the molecular structure and function of DNA and begins at the recognition of genetic information found within DNA. The technique deciphers the sequences found in the bases of DNA. Within molecular genetics, techniques such as PCR and next-generation sequencing (NGS) are commonly applied. PCR is used to amplify specific DNA material, whereas NGS can determine the precise nucleotides contained by the DNA. Molecular genetics allows for the identification of genetic aberrations, or mutations, underlying a person’s cancer.

Molecular genetics has opened a new realm of precision medicine and has revolutionized our understanding of leukekmia's complex etiology. Some mutations in genes such as *NPM1*, *TET2*, and *PML-RARA* are known to cause leukemia, inviting development of targeted treatments against these aberrant proteins.

These new generation technologies allow for high-throughput testing of many patient samples simultaneously, in addition to increased sensitivity of detection at a lower cost. This makes the identification of genetic alterations more probable and practical. Molecular genetics testing can later be combined with data from cytogenetics, further refining the classification of leukemia. This section will cover the principles of molecular genetics and its contribution to the field of diagnostics for leukemia.

3.1 Principles of Molecular Genetics and Common Gene Mutations

Advances in molecular genetics have mapped out the genetic landscape of leukemia much further than initially thought possible. This mapping allowed for identification of common genetic mutations in leukemia. These common mutations have improved the disease classification and contribute to the improved prognostic indicators. In this section, three of the most common genetic mutations found in leukemia will be discussed: *NPM1, FLT3, and CSF3R*.

Genetic mutations to the nucleophosmin 1 (NPM1) protein are frequently observed in AML. Mutations to *NPM1* are usually identified in patients with a normal karyotype. This common mutation is linked to a favorable prognosis, especially if the mutation is isolated. As a result, this mutation gives specific implications about the treatment approach to be taken.

FMS-like tyrosine kinase 3 (FLT3) is a mutation in leukemia which is one of the most prevalent mutations within AML. Two different types of *FLT3* mutations have been identified: *FLT3-ITD* and *FLT3-TKD*. *FLT3-ITD* is an internal tandem duplication which is typically accompanied by a worse prognosis. Leukemias with this mutation are oftentimes more aggressive, resulting in higher rates of patient relapse and lower rates of survival. Conversely, *FLT3-TKD* is often found in patients with a more favorable prognosis. *FLT3-ITD* is stated to be much more aggressive, and is often related to higher relapse rates, and a lower survival rate.

Colony-stimulating factor 3 receptor (*CSF3R*) mutations are common in CNL. This mutation causes aberrant activation of the CSF3R receptor, resulting in uncontrolled growth of neutrophilic cells. This contributes to the high levels of neutrophils commonly seen within CNL cases (Kelemen, 2022). Further information can be found in (Dwivedi & Greis, 2017).

3.2 Next-Generation Sequencing and Clinical Applications

NGS has revolutionized the ability of scientists to capture and identify rare, previously unknown genetic mutations in leukemia. This has significantly expanded our understanding of the genomic landscape of this disease. NGS has also been crucial in identifying therapeutically targetable mutations, particularly those for which treatment methods may already exist. The implication of NGS is a shift in DNA sequencing which goes from traditional sequencing methods to one that allows the simultaneous analysis of multiple DNA fragments. NGS has cut down the time, cost, and manpower needed to analyze DNA and even allowed for more extensive analysis than before. Adopting NGS has increased the ability to discover uncommon mutations.

3.3 Challenges and Future Prospects of Molecular Genetics

With the introduction of molecular genetics, cancer research has made remarkable advancements towards understanding and treating leukemia. As we look to the future of leukemia diagnostics, molecular genetic tools and techniques offer a good prospect towards the challenges. The ability to map out the entire genomic landscape and identify multiple different mutations is invaluable towards the discovery and treatment of rare disease subtypes. However, this widened understanding of disease is not without its limitations. The extent to which novel genetic mutations can be validated and then therapeutically targeted remains challenging. If a novel, rare mutation is discovered, much additional research and funding is required before being clinically actionable. As a result, no matter how good the diagnosis may be, it is challenged heavily by the treatment method applied. In addition, discerning the most relevant mutation for disease etiology is another significant limitation. As molecular genetic approaches capture numerous, if not all, present mutations in a sample, the challenge is to identify the main driver mutation(s) of disease, those that result in disease onset and/or progression, rather than ancillary passenger mutations. Identifying and validating driver mutation(s) requires extensive time and resources. As a result, many patients may not initially or directly benefit from their impact on improved diagnostics or therapies.

4.0 Epigenetics

A more recent field of study is that of epigenetics. Epigenetics is, in essence, the study of gene regulation and function; specifically, epigenetics describes heritable differences in an observed phenotype or behavior despite no changes in DNA sequence. Epigenetic modifications are utilized in cells to regulate gene expression patterns on a per cell basis. These mechanisms allow different cell types to produce different proteins while having the same DNA. However, epigenetic mechanisms can be dysregulated and research has focused on identifying aberrant epigenetic modification events that can result in leukemia formation or progression. Indeed, diagnostic tools capitalizing on this area of biology identify epigenetic changes that occur during the onset and progression of diseases such as leukemia. (Al Aboud et al., 2023) Knowledge of the aberrant epigenomic landscape present in leukemia also invites potential use of epigenetic-based anti-cancer therapies, as well as additional insight into leukemia's development progression, and heritability.

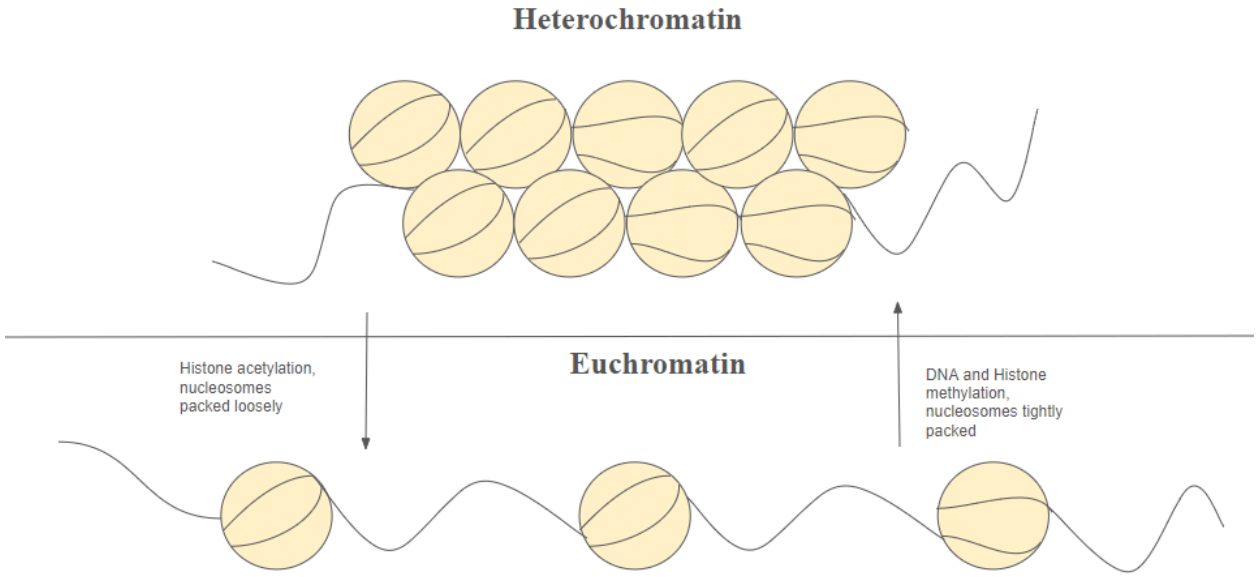
4.1 Epigenetic Aberrations in Leukemias

4.1.1 DNA Methylation

One type of epigenetic modification is DNA methylation. (Al Aboud et al., 2023) Typically this involves the addition of methyl groups to cytosine residue(s) within a particular section of DNA containing CpG dinucleotides (i.e., cytosine and guanine nucleotides linked together by a singular phosphate group). Methylation of cytosines in CpG dinucleotides is important because these regions regulate gene transcription. In general, DNA methylation results in gene inactivity or silencing. Aberrant DNA methylation at gene sites typically responsible for *suppressing* tumorigenic activity can thus contribute to the progression of disease. Indeed, certain patterns of DNA methylation in AML have been found to inform prognosis. Aberrant methylation of the gene *CDKN2B* is one such alteration that has been identified and related back to a specific prognosis. With an overall survival rate of 40% across all ages, the prognosis is relatively favorable for patients with this specific methylation pattern. (Chim et al., 2006)

4.1.2 Histone Modifications

Another type of epigenetic modification involved in gene regulation histone modifications (Below in model 1). In each of our cells, DNA is tightly wrapped around proteins called histones. Modifications of these proteins will either make the DNA more or less accessible for transcription, thereby influencing gene expression patterns. Generally, histone modifications may include acetylation (and its reverse de-acetylation), methylation, phosphorylation, or ubiquitination. Dysregulation of histone marks has been identified in multiple cancers, including leukemia. As the epigenetic field is relatively new, our understanding and utilization of this phenomenon for clinical purposes is still developing. Similar to molecular genetic tools, validation of identified epigenetic targets is required before informing patient prognostication. Fortunately, several epigenetic-based drugs, such as histone deacetylases (HDAC) inhibitors, are already clinically approved to treat other diseases. Regulators have been used clinically, especially histone deacetylases (HDAC) inhibitors, targeted therapy could become available.



4.1.3 Non-Coding RNA Dysregulation

A third and final method of epigenetic regulation involves non-coding RNA (ncRNAs). ncRNAs are typically sequences of transcripted DNA that do not get translated into functional proteins. Instead, these sequence fragments become long non-coding RNAs (lncRNA), among others. Similar to other methods of gene regulation, disruption in these processes can adversely affect the activity of tumor-suppressor or tumor-promoting genes. For example, a specific lncRNA, called HOTAIR, has been identified in AML. With much research focusing on these pathways, new ncRNAs will likely be identified in the near future, increasing our understanding of leukemia development and providing novel therapeutic targets.

4.2 Challenges and Future Prospects of Epigenetics

Epigenetics is a promising field for leukemia diagnostics. With improvements in navigating and managing the vast amounts of data provided by these methods, along with

standardization practices, the potential for methods exploiting epigenetic aberrations such as methylation and ncRNAs is vast. However, with these novel approaches also come specific obstacles that must be overcome for clinical adoption.

For one, there is currently no validation to assess the accuracy of epigenetics. Similar to molecular genetics, targets need to be validated to distinguish driver from passenger targets. Understanding the variations in the activity of lncRNAs and validating data regarding their ties to leukemia will allow for better diagnosis in the future. Although it may not help current patients right now, down the line it's going to help after data has been validated and sorted in a way that can clinically be applied. Furthermore, with multiple different methods validating specific indicators for cancer, the targeted methods can be produced in a manner that becomes clinically applicable. For one, there is currently no validation to assess the accuracy of epigenetics. Similar to molecular genetics, targets need to be validated to distinguish driver from passenger targets.

5.0 Conclusion

Convergence of the fields of cytogenetics, molecular genetics, and epigenetics opens up a realm where the biology of leukemia is much more comprehensively understood. It is of major importance that these methods get integrated together to provide a more holistic view into disease initiation and progression. This will allow more advanced patient risk stratification. By integrating or coupling these diagnostic approaches, the overall effectiveness of diagnostics will likely improve by diminishing challenges faced by each approach individually. Furthermore, it will provide a more holistic overview of a patient's tumor, allowing for more precise prognostic indicators and therapeutic opportunities.

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