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Mini Review

Pre-leukemic Cell Detection and Leukemic Transformation of a Normal Marrow Cell: A Mini-Review

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Abstract

The hematopoietic cells are multipotent primitive cells, which differentiate into either common myeloid and lymphoid progenitor. However, if there an abnormality in this process of differentiation, condition of leukemia arises, which is the 11th leading cause of cancer-related mortality worldwide in the year 2018. These abnormalities are brought about by array of mutations occurring at different cellular level. According to the two-hit model hypothesis, key oncogenic events are classified into two classes: class I mutations and class II mutations. Class I mutations are those that cause activation of the receptor tyrosine kinase (RTK), FLT3, c-kit (KIT), and Ras signaling pathways thereby increasing proliferation rate of progenitor cells. Class II mutations include recurrent chromosomal abnormalities such as t(8; 21), inv(16), and t(15; 17), which result in fusion transcripts of RUNX1/ETO, CBF/MYH11, and PML/RAR, respectively that eventually impair hematopoietic differentiation. The factors associated with leukemia can be biological, chemical or socio-economical. The advancement in the researches on the topic have aided to the development of various technologies such as detection of DNMT3A and xenografts assays, in order to detect these mutations in pre-leukemic cells. This review aims to provide an introduction to the condition, its types and provide brief summary on genes and mutations responsible for the condition. The factors associated with leukemia and technologies involved in the detection of leukemia are also reviewed.

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Keywords: Leukaemia; mutation; AML; CML; ALL; CLL; RAS signalling

Introduction

Leukemia is a common malignancy resulting in increased number of leukocytes in the blood. It is a clonal proliferation of hematopoietic stem cells in the bone marrow. It is the 11th leading cause of cancer-related mortality worldwide in the year 2018 (Lin *et al.*, 2021). Moreover, leukemia is most common cancer in children younger than five, with significant mortality (Du *et al.*, 2022). To understand the condition, leukemia, we must first understand the process of blood cell development. So initially, there is a Hematopoietic stem cell, which is also referred to as blood stem cell, and are found in several organs like, peripheral blood, bone marrow and umbilical cord blood. These hematopoietic cells being a multipotent primitive cell, then give rise to intermediate cells, Hematopoietic Progenitor Cells (HPCs) (Lee and Hong, 2020). These HPCs cells then differentiate into either common myeloid progenitor or common lymphoid

progenitor. The common myeloid progenitor cells are precursors to blood cells such as erythrocytes, platelets, mast cells, granulocytes, osteoclasts, monocyte-macrophages. The common lymphoid progenitor cells are precursors to lymphocytes such as T-cells, B-cells, Natural Killer Cells and Dendritic cells (Seita and Weissman, 2010). So, if there is any changes in this normal process of blood formation, then that condition is referred to as Leukemia, and on the basis of its progression and location where the change happened, type of leukemia is then classified in four types, namely Acute Myeloid Leukemia (AML), Chronic Myeloid Leukemia (CML), Acute Lymphocytic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL) (Chennamadhavuni *et al.*, 2022). In this paper, we reviewed the different types of Leukemia, briefly explained about leukemogenesis, associated genes and mutations, factors involve in leukemogenesis and concluding with the technologies involved in detection of pre-leukemic cell.

Acute Myeloid Leukemia (AML)

It is one of the most common forms of leukemia in adult population, and of about 80% cases of leukemia in adults are AML. This kind of leukemia is characterized by the clonal expansion of immature undifferentiated myeloid progenitors (blast cells) in peripheral blood and bone marrow which accounts for failure in the process of erythropoiesis (Vakiti and Mewawalla, 2022). The incidence of AML increases with age, as there are plausible evidences suggesting that with series of recurring hematopoietic stem cell genetic alterations which can accumulate with age (De Kouchkovsky and Abdul-Hay, 2016). AML can arise in patients as a consequence of prior cytotoxic chemotherapies or underlying hematological disorders. However, majority of cases of leukemia in healthy individuals are not accompanied by malignancy history. AML plausibly originated from the oncogenic transformation of a hemopoietic stem cells or its progenitors that have reacquired stem cell-like properties of self-renewal, which thus maintain the malignant clone (Short *et al.*, 2018). This oncogenic transformation is brought about by the genetic alterations of chromosomes such as t(8-21) in core binding factor AML, thus resulting in the formation of chimeric protein that alter the normal maturation process of myeloid progenitor cells (De Kouchkovsky and Abdul-Hay, 2016).

Chronic Myeloid Leukemia (CML)

It is a clonal hemopoietic stem cell disorder, characterized by the enhanced myeloid cell survival early during the chronic phase and uncontrolled mitogenesis during late blast crisis phase, where there is proliferation of immature white blood cells (Neshat *et al.*, 2000). Basically, there are three phases of CML, one being the chronic phase where there is accelerated accumulation of myeloid precursors and mature cells in bone marrow, peripheral blood and extramedullary site. Second being the accelerated phase that is characterized by the increased disease burden and increased frequency of myeloid progenitor cells rather than differentiated cells. Lastly, the third stage is the blast crisis, characterized by the rapid proliferative of differentiation-arrested myeloid cells (Calabretta and Perrotti, 2004). The central concept of CML pathogenesis is the fusion of Abelson murine leukemia (ABL) gene on chromosomes 9 with breakpoint cluster region (BCR) gene on chromosome 22, that results in the expression of an oncoprotein which is a constitutively active tyrosine kinase, BCR-ABL protein (Jabbour and Kantarijian, 2012). This activated tyrosine kinase is capable of autophosphorylation and uncontrolled signaling to activate effector pathways such as RAS, PI-3K/Akt, and STAT5, which all stimulates the growth and prevent apoptosis in hematopoietic cells. The effect of which can be seen in the late phase of CML, where cells become anti-apoptosis, and undergo rapid proliferation of immature myeloid cells. Conclusively, the expression of Bcl-Abl protein results in unlimited growth and leukemic transformation of hematopoietic cell lines (Calabretta and Perrotti, 2004). This tyrosine kinase is significant in both diagnosing the condition and also combating the condition. Several small molecule named tyrosine kinase inhibitors are available for the first line treatment of CML, some of the examples of TKIs are imatinib, nilotinib, and dasatinib, which all one way or another inhibit the functioning of expressed tyrosine kinase thus inducing apoptosis. The CML affects about one individual per 100000 population per year, and it accounts for approx. 15% of newly diagnosed cases of leukemia. The incidence of CML up until 2020 reached a plateau of almost 180000+ cases, however the annual overall mortality rate remains low at 2%, thanks to the success of TKIs (Jabbour and Kantarijian, 2020).

Acute Lymphocytic Leukemia (ALL)

The most frequent form of childhood malignancy, Acute Lymphoblastic Leukemia (ALL), makes up around 25% of adult cases of acute leukemia and has varying prognoses based on when it first manifests (Coccaro *et al.*, 2019). The incidence of ALL in the United States is thought to be 1.6 per 100 000 people (National Cancer institute, 2022).With about 6500 cases annually in the United States alone, Acute Lymphoblastic Leukemia (ALL) is the second most prevalent acute leukemia in adults. In adults, 75% of cases are caused by B-cell lineage precursors, with the remaining instances being caused by malignant T-cell progenitors (Terwilliger *et al.*, 2017). The poor result in adult ALL has been variously attributed to an increase in high-risk leukemia with more drug resistance, worse tolerance and compliance with therapy, reluctance to accept some transient adverse effects, and less effective treatment regimens, compared to pediatric ALL (Pui *et al.*, 2006). A bimodal distribution describes the incidence of ALL, with

the first peak occurring in childhood and the second peak appearing at age 50 (DeAngelo *et al.,* 2008).

When a bone marrow cell experiences alterations (mutations) in its DNA or genetic makeup, acute lymphocytic leukemia develops. The French American British (FAB) morphological criteria, which separated ALL into 3 subtypes (L1, L2 and L3) based on cell size, cytoplasm, nucleoli, vacuolation and basophilia, was the first attempt at categorizing ALL (Bennet *et al.,* 1976). In an effort to take into account the morphology and chromosomal profile of the leukemic blasts, the World Health Organization suggested a composite categorization in 1997 and classified three kinds of ALL: B lymphoblastic, T lymphoblastic, and Burkitt-cell Leukemia (Harris *et al.,* 1999). In Acute Lymphocytic Leukemia, the BCR-ABL fusion protein is a constitutive protein kinase that modifies signaling networks that regulate hematopoietic stem cell proliferation, survival, and self-renewal (Pane *et al.,* 2002).

Chronic Lymphocytic Leukemia (CLL)

Small Lymphocytic Lymphoma (SLL), also known as Chronic Lymphocytic Leukemia (CLL), is a slow-growing cancer that is characterized by an increase in mature but defective B cell production (Mukkamalla *et al.,* 2022). In Western nations, Chronic Lymphocytic Leukemia (CLL) continues to be the most common adult leukemia, yet it is rare in places like East Asia. About 7% of newly diagnosed non-lymphoma Hodgkin's cases are CLL/Small Lymphocytic Lymphoma (SLL) (Zelenetz *et al.,* 2015). As seen on a peripheral smear, CLL/SLL is classified as a monoclonal lymphoproliferative disease that is marked by the growth and accumulation of smudge cells, which are physically mature B-cell lymphocytes but are immunologically defective (Mukkamalla *et al.,* 2022). Peripheral blood, the spleen, lymph nodes, and bone marrow are the main illness sites. From a pathologic and immunophenotypic perspective, CLL and SLL are identical. Both CLL and SLL are B-cell lymphoid-derived diseases, although they appear in different ways depending on where the aberrant cells are located. This progressively proceeds to the lymphoma phase, which corresponds to SLL and is marked by the presence of lymph node-localized cells. The lymphoproliferative process that is restricted to the lymph nodes is known as SLL (Boddy *et al.,* 2018). Restrictions in the T cell receptor gene repertoire, T cell oligoclonal expansions, and common T cell receptor clonotypes among patients are all signs of CLL, which strongly suggests selection by constrained antigenic elements whose identities are still unknown (Roessner *et al,* 2020). For chronic lymphocytic leukemia cells to survive, proliferate, and migrate around the body, the B cell receptor (BCR) pathway is crucial.

Leukemogenesis, Genes and Mutations

Leukemogenesis is the process of how blast cells develop into leukemic stem cells, which retain the potential to self-renewal. According to the two-hit model hypothesis, key oncogenic events are classified into two classes: class I mutations confer a proliferation or survival advantage to blast cells, and class II mutations block myeloid differentiation and confer self-renewability (Dash and Gilliland, 2001). Mutations in certain genes, such as the fms-related tyrosine kinase 3 (FLT3), CCAAT/enhancer binding protein alfa (C/EBP), runt-related transcription factor 1 (RUNX1), myeloid-lymphoid or mixed lineage leukemia (MLL), Wilms tumor (WT1), and nucleophosmin (NPM) 1 genes, have significantly improved the understanding of leukemogenesis. Class I mutations are those that cause activation of the receptor tyrosine kinase (RTK), FLT3, c-kit (KIT), and Ras signaling pathways. Class II mutations include recurrent chromosomal abnormalities such as t(8; 21), inv(16), and t(15; 17), which result in fusion transcripts of RUNX1/ETO, CBF/MYH11, and PML/RAR, respectively. Therefore, AML patients' molecular events can be screened using the two-hit model of leukemogenesis, which combines an event blocking myeloid differentiation with a lesion activating tyrosine kinase pathways. This is supported by the frequent association of a FLT3-ITD mutation with fusion transcripts like AML1-ETO or PML-RARA (Takahashi, 2011). Several genes, including the myeloid-lymphoid or mixed-lineage leukemia gene (MLL), the fms-related tyrosine kinase 3 gene (FLT3), the CCAAT/enhancer binding protein gene (CEPBA), the nucleophosmin gene (NPM1), and the neuroblastoma RAS viral oncogene homolog, are mutated in cytogenetically normal acute myeloid (NRAS) (Schlenk *et al.,* 2008).

Three genes have been found till date that can be inherited in an autosomal dominant manner and specifically predispose people to the development of leukemia: CEPBA, RUNX1, and GATA2. There have been numerous case reports describing family pedigrees with an increased incidence of leukemia in the absence of a known inherited mutation (Gunz *et al.,* 2009). Germline mutations of CEBPA were discovered in 2004 (Smith *et al.,* 2004). According to a more recent investigation, patients who inherit germline mutations of the same gene occasionally get additional but separate somatic mutations in CEBPA, which likely contribute to the onset of AML (Pabst *et al.,* 2008). Since the initial publication in 2004, there have been several more documented pedigrees that support the hypothesis that familial AML is caused by the germline transmission of CEBPA mutations (Renneville *et al.,* 2008). Translocations are typically the most frequent somatic anomaly involving the RUNX1 gene in sporadic AML or ALL. However, the most frequent form of mutation in Myelodysplastic Syndrome MDS or FPD/AML linked to RUNX1 changes is a point mutation that causes

haploinsufficiency (Osato, 2004). The mutation in GATA2, a transcription factor that affects the integrity of hematopoietic stem cells as well as a regulator of macrophage phagocytosis have been linked to specific types of congenital neutropenia that progress to MDS or AML (Pasquet *et al.*, 2013).

Mutations and Development of AML

The development of AML is a multistep process and requires sets of mutations to obtain leukemia. These mutations happening in this multistep process is broadly classified into two types, namely, Class I and Class II. The Class I mutations activate signal transduction pathways and confer a proliferation on hematopoietic cells, and Class II mutations impacts the transcriptional factors, and disturb the regular hematopoietic differentiations (Takahashi, 2011). Common class I mutations that activates signaling pathways to prematurely proliferates hematopoietic cells are FLT3, PTPN11, NRAS, KIT and CBL mutations. FLT3 is a type III Receptor Tyrosine Kinase (RTK), and their mutation allows the malignant cells to proliferate (Gilland and Griffin, 2002). PTPN11 activates the RAS-MAPK signaling pathway, despite not having profound prognostic significance, this mutation was a poor risk factor for overall survival (OS) of AML patients. NRAS and KIT had adverse impact on the OS of AML patients. Similarly, common Class II mutations are Runx1, C/EBP α and MLL rearrangement. Runx1 is essential for normal hematopoiesis in order to differentiate myeloid progenitor cells to granulocytes, mutation in this gene, have straightaway effect to this mechanism of hematopoiesis. C/EBP α is a crucial transcriptional factor for the differentiation of granulocytes, a change in the transcribing gene can impact the process. The shuffle of MLL arrangements can alter DNA methylation patterns and gene expression profile additionally, there are unclassified mutations that are plaquing the clinical settings, such as DNA methyltransferase (Dnmt) 3a mutations, ten-eleven-translocation oncogene family member 2 (TET2) mutations, isocitrate dehydrogenase (IDH) 1 gene mutations, wilms tumor (WT1), nucleophosmin (NPM) 1 genes, and ASXL1. (Takahashi, 2011). Dnmts mutations brings about a problem in the process of methyl group addition to the cytosine residue of CpG dinucelotides, and plausibly contribute to the pathogenesis of cancer (Estellar, 2008). IDH 1 and 2 gene mutations disrupts the lipid synthesis, lowers the defense against oxidative stress, thus leading to error in oxygen-sensing transduction, can impair hematopoietic differentiation (Reitman and Yan, 2010). NPM1 mutations allows hematopoietic cell to be prone to apoptosis, and make cell more sensitive to stress. WT1 mutations implicates cell survival, proliferation and differentiations. All these barrage of mutations co-exists to develop AML (Takahashi, 2011).

Leukemia-Specific Mutations in HSCs

Leukemia might develop from committed progenitors as a result of mutations or the selective expression of genes that increase their otherwise constrained capacity for self-renewal. For the majority of malignancies, the cell that undergoes transformation is unclear, however evidence points to the possibility that some leukemias are brought on by mutations that accumulate in HSCs (Passegue *et al.*, 2003). Restricted haematopoietic progenitor cells are less likely to undergo neoplastic transformation than stem cells because they multiply for a considerably shorter length of time before terminal differentiation. Nonetheless, limited progenitors could be altered either by acquiring mutations that lead those to self-renew like stem cells or by inheriting existing mutations from stem cells (Reya *et al.*, 2001). Changes in the CEBPA gene or the core-binding factor (CBF) complex genes, including RUNX1, can cause differentiation blocks, resulting in distinct subtypes of AML (Renneville *et al.*, 2008). In most AML subtypes, the cells capable of initiating human Acute Myeloid Leukemia (AML) in NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice exhibit a CD34+ CD38- phenotype, making them identical to normal HSCs. Despite having a leukaemic blast character, leukaemic cells cannot transmit illness to mice in the great majority of situations. This shows that leukaemia is targeting normal HSCs rather than committed progenitors (Reya *et al.*, 2001). Mutations accumulate in functionally normal hematopoietic stem cells (HSCs) over a lengthy "pre-leukemic" period. Pre-leukemic HSCs are intermediate HSCs that have some, but not all, leukemia-specific mutations (Corces-Zimmerman and Majeti, 2014).

The Ras Functioning

Class I mutations leading to the activation of signal transduction pathways by binding to the receptor tyrosine kinase or cytosolic kinase increases the multiplication and survival of hematopoietic progenitor cells (Schlenk *et al.*, 2008). The Ras signaling pathway is an active yet complex circuit involving intracellular signaling network of various cells and molecules, thereby playing important roles in cell proliferation, growth, migration, cytoskeletal changes, differentiation, senescence and apoptosis processes (Renneville *et al.*, 2008). The three functional RAS oncogenes namely H-RAS, N-RAS and K-RAS are the first human oncogenes discovered (Fernandez-Medarde and Santos, 2011) and the most common mutated genes in tumors (Pomeroy and Eckfeldt, 2020). Mutation in Ras oncogenes occur by one base substitution at in 12, 13 and/or 61 codons resulting in impaired GTP hydrolysis leading to constitutive activation of downstream effector pathway. Existing as an equilibrium between GTP-bound (active) and GDP-bound (inactive) states, Ras GTPase proteins switches and modulates signaling by cycling between these states, transducing signals to various effector enzymes with the

coordination of two factors called guanine nucleotide exchange factors (GNEFs) and GTPase activating proteins (GAPs) (Downward, 2003). This modulation is activated by protein factors called GNEFs that promotes the exchange between GDP and GTP and activates effector enzymes as well as membrane localization. In contrary, this modulation is negatively regulated by GAPs to enhance the inefficacious intrinsic Ras GTPase activity (Pomeroy and Eckfeldt, 2020).

The conversion of inactive form (RAS-GDP) to active form (RAS-GTP) is facilitated by GNEFs which stimulates the cascade of signal transduction pathways and effector molecules that includes RAF-MAPK/ERK kinase (MEK), extracellular signal regulated kinase (ERK), phosphoinositide-3 kinase/protein kinase B (PI3K/AKT), phospholipase C (PLC)-protein kinase C (PKC), NORE1-RASSF1 (RAS association domain family member 1), RLDGSRAS-Like (RLDGS-RAL); and TIAMI-RAC-p21-activated kinase (PAK) (Khan *et al.,* 2019).

Ras Mediated Signaling Pathways

The mitogen-activated protein kinase (MAPK) and phosphatidylinositiol-3-kinase (PI3K) signaling pathways are markedly regulated by the Ras effector pathways. This pathway involves the binding of Ras-GTP of RAF kinases receptors to growth factors followed by dimerization and cross phosphorylation of tyrosine residues in cytosolic domains (Rajasekharan and Raman, 2013), resulting in plasma membrane localization. The phosphorylation activates the mitogen-activated kinases called MEK1 and MEK2 (Gurung and Bhattacharjee, 2015) which in turn phosphorylates extracellular signal regulated kinases, ERK1 and ERK2. The ETS domain transcription factor families, JUN are targeted by primary ERK that ultimately drives AP1 mediated cell proliferation. Furthermore, the P13K signaling pathway is induced and activated by Ras-GTP through interactions with the subunits of type I P13K proteins. This results in membrane localization and activation of kinase, subsequently leading to phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2). The phosphorylation of PIP2 leads to production of phosphatidylinositol-3,4,5-trisphosphate (PIP3). As PIP3 acts as a second messenger, it activates two signaling pathways; AKT (Protein kinase B)-dependent and AKT-independent pathways that regulate diverse cellular processes including proliferation, survival, motility, and metabolism (Pomeroy and Eckfeldt, 2020; Thorpe *et al.,* 2015; Rodriguez-Viciana *et al.,* 1994).

Factors Associated with Leukemia

Leukemia being hematological malignancies belongs to fifth most prevalent cancer type (Ternak *et al.,* 2022) that is affected by several risk factors on multiple degrees. Similar to many other human diseases, the aetiology of the leukemia has been linked to both environmental and genetic causes (Lightfoot and Roman, 2004). The etiological agents such as genetic susceptibility, infections, chemicals, irradiations etc. have been properly identified in pathomechanism of those malignancies (Filippini *et al.,* 2015; Ternak *et al.,* 2022). Electric fields and non-ionizing electromagnetic radiation are other environmental elements for which there is some evidence, albeit it is unclear how these factors contribute to leukaemogenesis (Eden, 2010). More recently, the contribution of the altered gut flora, dysbiosis, has also been identified as a potential additional factor to the existing ones (Ternak *et al.,* 2022). Leukemia's etiology has proven challenging to pinpoint, and little is known about the disease's origins or how potential risk factors interact (Filippini *et al.,* 2015; Schmidt *et al.,* 2021).

Biological Factors

Genetics

The genetic variation has been the medium for the causation of various cancer even within the leukemia. The early stages of leukaemogenesis frequently entail mutations in genes involved in epigenetic regulation (Khwaja *et al.,* 2016). The etiology of Acute Lymphoblastic Leukemia (ALL), a subtype of Leukemia, has been clarified as a result of the recent discovery of unique genetic variations and sequence mutations (Imai, 2017). The modification of risk for genomic damage due to the polymorphism in genes that are involved in detoxification and DNA-repair pathways has been the leading cause for development of malignant disease including leukemia (Voso *et al.,* 2007). The most plentiful cytochrome P450 3A4 (CYP3A4) responsible for the detoxification of drugs and metabolism of many carcinogens or their metabolic deactivation has been associated with leukemia and other types of cancer after polymorphism as CYP2A4-290G has been proved as the genotypic factor which causes increase of CYP3A4 enzymatic activity leading to increase of risk of Acute Myeloid Leukemia (AML) by 18.9-fold (Ali *et al.,* 2014). Numerous recurring mutations, including DNA methylation effectors, chromatin modifiers, and spliceosomal machinery, have been discovered throughout a spectrum from clonal hematopoiesis to myelodysplasia to overt AML (Kishtagari *et al.,* 2020). Leukemia further have been linked to a wide range of genetic abnormalities, such as chromosomal translocations, gene deletions, amplifications, and point mutations (Zhao *et al.,* 2010).

MicroRNA

The small non-coding RNAs, microRNA (miRNA) regulates the gene expression that are involved for differentiation of hematopoietic stem cells and tumorigenesis and as with the dysregulation of miRNAs, hematological malignancies including human Acute Myeloid Leukemia (AML) has been evidential factor (Liao *et al.,* 2017). Mixed lineage leukemia 1 (MLL1) is a master regulator for transcription of important genes, such as Hox genes, for hematopoiesis and embryonic development and

with overexpression of certain Hox genes due to dysregulation of MLL1 eventually causes leukemia initiation (Li and Song, 2021).

Microbiology

Some other etiological agents of human leukemias are viruses from the herpesvirus and retrovirus families (Jarrett, 2010). In regards to adult T-cell leukemia which is an aggressive form of T-cell malignancy, is found to be caused by the human T-cell leukemia virus type 1 (HTLV-1) (Takachi *et al.,* 2015). While there is no evidence of viral genomic inclusions within leukemic cells, and no single virus has been firmly connected with childhood leukemia (McNally and Eden, 2004).

Chemical Factors

Polluting Agents

Many pollutants that have been studied to show increase of risk for leukemia and some of which includes benzene, cadmium, diesel exhaust, 1,3-butadiene and several polycyclic aromatic hydrocarbons (Elliot *et al.,* 2017). Benzene is a common environmental carcinogen that is present in a variety of consumer products which is known to induce acute myeloid leukemia in adults (Lu *et al.,* 2020). The occupational exposure of parents to benzene before conception has been evidential link for the risk of childhood Acute Lymphoblastic Leukemia (ALL) (Heck *et al.,* 2019). The exposure to petrochemicals which is a major source of hazardous and toxic air pollutants such as toluene, benzene, ethylbenzene, and xylene have been incident for 30% higher risk of developing leukemia (Jephcote *et al.,* 2020). Tobacco smoke is a proven risk factor for adult myeloid leukemia and includes at least 60 known human carcinogens of which the main chemical groups in tobacco smoke are volatile hydrocarbons, aldehydes, aromatic amines, polycyclic aromatic hydrocarbons, and nitrosamines (Chang, 2009). In contrast to maternal smoking, paternal smoking was much profoundly associated with significantly elevated risk of childhood Acute Lymphoblastic Leukemia (ALL) during pregnancy but not for Acute Myeloid Leukemia (AML) while the higher consumption was directly involved for increase of childhood ALL or AML (Chunxia *et al.,* 2019). Additionally, pesticide have been linked with childhood leukemia as potential risk factor as the evaluation of prenatal and/or early life exposure to pesticides have provided close proximity as risk factor for childhood leukemia (Nguyen *et al.,* 2021).

Oxidative Stress

The imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms, which can activate different transcription factors and alter their transcriptional pathways, results in oxidative stress. Leukemia's occurrence and progression, as well as its treatment and prognosis, are all directly impacted by oxidative stress (Dong *et al.,* 2021).

Chemical Medications

Leukemia occurrence has been studied even for the use of antibiotics of which there has been no profound positive or negative association with any antibiotic classes specifically but the increased risk of leukemia was showed with the consumption of macrolides (Ternak *et al.,* 2022). Researchers have found that regularly consuming flavonoids lowers the risk of developing various malignancies, particularly leukemia, as well as their incidence while consumption of some vitamins has been positively associated with leukemia (Saraei *et al.,* 2019; MacArthur *et al.,* 2008). The deficit of risk was observed for the index child when treated with immunosuppressant medication while the pelvic radiotherapy used was found to associate for increase in risk of secondary leukemia (MacArthur *et al.,* 2008; Wright *et al.,* 2010).

Socio-Economic Factor

Age

While the leukemic condition can affect anyone, it mostly occurs predominantly in persons over the age of 60, while being one of the most frequent malignant diseases affecting children (Filippini *et al.,* 2015; Khwaja *et al.,* 2016). In the coming years, it is anticipated that the disease's prevalence among senior people would rise even more due to the general population's progressive aging. Age is the most important prognostic factor in AML, along with cytogenetics upon diagnosis, as the outcome gets worse as people age (Ferrara, 2014). Maternal and paternal age are significant risk factors for childhood leukemia, albeit their impact varies depending on the subtype of the disease (Sergentanis *et al.,* 2015). Maternal age increased with risk of ALL, but not paternal age while there was weak association with number of older siblings that disregarded decrease of disease in incidence, suggesting a delay in disease manifestation (Feller *et al.,* 2010).

Occupation

Leukemia's etiology has been linked to industrial and occupational exposures; however, it is unclear which jobs are particularly high risks. It has been discovered that a number of jobs, particularly those connected to the production of electronics, munitions, plastics, rubber, petroleum products, and nuclear energy may raise the risk of acute leukemia (Terry *et al.,* 2005).

Nutrition

A predictive factor for leukemia in children and adolescents has been found to be malnutrition. Young people with leukemia have lower survival chances when they are undernourished (Barr *et al.,* 2016). A minor increase in the risk of acute childhood leukemia may be related to feeding human milk for brief periods of time or not at all (Gungor *et al.,* 2019). Breastfeeding for longer than six months has

been found to be preventative of leukemia (MacArthur *et al.*, 2008).

Technologies Involved in Detection of Pre-leukemic Cells

Cancer cells have been discovered to be evolved through cloning from a single cell. However, the trajectory of the evolution of somatic mutation in a single cell leading to the formation of cancer cells is not well plotted (Corces-Zimmerman MR *et al.*, 2014). Clonal expansion of single cell being the first stage in leukemogenesis, pre-leukemic cells are known to initiate leukemogenesis (Jan *et al*., 2012; Lal *et al.*, 2017).

Detection of DNMT3A (DNA methyltransferase 3 alpha) Mutations in Leukaemia

DNMT3A is a new DNA methylation that goes under frequent mutations in less developed and developed hematologic neoplasm (Brunetti *et al*., 2017). Through a dysfunctional cooperative connection with Polycomb suppression complex 1, R882 mutant DNMT3A suppresses the expression of differentiation-related genes, preventing the differentiation of hematopoietic stem cells and leukaemia cells. (Furuya and Kurokawa, 2018; Koya J and Kurokawa M, 2018). Evidence from AML patients suggest DNMT3 mutations occur prior to cancer development so can be used to detect early acute myeloid leukemia (Brunetti *et al*., 2014).

Xenograft Assays

The in vivo xenotransplantation assay in NOD/SCID IL-2Rγ common chain null (NSG) mice are currently the model most frequently used to study the biology of leukemia-initiating cells (LICs) (Wunderlich *et al.*, 2010). These leukemia-initiating cells from primary acute myeloid leukemia samples can be functionally analyzed using a xenograft test (Griessinger, 2017).

Clonal Tracking from Single-Cell Transcriptomes

Muta seq (workflow) amplifies nuclear mutations from cDNA, mitoclone (computational tool) clones by mutations in mitochondrial markers, allowing us to identify and detect transcriptomics consequences of leukemic and pre-leukemic mutations with concurrent mapping of genomic and mitochondrial mutations in single-cell transcriptomes. Identifying cancerous clones leaving the healthy clones can be achieved by clonal tracking with the aid of genomic and mitochondrial mutation (Velten *et al*., 2021). Deep DNA sequencing and single-cell RNA sequencing can be applied to detect the somatic mutations and virus directly and characterize the transcriptional readouts in respective subclones in case of Adult T-cell Leukaemia-lymphoma (ATL) (Yamagishi *et al*., 2021).

Single-Cell Gene Expression Profiling

Single-cell profiling using flow cytometry for immunophenotyping can be used as a routine hematological diagnostic assay (Herzernberg *et al*., 2002). Single cell genotyping of *FLT3*-ITD and the *NPM1* mutation can be applied to study the evolution of clonal diversity (Paguirigan *et al*., 2015). Through the detection and characterization of suspected subclonal populations, single-cell genomic profiling may enhance clinical diagnosis by allowing for the differentiation of tumour heterogeneity (Yan *et al.*, 2017).

Proteomics -Reverse Phase Protein Arrays (RPPA)

For the analysis of protein expression levels and protein activation states in primary AML samples as well as normal and AML stem cells, RPPA offers a quick, high-throughput, dependable, and reproducible approach. To understand more about normal and leukemic stem cells and the methods to eradicate leukemic stem cells, the immense potential of RPPA must be completely utilized (Tibes *et al.*, 2006).

Conclusion and Future Perspective

It has been demonstrated that the development of genetic changes in a single lineage of cells over time causes cancer either proliferation or myeloid differentiation of blasts cell form leukemic cells. Since Pre-leukemic hematopoietic cells in leukemia place themselves at valuable position, the study of clonal evolution through single-cell transcriptomics, genotyping and protein arrays creates an idea of relapsed disease. Various factors have been studied such as age, genetic factors, and occupational exposures where chemical exposures are believed to impact transcriptional pathways. The study of pre-leukemic genetics can lead to an understanding of patterns of mutation acquisition, which has substantial implications for the early detection and treatment of leukemia. Further studies regarding leukemic transformation and implementation of effective detection technology for pre-leukemic cells may eradicate immature death cases through chronic leukemia. Detection techniques such as Detection of DNMT3A mutations, Xenograft assays, Clonal tracking from single-cell transcriptomes, gene expression profiling and Proteomics -reverse phase protein arrays (RPPA) can be used for early stage detection of pre leukemic cells and can progressively prevent the transition to chronic stages.

Author's Contribution

All authors contributed equally at all stages of manuscript preparation, critical revised and finalized the manuscript. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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