Germline Mutations: Many Roles in Leukemogenesis

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Abstract

Purpose of review: The purpose of this review is to summarize the current understanding of germline mutations as they contribute to leukemia development and progression. We also discuss how these new insights may help improve clinical management of germline mutations associated leukemia.

Recent findings: Germline mutations may represent important initial mutations in the development of leukemia where interaction with somatic mutations provide further hits in leukemic progression. In addition, germline mutations may also contribute to leukemogenesis by impacting bone marrow stem cell microenvironment and immune cell development and function.

Summary: Leukemia is characterized by the clonal expansion of malignant cells secondary to somatic or germline mutations in a variety of genes. Understanding somatic mutations that drive leukemogenesis has drastically improved our knowledge of leukemia biology and led to novel therapeutic strategies. Advances have also been made in identifying germline mutations that may affect leukemic development and progression. This review will discuss the biological and clinical relationship of germline mutations with clonal hematopoiesis, bone marrow microenvironment, and immunity in the progression of leukemia.

Keywords
Germline mutations; Leukemogenesis; Hematopoietic stem cell; Clonal hematopoiesis of indeterminate potential; Microenvironment; Antitumor immunity

Introduction

Despite dramatic improvements in therapy for some, leukemia remains a clinical challenge, particularly in older adults for whom the prognosis can be quite dire. Leukemia is characterized by the clonal expansion of malignant cells driven by mutations in a variety of...
genes (1–6). Our understanding of somatic mutations that drive leukemogenesis has had a profound impact in better understanding leukemia biology and, to some extent, informing therapeutic interventions. Additionally, notable advances have been made in identifying germline mutations which predispose towards the development of hematologic malignancies (7, 8). Of note, several of these germline genes, including TP53 and PTEN, are also targeted in somatic mutations involved in leukemic transformation (9). Importantly though, the penetrance of leukemia in pedigrees that carry a pathogenic variant is incomplete. In contrast, a unique and more indolent form of clonal expansion of hematopoietic cells with specific somatic mutations has been identified and termed clonal hematopoiesis of indeterminate potential (CHIP) (10). While germline mutations are well known to predispose towards the development of solid and hematologic malignancies through cell autonomous effects, this review will discuss the relationship of germline mutations with common CHIP mutations and the bone marrow microenvironment. Moreover, as immune cells are integral in the leukemic microenvironment, we will also discuss the role of germline mutations and clonality in immune evasion and anti-tumor immunity.

**Functional Interactions of Germline Mutations with CHIP**

Clonal hematopoiesis is an aging associated process where genetically distinct populations of blood cells clonally derived from a single hematopoietic stem/progenitor cell (HSPC) begin to populate the hematopoietic system. While often a benign process, somatic mutations in leukemia-associated genes such as DNA Methyltransferase 3a (DNMT3A), ASXL Transcriptional Regulator 1 (ASXL1), or Ten-Eleven Translocation-2 (TET2), have been well characterized to develop into a condition known as CHIP (11–17, 18*, 19). CHIP is defined as clonal expansion driven by somatic mutations which provide a growth advantage to a subset of HSPCs – at a variant allele fraction ≥2% – in the absence of cytopenias and dysplastic hematopoiesis (10, 20*). Moreover, CHIP is identified as a precursor condition, or an indolent pathological state associated with an increased risk of myeloid malignancy (10, 20*). As a benign precursor, CHIP is similar to monoclonal gammopathy of undetermined significance and monoclonal B-cell lymphocytosis. Importantly, most individuals who develop CHIP do not develop myeloid malignancy, with only a 0.5% to 1% annual risk, suggesting that additional perturbations are necessary for leukemogenesis (10, 20*).

A better understanding of the relationship of germline mutations and CHIP-associated mutations may elucidate the progression to overt hematological malignancy. Among the common CHIP-associated mutations, germline variants in TET2 have recently been associated with hematologic malignancy. Heterozygous germline TET2 frameshift mutation has been described in families in which select family members developed lymphoma, acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), and polycythemia vera (21*, 22*, 23). TET family proteins are involved in DNA methylation and play a critical role in normal hematopoiesis including self-renewal of hematopoietic stem cells, lineage commitment, and terminal differentiation on monocytes (24, 25). Mutations in TET2 are found in 20–25% of myelodysplasia syndrome (MDS) and AML and 40–50% of CMML (22*). Interestingly, TET2 mutations have been shown to cooperate with JAK2 and SRSF2 mutations – both of which are less commonly identified CHIP genes – to promote...
hematologic malignancies (26). Thus, germline and somatic mutations in *TET2* cooperate with mutations in other CHIP-associated genes to promote leukemogenesis.

To further understand the relationship of germline and CHIP-associated mutations, analysis of the somatic mutations in individuals with known germline, leukemia-predisposing mutations may provide insight. Germline mutation of the Runt-Related Transcription Factor 1 (*RUNX1*) gene is the cause of Familial Platelet Disorder with Predisposition to Myeloid Malignancy (FPD-MM) (9). Characterized by thrombocytopenia and varying degrees of platelet dysfunction, individuals with FPD-MM are at an increased risk of developing AML, MDS, and T-ALL with a lifetime risk of 30–40% (9, 27). Patients with germline *RUNX1* mutations have also been found to have additional mutations in CHIP-associated genes, including but not limited to *JAK2*, *SRSF2*, *ASXL1*, *TET2*, and *DNMT3A* (27, 28). Similarly, among 72 individuals with FPD-MM due to germline *RUNX1* mutation, somatic mutations in CHIP-associated genes were found in 22% without leukemia and in 40% with myeloid malignancies, including *DNMT3A* and *TET2* (29*). However, comparing somatic mutations in AML patients from those with FPD-MM to those with sporadic disease with at least one somatic *RUNX1* variant revealed that individuals with FPD-MM had fewer mutations in *DNMT3A*, *NRAS*, *SRSF2*, and other CHIP-associated genes, suggesting that the early acquisition of *RUNX1* mutation may influence which cooperating mutations promote transformation (29*).

GATA binding protein 2 (*GATA2*) mutations are associated with increased risk of bone marrow failure, MDS and AML (30). A strong association between germline *GATA2* and *ASXL1* has been described (31, 32, 33*). In patients with germline *GATA2* mutations, studies have found that about one third of patients with germline *GATA2* mutations also have mutations in *ASXL1*, with a small percentage of the patient sample having *DNMT3A* and *TET2* mutations (31, 32, 33*). Taken together, though the data remains limited, there appears to be overlap and functional interaction of leukemia predisposition germline mutations with CHIP-associated gene mutations. Nevertheless, many factors affect the ability of CHIP-associated mutations to promote leukemogenesis including HSPC microenvironment and changes to anti-tumor immunity that are simultaneously impacted by germline mutations.

**Microenvironmental Impact of Germline Mutations on Leukemogenesis**

HSPCs interact with the bone marrow microenvironment (BMME) for normal processes of self-renewal, proliferation, and differentiation (34, 35). Divided into mesenchymal stem cells, pericytes, osteoblastic cells, and endothelial cells, the BMME has more recently been linked to dysregulated hematopoiesis, MDS, and leukemia (34–39, 40, 41). Somatic mutations resulting in the dysregulation of the transcription factor, Forkhead Box C1 (Foxc1), in mesenchymal cells has been associated with increased BM adipocyte populations and decreased Stromal Cell-Derived Factor 1 (*CXCL12*) expression, which are associated with a poor prognosis in leukemia patients (43). Osteoblasts represent a protective niche as osteoblast ablation has been correlated with accelerated leukemia development and decreased overall survival (44). Endothelial cells sustain angiogenesis and promote leukemia cell adhesion through E-selectin, thereby inducing chemotherapy resistance (42, 46).
Immune cells also represent a substantial population in the BMME and will be discussed in the following sections. Despite microenvironmental heterogeneity, genetic alterations, including germline mutations, and epigenetic changes effecting the BMME promote hematopoietic dysregulation (34–36, 45*).

For example, dominant germline mutations in PTPN11 (SHP2) cause about 50% of cases in Noonan syndrome (NS), a developmental disorder associated with an increased risk of leukemia (47). Although these mutations drive leukemogenesis primarily through hyperactivation of the Ras signaling, they can have significant impact on the bone marrow stem cell microenvironment (47). On a molecular level, hyperactivity of mutant SHP2 in bone marrow mesenchymal stem cells and osteoprogenitors is correlated with increased production of the chemokine CCL3, which recruits monocytes to the vicinity where hematopoietic stem cells also reside (48). Consequently, hematopoietic stem cells are hyperactivated by IL-1β and other proinflammatory cytokines produced by monocytes, accelerating the development of myeloproliferative neoplasm (MPN) in the setting of NS and leading to donor cell-derived MPN following stem cell transplantation for NS-associated hematological malignancy in the mouse model (48). Another key germline mutation involved in the leukemic progression is TP53. TP53 mutation has been detected in patient bone marrow MSC populations with no other leukemia-specific, cooperating mutation detected (49**). Importantly, TP53 alterations are observed in tumor as well as non-tumor cells in 43.3% of childhood low hypodiploid ALL, suggesting underlying Li-Fraumeni syndrome (50*). While germline mutations in PTPN11 and TP53 may promote the developing hematological malignancy in part by impacting stem cell microenvironment, altered immune cell populations and aberrant anti-tumor immunity directly caused by other germline mutations might be also significant players in leukemic progression.

Germline Mutations Affecting Anti-Tumor Immunity in Leukemia

Impaired immunity and immune tolerance have been identified as a key feature of leukemia (52–54). Indeed, primary immune deficiencies such as ataxia-telangiectasia, Nijmegen breakage syndrome, and autoimmune lymphoproliferative syndrome are associated with high-risk of hematologic malignancy (51, 56*, 58). The immune system is comprised of the innate and adaptive immune branches. The ability of innate immune cells to present germline-encoded pattern recognition receptors to recognize “non-self” conserved microbial components or, in the context of leukemia, malignant cell clones is key to the adaptive immune response (55). In a functioning immune system, antigen-specific lymphocytes known as T-cells undergo clonal expansion to populate the adaptive immune system and mature into effector cells to induce target cell death or become long-lived central memory cells for sustained adaptive immunity (55). The adaptive immune system also consists of B-cells which serve as antigen-presenting cells that activate T-cells during an immune response (54). Manipulating the adaptive immune response with immunotherapy via immune checkpoint blockade has been promising in solid tumors (57). Thus, understanding the immune landscape in the context of germline mutations associated leukemia is essential to progress immunotherapy as a viable therapeutic option for these leukemia patients.
Germline mutations in cytokine signaling pathways causing primary immune deficiencies can increase risk of developing hematologic malignancies, including lymphoma and leukemia. Germline loss-of-function STAT3 mutations have been associated with immunodeficiency and impaired Th17 differentiation, while germline gain-of-function STAT3 mutations have been associated with early-onset autoimmunity and lymphoproliferation (53). Additionally, a germline mutation in interleukin-2-inducible T-cell kinase (ITK) has been described in a child manifesting B-cell lymphoproliferative disorder, classical Hodgkin lymphoma, and hemophagocytic syndromes (58). Perhaps a more prevalent example of childhood immunodeficiency and high-risk hematological malignancies is found in Down’s syndrome. Significantly, individuals with Down’s syndrome display higher levels of pro-inflammatory cytokines, including IL-6, IL-22, TNF-α, and MCP-1 (59). In a Down’s syndrome model, germline trisomy 21 has been associated with progenitor B-cell self-renewal in-vitro, maturation defects in-vivo, and the development of CRLF2-rearranged and JAK2 pathway-activated B-cell precursor ALL (59). Overall, germline mutations contributing to the immunosuppressive, pro-inflammatory BMME in individuals with hematological malignancies indicate that modulating cytokine signaling pathways may increase anti-tumor immunity in individuals with lymphoid and myeloid leukemias.

As germline mutations, especially those associated with childhood syndromes, such as TP53 in Li-Fraumeni syndrome, can influence leukemic progression, understanding the clinical significance of germline mutations is key to improving management of leukemia in patients with germline mutations.

**Clinical Implications and Management of Leukemia Predisposition**

The impact of germline and somatic mutations on cell-autonomous and non-cell-autonomous contributions to leukemogenesis needs to be considered in the management of cancer predisposition. Existing guidelines for management of leukemia predisposition is predicated on careful observation of symptoms, laboratory evaluation, and continued surveillance measures based on the risk of disease progression (5, 60). Others have described their practices of managing patients with clonal hematopoiesis which includes a thorough history and exam, baseline complete blood count, bone marrow evaluation and multidisciplinary care including cardiology (61–63). Additional clinical trials will help demonstrate whether serial assessment of variant allele frequencies using deep sequencing in those with germline leukemia predisposition and/or CHIP can provide the opportunity for detection of clonal evolution. Nevertheless, as germline mutations and their interaction with CHIP can affect disease occurrence, progression, and, in the instance of TP53 mutations in AML, drug response and prognosis, extensive germline mutation sequencing in addition to sequencing of currently known leukemia-associated genes should be considered for patients with or at risk for leukemia (64*).

Management of non-cell-autonomous contributions to leukemogenesis is guided by the underlying defect. For some immunodeficiencies, immunoglobulin therapy may be indicated, although the extent to which this may modify risk of malignancy is not known (65). More severe immune deficiencies require hematopoietic stem cell transplantation.
(HSCT) to abrogate the risk of severe infections (e.g. severe combined immunodeficiency) and/or correct hematologic defects (GATA2 deficiency) (66). Indeed, the prospect of preemptive HSCT, prior to the onset of disease manifestations, should be considered for highly penetrant diseases such as N-terminal CEBPA mutation and Fanconi Anemia, recognizing that doing so can alter the natural history of disease such that other manifestations, such as solid tumor risk, may become apparent (67–69). Moreover, as a healthy BMME predicts successful HSCT, screening for germline mutations (e.g. TET2) in the BMME should be conducted to address potential germline mutation associated BMME impairment prior to HSCT (63, 70*). As cellular engineering technologies are rapidly evolving, it is conceivable that more refined cellular therapy approaches may be applied for the prevention or treatment of leukemias in those at the highest risk.

**Conclusion**

Germline mutations, whether by interacting with CHIP mutations or by affecting the BMME, are significant contributors to leukemic initiation and progression. As such, germline mutations represent a clinical challenge in the treatment and management of hematological malignancies. Continued research in the realm of germline mutations’ contribution to leukemogenesis will advance preemptive testing and treatment options for those with germline mutations associated with leukemia and lymphoma. With the advancement of personalized medicine, identifying germline mutations is invaluable to providing holistic care to individuals who may be at risk for hematological malignancies.

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Key Points

- Germline mutations may functionally interact with CHIP-associated gene mutations
- Germline mutations can contribute to leukemogenesis by disrupting supportive stem cell microenvironment
- Germline mutations may also suppress immune cell development and cause a proinflammatory microenvironment to predispose individuals to leukemia
- Clinical management is predicated on early detection, appropriate surveillance, and a multidisciplinary approach