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Review

The origin of leukemia: Genetic alterations and inflammatory factors in the development of premalignant clonal hematopoiesis

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ABSTRACT

Clonal hematopoiesis of indetermined potential (CHIP) is increasingly common with age and identified in more than 1 in 10 healthy individuals at the age of 70. Mutations in epigenetic and splicing factors are recurrent genetic events in CHIP, and experimental data suggest that microbial and inflammatory factors may contribute to the selective expansion of hematopoietic stem cells carrying these mutations. In parallel, CHIP is associated with an increased incidence of cardiovascular disease and studies in mice support a causal relationship where mutated hematopoietic cells contribute to inflammation and atherosclerotic plaque formation. Collectively, current clinical and experimental data suggest a complex network where genetic alterations and inflammatory factors contribute to the development of the early stages of hematological malignancy.

Introduction

Most of our blood cells are constantly lost and replenished in a process where hematopoietic stem- and progenitor cells develop into 1 or more mature blood cell lineages. Acquired genetic alterations in hematopoietic stem cells (HSCs) that increase self-renewal or impair differentiation may disrupt this tightly controlled process. This ultimately results in clonal expansion and deregulated differentiation of hematopoietic cells, which are hallmarks of hematological malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Development of myeloid malignancy is a multistep process with stepwise accumulation of genetic alterations that precede leukemia phenotypes [1]. In line with this, premalignant clonal expansion of cells that carry somatic alterations associated with myeloid malignancy is relatively common in the blood of healthy individuals [2-4]. This benign clonal hematopoiesis is associated with age and with an increased risk of hematological malignancy [2,3]. In common with other cancers, the risk for MDS and AML is increased among individuals with a history of infectious or autoimmune diseases [5] and deregulation of inflammatory signaling is common in myeloid malignancy [6,7]. Furthermore, clonal hematopoiesis is identified as a risk factor for cardiovascular disease and experimental studies support a role for clonally expanded mutated cells in pro-inflammatory processes [8,9]. The aim of this review is to summarize clinical and experimental evidence of how genetic alterations inherent to HSCs and extrinsic inflammatory factors contribute and synergize in the development of premalignant clonal hematopoiesis and subsequent transformation to myeloid malignancy.

CHIP

Prevalence and clinical relevance

Genetic events are accumulated stepwise in the development of AML [1], indicating that premalignant somatic events precede development of leukemia pathogenesis. Early evidence of the presence of genetic alterations associated with hematological malignancy in healthy individuals emerged through development of highly sensitive methods that detected presence of the BCR-ABL1 fusion transcript, characteristic for chronic myeloid leukemia, in healthy controls [10]. Similarly, low levels of lymphoma-associated BCL2 translocations were detected in B-cells from healthy individuals [11]. During the same time, the first evidence of clonal blood mosaicism came from signs of skewed X-allele inactivation in the blood of healthy elderly women [12]. Genome wide SNP-array data later detected clonal mosaic duplications, deletions and uniparental disomy in the blood of over 2% of older healthy indi-

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viduals [13,14]. These studies were also the first to report an increased risk for hematological malignancy among individuals with clonal hematopoiesis. Later on, somatic TET2-mutations were identified in a substantial fraction of women with skewed X-allele inactivation [15]. In addition, deep sequencing of sorted HSCs, T-cells and leukemic cells from AML patients identified the presence of premalignant pluripotent HSCs carrying only a subset of the mutations identified in the AML cells [16].

These results collectively spurred the hypothesis that somatic genetic events in hematopoietic stem- and progenitor cells can infer a clonal advantage resulting in outgrowth of a dominant clone without concomitant malignancy. Evidence of such somatic events were presented in three parallel studies analyzing exome-sequencing data from individuals without a record of hematopoietic malignancy [2-4]. Despite different cohort characteristics and methodologies, these studies present very similar results where clonal hematopoiesis evident by somatic alterations is identified in less than 1% of individuals at the age of 40 but in over 10% at the age of 80 [2-4]. Presence of clonal hematopoiesis was also associated with a 10-fold increased risk of developing hematological malignancy [2,3]. This is equivalent to an annual transformation rate of 0.5% to 1% and means that the vast majority of individuals with clonal hematopoiesis never develop malignancy and the phenomenon is therefore termed clonal hematopoiesis of indetermined potential (CHIP). CHIP shares traits with other clonal states such as monoclonal gammopathy of undetermined significance (MGUS) and monoclonal B-cell lymphocytosis (MBL). MGUS and MBL are conditions of symptomless clonal expansion of plasma cells and B-cells respectively, both with increased risk of malignant progression. The risk of progression to multiple myeloma in MGUS is about 1% per year and the risk for chronic lymphocytic leukemia is 1% to 2% per year in individuals with MBL [17,18].

As HSCs acquire somatic mutations over time, random genetic alterations in hematopoietic cells accumulate with age in all individuals [1,19]. The number of HSCs that contribute to hematopoiesis in a healthy adult is estimated to be at least 50 000 [19], meaning that without clonal selection, the average fraction of blood cells that derive from a single HSC (and carry its mutational history) may be approximately 0.002%. Natural drift within the HSC compartment [20] as well as changes in HSC properties with age [21] could cause substantial deviations from this estimate, possibly resulting in detectable clonal hematopoiesis at low levels also in the absence of genetic alterations that drive clonal expansion. Furthermore, somatic mutations that occur during early embryogenesis can result in blood mosaicism if the mutated embryonic cell's progeny contribute partly to hematopoiesis [22]. To avoid misinterpretation of findings as evidence of clonal hematopoiesis, the proposed definition for CHIP in individuals without hematological dysplasia or cytopenia is identification of a somatic variant at a mutant allele frequency of at least 2% in any of 18 genes that are recurrently mutated in hematological malignancy [23]. A recent study, using sequencing methods able to detect clonal mutations at an allele frequency down to 0.01%, also showed that an increased risk of developing AML was seen only at mutant allele frequency above 1%, while the risk at lower allele frequency was more uncertain [24].

Genetic alterations in CHIP

The three genes most commonly mutated in CHIP are the epigenetic modifiers *DNMT3A* (DNA methyl transferase 3 alpha), *TET2* (tet methylcytosine dioxygenase 2), and *ASXL1* (ASXL transcriptional regulator 1) [2-4]. How mutations in these genes and the resulting deregulated DNA methylation or altered chromatin structure result in clonal advantage is not fully determined but may include upregulation of genes involved in HSC self-renewal or disruption of gene expression programs necessary for differentiation [25]. Missense mutations in the two splicing factors *SRSF2* (serine and arginine rich splicing factor 2) and *SF3B1* (splicing factor 3b subunit 1) are also relatively common in CHIP and may, similarly to deregulation of epigenetic marks, affect proper expression of many genes. The long list of less frequently mutated genes in CHIP include the tumor suppressors *TP53* (tumor protein p53) and *PPM1D* (protein phosphatase, Mg2+/Mn2+ dependent 1D), the tyrosine kinase *JAK2* (Janus kinase 2), and the ubiquitin-protein ligase and signal transducer *CBL* (Cbl proto-oncogene) [2-4].

Without doubt, many of these genetic alterations act in a cell-autonomous manner to increase HSC self-renewal and/or to impair normal differentiation resulting in a potential for clonal expansion. Loss-of-function mutations in *DNMT3A* and *TET2* are the alterations most widely studied in experimental systems and show a competitive advantage in transplant settings in both mouse [26,27] and human cells [28], supporting cell-autonomous mechanisms.

Risk factors for CHIP and risk factors for malignant transformation in CHIP

Smoking is a well-established risk factor in the development of an array of malignancies, including hematologic malignancies [29] and an association between smoking and clonal hematopoiesis has also been observed [2]. In support of this, Coombs et al found an increase in C>A transversions, which are characteristic for tobacco exposure, in smokers with clonal hematopoiesis [30]. Exposure to radiation or chemotherapy is also recognized as a risk factor for developing CHIP [30]. Patients with thyroid cancer treated with radioactive iodine have an increased prevalence of CHIP [31] as well as higher risk of developing leukemia [32]. Furthermore, a recently published study on survivors of breast cancer and aggressive lymphoma showed a 4 times increased frequency of CHIP after treatment with myelotoxic chemotherapy [33]. The mutational pattern was similar to that found in a general CHIP-population and the authors concluded that rather than a direct mutagenic effect from chemotherapy, the increased prevalence of CHIP is likely a result of competitive advantage of already present clones during chemotherapeutic stress or changes in the immune microenvironment [33]. In line with this, clones harboring mutations in DNA damage response genes such as *TP53* and *PPM1D* expand following cytotoxic therapy, which likely explains the over-representation of *TP53* mutations in therapy-related MDS and AML [34]. Notably some mutations may result in different responses depending on the type of cellular stress. *PPM1D* mutations for example, confer a selective advantage in an experimental setting of chemotherapeutic stress [35] but not in the setting of bone marrow transplantation [36].

The risk of developing AML among individuals with CHIP increases with the complexity of the mutational landscape and higher allele frequencies of the mutations, shown by retrospective analysis of blood samples obtained years before AML diagnosis [37,38]. Furthermore, CHIP with mutations in certain genes, including *TP53*, *U2AF1*, *IDH1*, and *IDH2*, showed an increased risk of AML-development [37,38] and a recent study showed that CHIP with mutations in the R882 hotspot residue in *DNMT3A* more often progressed to AML compared to CHIP with *DNMT3A* W806R mutations [24]. These results highlight that the risk for malignant transformation in individuals with CHIP may depend on the nature of the individual mutations. Lastly, clonal hematopoiesis is generally associated with short telomere length [39] though the role of telomere length and telomerase activity in CHIP is a topic not widely studied but which may prove to be of interest. It has been speculated that CHIP clones with longer telomeres would be a risk factor for AML progression by allowing a higher number of cell replications

and thus increasing the risk for additional mutational hits and malignant development [40].

Inflammation and CHIP: consequence, cause or both?

Besides an increased risk of hematological malignancy, several studies have linked CHIP to an increased risk of mortality related to cardiovascular events [3,9,41]. This has led to the hypothesis that expansion of mature immune cells carrying CHIP-mutations could result in an inflammatory state contributing to atherosclerotic plaque formation. This is supported by 2 mouse studies where inactivation of Tet2 in hematopoietic cells increased atherosclerotic plaque formation in atherosclerosis-prone mice [8,9]. In addition, 1 study showed that inactivation of Tet2 or Dnmt3a in hematopoietic cells worsened cardiac dysfunction after Angiotensin II challenge in mice [42], supporting a causal role for hematopoietic loss-of-function mutations in *TET2* or *DNMT3A* in the development of cardiovascular disease. These results are also corroborated by 1 human study showing increased serum levels of the pro-inflammatory cytokine IL-6 and the chemoattractant Eotaxin-1 in individuals with CHIP and *TET2* or *DNMT3A* mutations [43]. The mechanisms by which inactivation of these genes may deregulate inflammation include repression of pro-inflammatory signaling by TET2 and involvement of DNMT3A in interferon-responses [8,44,45].

Experimental studies thus support a causal role for CHIP in deregulated inflammation and in the development of cardiovascular events. Alternatively (or additionally), subclinical chronic inflammation with increased pro-inflammatory cytokines – a well-known risk factor for cardiovascular disease [46] – may also increase the risk for developing CHIP. A chronic inflammatory state could contribute to CHIP development in at least 2 ways; (1) Through imposed DNA damage and genotoxic stress resulting in a higher risk for mutations, and (2) Through a selective advantage of hematopoietic cells with CHIP-mutations in a pro-inflammatory environment. The first of these mechanisms is supported by data showing increased oxidative DNA damage in lymphocytes from patients with autoimmune diseases such as rheumatoid arthritis and Behçet's disease [47]. The second mechanism, where CHIP clones may take advantage of an inflammatory environment, is supported by experimental data showing that expansion of hematopoietic cells with loss-of-function mutations in Tet2 depend on pro-inflammatory signaling [48,49].

Regardless of whether an inflammatory state increases HSC mutagenesis or boosts the outgrowth of mutated hematopoietic cells, these mechanisms would increase the incidence of CHIP among individuals with increased pro-inflammatory cytokines resulting from autoimmune disease or metabolic syndrome. A causal role for chronic inflammation in the development of CHIP could also contribute to the increased risk of myeloid malignancy in subjects with a history of infectious and autoimmune diseases [5]. Support for this comes from 1 recent study of patients with rheumatoid arthritis that detect CHIP in 18% of patients in the age span 30 to 80 years [50]. The study did not include a control group and use of different methodologies makes comparison to other studies complicated. Nevertheless, the larger CHIP studies show a prevalence of around 10% in the same age group [2,3], possibly indicating an increased incidence of CHIP among individuals with rheumatoid arthritis. Furthermore, type 2 diabetes, which is associated with increased levels of pro-inflammatory cytokines, is associated with increased prevalence of CHIP [3,41]. Metabolic syndrome and obesity also lead to chronic inflammation with increased pro-inflammatory cytokines. The frequency of CHIP in obesity remains to be determined but obesity is associated with an increased risk of hematological malignancy [51]. Interestingly, higher red blood cell distribution width, which is associated with inflammation as well as with anemia, is also associated with CHIP [3] and with risk of progression to AML [38].

Inflammatory signaling in hematopoietic malignancy

Cell-intrinsic inflammatory signaling

The innate immune system is our first line of defense against infectious agents such as viruses and bacteria. Inflammation results from the recognition of pathogen-associated molecular patterns (PAMPs) or host derived danger-associated molecular patterns by pattern recognition receptors. Pattern recognition receptors include Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors and their activation lead to upregulation and secretion of pro-inflammatory cytokines and interferons [52]. Inflammatory signaling is implicated in the pathogenesis of hematological malignancy and in MDS and myeloproliferative neoplasms (MPNs) in particular [6,7]. TLR-signaling is upregulated in many patients with MDS [53,54] and upregulation of the TLR effector TRAF6 induces MDS-like symptoms including bone marrow failure in an MDS mouse model [55]. Somatic activating mutations in TLR2 are also identified in a subset of MDS patients and TLR2 ant agonists improve hematopoietic function of hematopoietic progenitors from MDS patients in vitro [56]. Elevated inflammatory cytokines are also believed to contribute to disease progression in MPN [6]. Furthermore, mutations in *U2AF1*, *SRSF2*, *SF3B1*, *ASXL1*, and *TET2* that are recurrent alterations in MDS, MPN, and in CHIP induce signaling normally activated downstream of the NOD receptor NLRP3 [57,58].

Systemic inflammation

TLRs are highly expressed by mature innate immune cells but also by HSCs and hematopoietic progenitors [59] and experimental studies support mechanisms where PAMPs stimulate HSCs directly towards proliferation and differentiation [59,60]. In parallel, pro-inflammatory cytokines and interferons affect self-renewal and proliferation of HSCs [61-64]. In line with this, sustained LPS administration or repeated infections that induce chronic IFNγ-stimulation deplete the HSC pool in mice [65,66]. How inflammatory factors influence the development of myeloid malignancy is not fully known but as stated above, infectious or autoimmune disease as well as obesity is associated with myeloid malignancy [5,51]. Speculatively, repeated episodes of infection, chronic infection or chronic inflammatory states associated with autoimmune disease or metabolic syndrome could create an exhausted HSC pool, enabling outgrowth of a CHIP-mutated clone. Indeed, experimental work has shown accelerated myelodysplasia in a mouse model of MDS upon LPS administration [67]. Furthermore, TLR signaling may be continuously activated by bacterial PAMPs that enter the blood stream from the intestinal tract and both steady-state hematopoiesis [68,69] and hematopoietic recovery [70] are influenced by the intestinal flora. Interestingly, inflammatory bowel disease is associated with higher incidence of hematological malignancy [71] and may give rise to increased intestinal translocation of microbial factors [72]. In addition, the recent experimental evidence supporting a role for factors derived from the intestinal flora in the outgrowth of *TET2*-mutated cells [49] indicates that inflammatory signals of noninfectious origin may have a role in development of CHIP and myeloid malignancy.

The bone marrow microenvironment

Besides hematopoietic cells, the bone marrow also contain many other cell types including mesenchymal stromal cells, osteoblasts, osteoclasts, and adipose cells. These cells form a mi-

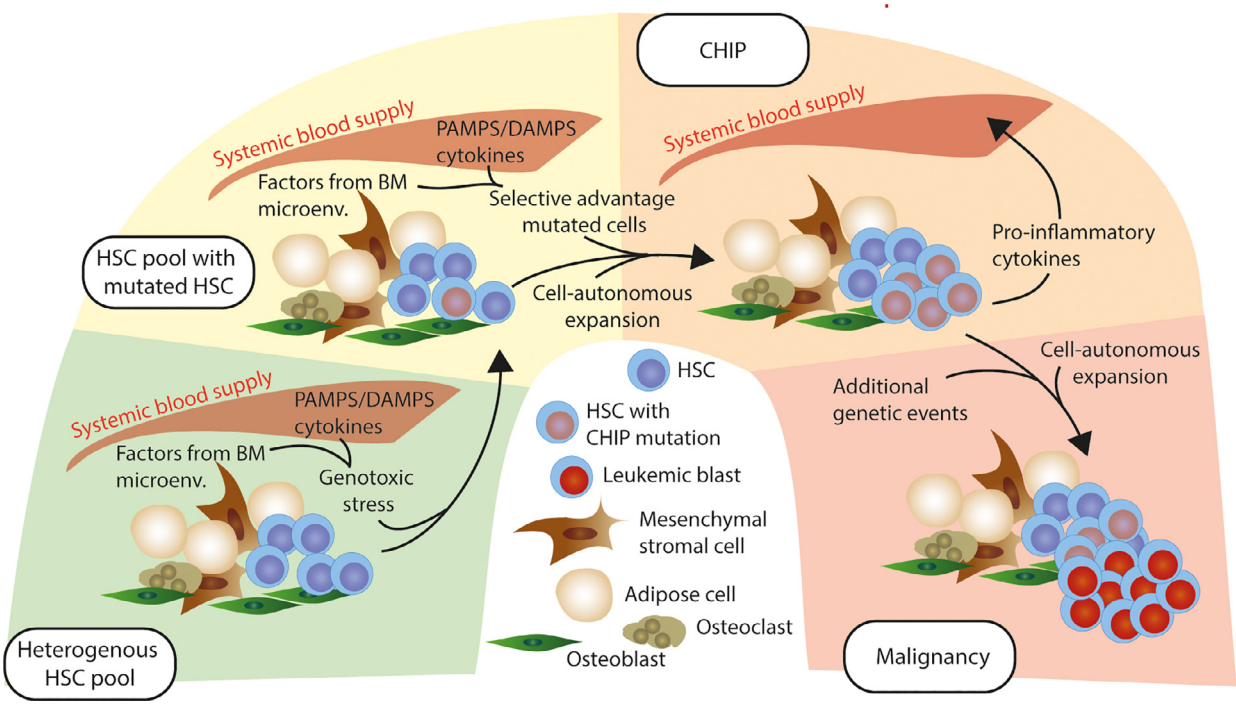

Figure 1. Outline of potential crosstalk between cell-extrinsic inflammatory processes and cell-intrinsic genetic alterations in the development of premalignant clonal hematopoiesis and hematopoietic malignancy. Inflammatory factors derived from systemic injury, infection, inflammation, or from a local inflammatory bone marrow (BM) microenvironment may impose genotoxic stress increasing the likelihood for somatic mutations. These factors may also contribute to a selective advantage of mutated HSCs enhancing the clonal outgrowth of cells with genetic alterations. In addition, clonally expanded mutated clones may express inflammatory factors and increase systemic inflammation.

croenvironment, often termed the HSC niche, that regulates HSC integrity [73]. Factors such as interleukin 7, stem cell factor, the chemokine CXCL12, and delta like ligand 4 (DLL4) produced by niche cells are important cues for HSCs and other early hematopoietic progenitors [73].

The bone marrow microenvironment and local inflammation may also have a role in initiation of clonal hematopoiesis and hematopoietic malignancy. Deficiency of the transcription factor RARγ or deregulated ribosome biogenesis in nonhematopoietic cells within the bone marrow microenvironment drive myelodysplasia in experimental systems [74-76]. In both these models, upregulation of inflammatory signaling within the bone marrow niche contributed to the disease phenotypes [74,76]. Furthermore, bone marrow adiposity is dynamic and accumulation of adipocytes in the bone marrow is associated with age and obesity but also with starvation and caloric restriction [77]. Administration of PPARy agonists to mice induce bone marrow adipogenesis and improve normal hematopoiesis while inhibiting leukemogenesis [78]. In line with this, experimental studies show that caloric restriction may improve normal HSC function [79] and impair leukemogenesis in experimental mouse models [80].

Conclusions and future perspectives

Acquired genetic alterations drive leukemogenesis and clonal expansion of preleukemic cells with CHIP mutations depend on cell-intrinsic mechanisms [26-28]. Both experimental and clinical studies however suggest that inflammatory processes derived from autoimmune disease, chronic infection, or the intestinal flora may also contribute to development of clonal hematopoiesis and preleukemia [3,5,41,48-50]. In parallel, clinical and experimental evidence, support a causal role for preleukemic clonal hematopoiesis in elevated inflammatory processes and cardiovascular disease [3,8,9,41-43]. Fig. 1 summarizes these potential mechanisms.

If recognized beyond the proposed definition of a variant allele frequency of 2%, CHIP seems to be an almost inevitable state in aged individuals as studies utilizing error corrected sequencing to enable detection of CHIP down to a variant allele frequency of 0.01%, identify somatic alterations in almost all individuals at the age of 60 [24,81]. Furthermore, the size of mutated clones may be stable (at least in peripheral blood) over long periods of time [24], which to some extent challenges the view that HSCs with CHIP mutations have a dominant cell-intrinsic growth advantage. Experimental work show a selective advantage of Tet2-mutated cells under inflammatory stress [48,49] and it may be hypothesized that external cues such as a pro-inflammatory environment contribute to the growth advantage of these preleukemic clones. Low-grade inflammation associated with ageing ("inflammaging") [82] together with the time-dependent risk of acquiring a CHIP-associated mutation may thus explain the exponential increase in CHIP incidence (and myeloid malignancy) with age. Similarly, pro-inflammatory factors derived from inflammatory, infectious or, autoimmune diseases may be chronic or temporal factors in the development of CHIP and subsequent malignant transformation.

Large cohort studies as well as experimental data will be necessary to define how cell-intrinsic factors such as epigenetic alterations cooperate with external cues in leukemogenesis. In addition, although the majority of mutations identified in CHIP affect epigenetic modifiers or splicing factors, the mutational landscape in CHIP is heterogeneous and the crosstalk between different genetic alterations and extrinsic factors likely vary. Nevertheless, unravelling the causal and co-operative relationships of this network will be fundamental for understanding both the risks associated with CHIP and the early pathogenesis of hematopoietic malignancy.

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Conflicts of interest

The authors declare that they have no conflicts of interest or competing financial or personal relationships that could inappropriately influence the content of this article.

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