

Review Paper

A Review of Immune Landscape of Clonal Hematopoiesis: Progression and Prospects for the Future



Majid Teremmahi Ardestani¹ , Marzieh Norouzian^{1*} 

1. Department of Laboratory Sciences, School of Allied Medical Sciences, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.



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ABSTRACT

Context: Clonal hematopoiesis (CH), characterized by the clonal proliferation of mutant hematopoietic cells, is frequently observed in older individuals. It is widely recognized that ageing leads to somatic mutations in hematopoietic stem cells, increasing the risk of developing various diseases later in life. However, the impact of these clonal hematopoiesis-related mutations on the activation and regulation of the immune response remains uncertain. **Evidence acquisition:** To address this knowledge gap, a thorough review of the existing literature on clonal hematopoiesis and immune dysregulation was conducted. Relevant studies were systematically searched and their key findings were carefully analyzed to provide a comprehensive overview.

Results: The review shows that mutations associated with clonal hematopoiesis have a significant impact on the modulation of immune responses in the body. These mutations are found to contribute to immune dysregulation, which has important implications for disease progression in the elderly.

Conclusion: This review highlights the importance of studying immune dysregulation in the context of clonal hematopoiesis. By unravelling the relationship between CH-related mutations and immune responses, we can improve our understanding of disease progression in the elderly and identify promising therapeutic targets. This knowledge can inform the development of innovative interventions for improved disease management.

* Corresponding Author:

Marzieh Norouzian, Assistant Professor.

Address: Department of Laboratory Sciences, School of Allied Medical Sciences, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Tel: +98 (76) 3366367

E-mail: marzieh.norouzi@gmail.com

Introduction

Hematopoietic stem cells (HSCs) are characterized by their ability to generate themselves and differentiate into multiple cell types in response to extracellular signals [1]. Preserving hematopoietic stem cells in the non-dividing phase reduces energy demand, prevents oxidative damage, and provides a pool of stem cell storage for sustaining human life [1]. The intricate balance of quiescence, self-renewal, and differentiation of hematopoietic stem cells is governed by intracellular mechanisms, including transcription, epigenetics, metabolic changes, and cell cycle regulators [2]. On the other hand, stressors such as infection and pathological or physiological conditions (aging) affect the self-renewal of hematopoietic stem cells [3]. It is essential to understand the sustaining hemostasis of hematopoietic stem cells under normal conditions or its disruption under stress conditions. Constant cell contact with DNA-damaging factors leads to the accumulation of somatic mutations in the cell during aging [4-6].

Several mutations, termed driver mutations, provide a competitive advantage to the cell, leading to the emergence of a dominant cell clone, under certain conditions, such as chronic inflammation or environmental stimuli [7]. Then, the ancestors of this dominant cell clone can undergo other mutations, potentially culminating in cancer. So, in the absence of cancer and within the normal tissue, we will see cell clone proliferation. Clonal hematopoiesis (CH) is a term used to describe the clonal expansion of HSCs with somatic mutations frequently resulting from aging [8]. CH has been recognized as a risk factor for the formation of blood malignancies, especially myeloid neoplasms, such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN) [9-11]. Evidence suggests an association between clonal hematopoiesis and other illnesses, such as cardiovascular and autoimmune diseases [12].

Clonal hematopoiesis of indeterminate potential (CHIP) is the new term defined by the existence of leukemia-associated driver mutation with an allelic frequency of at least 2%, coupled with the absence of hematologic malignancies [13]. Chemotherapy and radiotherapy are important risk factors in the development of the CHIP [8, 11, 14]. This potential has also been observed in patients undergoing autologous bone marrow transplantation due to non-myeloid blood malignancies such as multiple myeloma or lymphoma [13, 15]. CHIP has been identified in other hematologic diseases, such as hereditary bone marrow failure syndrome and aplastic anemia [11, 14].

Common driver mutations in CHIP

Most mutations in clonal hematopoiesis are heterozygous and associated with protein function loss. In general, three groups of genes are involved in the formation of clonal hematopoiesis: Epigenetic regulators, including DNA methyltransferase 3A (*DNMT3A*), Ten-eleven-translocation 2 (*TET2*), and additional sex combs-like 1 (*ASXL1*); transcription factors; and genes responding to DNA damage, including tumor protein 53 (*TP53*), and protein phosphatase, Mg²⁺/Mn²⁺ dependent 1D (*PPM1D*). It has already been shown that mutations in epigenetic, splicing, transcription, and signal transduction factors are related to the development of clonal hematopoiesis [16-19].

DNMT3a mutations

Epigenetic regulatory mutations are the predominant type of clonal hematopoiesis mutations, comprising 50% of clonal hematopoiesis associated with *DNMT3a* variants [20]. Mutations in the *DNMT3a* gene and decreased activity are associated with enhanced hematopoietic stem cell regeneration. An essential cause of this phenomenon is the reduced methylation of regulatory regions of genes related to the self-renewal property of hematopoietic stem cells, including *Meis1*, *Evi1*, and *HOXA9* [7, 21].

The range of mutations observed in clonal hematopoiesis differs from those seen in myeloid leukemia. The R882 mutation hotspot of *DNMT3a* is a predominant mutation in acute myeloid leukemia, which, with a dominant negative effect, even in a heterozygous state, reduces *DNMT3a* activity drastically. Other mutations in the *DNMT3a* gene lower its activity by up to 50% of the average level [22].

TET2 mutation

TET2 is another epigenetic regulator whose mutations are common in patients with clonal hematopoiesis. *TET2* appears as a DNA demethylating factor by converting 5-methyl cytosine to 5-hydroxymethyl cytosine [17]. *TET2* mutations act as loss-of-function mutations, and unlike *DNMT3a*, they are involved in the self-renewal of hematopoietic stem cells and hematopoietic progenitor cells. In its normal state, *TET2* sometimes alters gene expression, leading to differentiation and inhibition of self-renewal [20].

ASXL1 mutation

ASXL1, as an epigenetic regulator, is critical in controlling gene expression by affecting histone methylation [23]. *ASXL1* mutations in patients with myeloid malignancies predict poor prognosis. *ASXL1* mutations can also affect HSCs' regenerative capacity. Previous studies, however, demonstrate the inhibition of HSCs' differentiation in *Asx1l*-deficient or mutant mice. *ASXL1* mutations stimulate abnormal proliferation of HSCs by disrupting epigenetic alterations, potentially contributing to CH in the long run [24].

PPM1D and TP53

TP53 and *PPM1D* (a P53-induced serin phosphatase) are stress-response regulators essential in cell cycle control, DNA repair, and tumor metabolism [25]. Exogenous selective pressures such as chemotherapy and radiation are higher relative risks in CH progression [26, 27]. The increased mutation rates in *TP53* and *PPM1D* are observed in patients with therapy-related AML and MDS [28]. Studies reveal a significant increase in cancer patients treated with chemotherapy and radiation who harbor CH-related *TP53* or *PPM1D* mutations [29].

Immune landscape of clonal hematopoiesis

Clonal hematopoiesis, characterized by somatic mutations in hematopoietic stem cells, can affect immune cells, such as macrophages, monocytes, neutrophils, and lymphocytes [30]. Under the influence of CH mutations, immune cells undergo qualitative [31, 32] and quantitative changes by affecting the differentiation of hematopoietic progenitor cells into specific lineages [33, 34]. These immune effector cells can negatively impact many disease processes, particularly those associated with chronic inflammation [12].

Objectives

Here, we review the immune landscape of age-associated clonal hematopoiesis and discuss the role of dysregulated immune responses that contribute to the outcome of CH-related disorders.

Evidence acquisition and results

Dysregulation of innate immune response in clonal hematopoiesis

The immune cells are part of the hematopoietic system. Alteration in immune functioning related to aging may have a role in clonal hematopoiesis and CH-related

disorders. Evidence from both human and animal models suggests that increased inflammation, "inflammageing," occurs during aging and correlates with changes in HSCs, including a decline in regenerative capacity and differentiation towards myeloid cells [35, 36]. Several studies support the increased level of inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF- α), IL-1, and transforming growth factor- β (TGF- β) in aged mice bone marrow [37-40]. Accordingly, old mice treated with inhibitors of IL-1 β and TNF- β responded with a significant reduction in bone marrow lymphopoiesis [41]. A study of older people demonstrated that serum levels of IL-6 and TNF- α were significantly higher in individuals with CH than those without it [42].

Data indicate that chronic inflammation can promote clonal hematopoiesis by selecting mutated hematopoietic stem cells in the context of aging [43]. The clonogenic potential of *TET2*-mutant hematopoietic stem cells in an in vitro inflammatory environment such as TNF- α has been reported in mice and humans, associated with myeloid deviation and evasion of apoptosis [44]. In line with this finding, Cai et al. reported that *TET2*-mutant hematopoietic stem and progenitor cells express high levels of IL-6 under inflammatory stress (with lipopolysaccharide [LPS] stimulation), promoting mutant HSC proliferation and survival [45]. A similar observation was reported regarding other HSC mutations. Activity of inflammatory signaling pathways in monocytes and T cells increased in subjects with *DNMT3* mutations [46]. Secretion of IL-6, TNF- α , and IL-13 was significantly higher in mouse mast cells lacking *DNMT3a* compared to wild-type cells [31].

As previously mentioned, inflammatory cytokines are associated with the proliferation of mutated hematopoietic stem cells in the clonal hematopoiesis context. However, *TET2* and *DNMT3* mutants exhibit different cytokine expressions; serum IL-6 increases in people with CH carrying *TET2* mutation, whereas serum TNF α levels are elevated in those with *DNMT3A* mutation [42].

Dysregulation of innate immune and inflammatory signaling pathways has been observed in people with clonal hematopoiesis and hematologic diseases like myelodysplastic syndromes [47]. Recent studies have focused on the role of mutation-driven clonal hematopoiesis in innate immune cell function [31, 42, 48]. Macrophage cells, or phagocytic cells, are specialized components of innate and adaptive immune systems found in bone marrow and tissues. Most knowledge about the association between inflammation and CH was obtained from studies on mouse macrophages and monocytes [49-52]. In

other words, the inflammatory profile in macrophages carrying mutations of CH and their role in diseases during aging have been well described in mice models. So, in addition to the lack of human studies supporting this data, the exact role of other immune cell types affected by these mutations has remained poorly understood.

In studies of mice with mutations in the *TET2* gene, the increased expression of NLR family pyrin domain containing 3 (NLRP3) inflammasome and IL-1, IL-6 inflammatory cytokines was shown in macrophages and monocytes, respectively [48-52]. Also, the increased expression of IL-6 in *Tet2*-deficient macrophage was demonstrated in patients with myelodysplastic syndromes and chronic myelomonocytic leukemia [48]. Frisch et al. uncovered that macrophages within the bone marrow of aged mice have a defect in the clearance of apoptotic neutrophils and induce platelet bias in HSC via increased production of IL-1 β [38]. In addition, evidence supports that *DNMT3A* deficiency may alter innate immune function, including enhanced inflammatory activation of mast cells and inhibition of immunosuppressive function in suppressive myeloid cells [31, 53]. Several studies have shown that clonal hematopoiesis bearing *DNMT3A* and *TET2* mutations contributes to the development and progression of multiple diseases through inflammatory mechanisms [14]. Recent studies have shown that mutations in *TET2* and *DNMT3A* reduce inflammation and produce type I interferon, respectively [54, 55].

Beyond macrophages, neutrophils are typical innate cells that comprise 40%-70% of human white blood cells. These cells are involved in the immune response against extracellular pathogens by producing reactive oxygen species and neutrophil extracellular traps. Some studies propose alterations in neutrophil function in the context of CH that contribute to CH-related disease progression [56-58]. A recent study of patients with AAV (anti-neutrophil cytoplasmic antibody [ANCA]-associated autoimmune vasculitides) indicates that oxidative burst of neutrophils was significantly decreased in AAV patients with CH compared to those without it [56]. Also, the direct association between dysregulated NET formation in neutrophils with *Jack2* mutation, as one of the most common mutations in CH and increased risk of thrombosis, has been reported in patients with myeloproliferative neoplasm which further supports the idea of potential correlation between CH and clinical and pathological outcome of autoimmune diseases [57]. However, the impact of other mutations, such as *DNMT3* and *TET2*, on neutrophils remains to be elucidated.

In sum, it can be hypothesized that mutated hematopoietic stem cells differentiate into leukocytes, including monocytes, macrophages, and lymphocytes, which are functionally impaired and release increased levels of proinflammatory cytokines. CH-induced inflammation creates a microenvironment favorable for clonal development and aggravates systemic inflammation, which can help the progression of age-related diseases. A comprehensive understanding of the mechanism of immune system dysfunction and inflammatory processes in clonal hematopoiesis holds promise for the early detection and prevention of hematologic malignancies and other associated disorders.

Dysregulation of adaptive immune response in clonal hematopoiesis

However, CH mutations are also known to have associations with myeloid and lymphoid malignancies [59-61]. A recent cohort study of 109 patients with T-cell lymphomas demonstrated that *TET2* mutations in T cells skew deviation toward Th follicular (TFH) cells related to unfavorable disease outcomes [60]. Likewise, *Tet2* knockdown mice exhibited the increased expansion of Th follicular cells in the spleen of aged mice compared with young mice [62]. In separate experiments, the increased generation of CD8⁺ memory T cells and functional suppression of regulatory T cells were shown to be affected by *TET2* loss of function mutations [63, 64]. DNMT3 mutant CD8⁺ T cells exhibit reduced exhaustion in response to chronic stimulation [65]. Increased expression of IFN- γ and high Th17/Tregulatory ratio have been reported in DNMT3 mutant T cells [33, 66].

However, the effects of CH mutations on B cells, as one of the crucial components of adaptive immune response, remain relatively less explored. Knockout mouse studies showed that alterations in *TET2* impacted the B cells' development and activation, resulting in abnormalities in B1 cell subsets, inhibition in plasma cell differentiation, and enhanced risk of cancer [67-69]. In contrast, loss of DNMT3 in mice B cells led to increased activation of germinal center B cells and plasma cell differentiation upon in vivo stimulation with PE-CFA antigen [70].

The potential role of clonal hematopoiesis has been shown in the progression of hematologic malignancies, cardiovascular disease, and autoimmune disease in older adults [11, 50]. As described above, some CH-related mutations disturb the immune system's homeostasis, which can play a role in developing CH-associated diseases. For example, experimental models in cardiovascular disease demonstrated the infiltration of the heart with *Tet2*-defi-

cient inflammatory cells followed by enhanced expression of IL-1 β and deterioration in heart function [49, 52]. Consistently targeting and inhibiting inflammatory pathways by NLRP3 inhibitor ameliorated atherosclerotic lesions mediated by clonal expansion of mutated immune cells [71]. Moreover, studies investigating the relationship between other CH-related mutations like *DNMT3*, *JAK2* mutations, and coronary heart diseases showed an inflammatory shift in macrophages and T cells of heart failure patients carrying *DNMT3* mutation, which may contribute to the exacerbation of their disease [46, 72].

Mouse melanoma models found that deletion of *TET2* resulted in restraint immunosuppressive function of macrophages and myeloid-derived suppressor cells and improved antigen-specific T cell response [73]. Subsequent investigations in clinical settings support CH's positive role in promoting anti-tumor immunity. Patients with hematologic malignants who were transplanted with *DNMT3A* mutated HSC showed reduced incidence of relapse or progression of tumor and increased risk of GVHD (graft versus host disease) [15]. This outcome may be because mutated clones might promote an immune response against normal and tumor cells. In CAR-T cell immunotherapy of patients with chronic lymphocytic leukemia, it was shown that *TET2* deletion in CAR-T cells changed the differentiation of T cells to central memory T cells, with increased cytokine expression more effective in inhibiting tumor cells [74].

Therapeutic approaches

Up to now, several treatment strategies have been developed for specific CH-related mutations. For example, *Tet2*-deficient mice treated with high vitamin C had promising results like restoration of aberrant self-renewal in HSC of treated mice and improved blood hemostasis [75]. In a study on aged *Asx11*-mutant mice, treatment by rapamycin (mTOR inhibitor) restrained the cell division of impaired HSCs and led to the prevention of CHIP progression [76]. A recent study has reported that inhibiting the innate immune signaling pathway may affect myeloid leukemia [77]. Moreover, targeting age-associated alterations in bone niches, such as the TGF- β signaling pathway, has been suggested as a promising therapeutic option for CHIP [37]. Above all, targeting inflammatory molecules more closely related to CH's consequences might help treat CH-related diseases, particularly atherosclerosis [50]. However, therapies to promote the function of the aging immune system have become attractive modalities to suppress the mutant clones; whether such specific treatments will be clinically possible or not remains to be elucidated.

Conclusion

CHIP has been reported to be prevalent among older adults, but it hurts health. However, whole exome sequencing in healthy adults demonstrated that CH occurs in 1% of the population younger than 40. However, only a fraction of individuals may reach a condition where the mutant clone expands significantly. These findings suggest that in addition to well-known mutations for CH, non-mutational mechanisms such as epigenetic alterations and environmental agents can be associated with the expansion of mutant clones in CH. So, further research is needed to find a cause-effect relationship between these factors and the development of CH. In the years to come, several surprising associations between these mutations and aging diseases will be revealed. Conducting biobank research will also provide more insights into diagnosing other factors that affect CHIP pathogenicity.

Evidence supports that inflammation and dysregulation of immune cell pathways contribute to the progression of clonal hematopoiesis and associated diseases, including cancers, atherosclerosis, autoimmune diseases, etc. However, the extent of immune dysfunction in a hematopoietic system with clonal expansions of mutated hematopoietic cells and the precise mechanisms of mutations leading to dysregulation in immune cells are largely unknown. Detailed phenotypic and functional studies are needed to shed more light on the role of dysregulated immune response on the development of clonal hematopoiesis and related diseases, which can help in designing novel therapeutic approaches.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors contributed equally in preparation of this paper.

Conflict of interest

The authors declared no conflict of interest.

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