

THEMED ISSUE REVIEW

Micro-environment alterations through time leading to myeloid malignancies

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The micro-environment plays a critical role in haematopoietic stem cell (HSC) development, self-renewal, differentiation and maintenance by providing a supportive cellular framework and essential molecular cues to sustain homeostasis. In ageing and development of age-related clonal haematopoiesis, the combined contribution of intrinsic alterations in haematopoietic stem cells and their surrounding micro-environment can promote myeloid skewing and release of pro-inflammatory cytokines. A pro-inflammatory micro-environment is a common feature in the initiation and sustenance of several myeloid malignancies. Furthermore, remodelling of the micro-environment is recognized to potentiate the survival of malignant over normal cells. This review explores micro-environmental interactions in the haematopoietic system of adults, especially how the bone marrow micro-environment is impacted by ageing, the onset of age-related clonal haematopoiesis and the development of myeloid malignancies. In addition, we also discuss the possible role age-related clonal haematopoiesis and chronic inflammatory conditions play in altering the bone marrow micro-environment dynamics. Finally, we explore the importance of *in vitro* models that accurately mimic different aspects of the bone marrow micro-environment in order to study normal and malignant haematopoiesis.

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ageing, bone marrow microenvironment, haematopoiesis

1 | INTRODUCTION

In adults, haematopoiesis occurs in the bone marrow (BM), which is the main site of haematopoiesis throughout life. There are several distinct niches comprising of unique cellular networks/milieu within the

bone marrow micro-environment that influence the regulation, repopulation capacity and maintenance of haematopoietic stem cells (HSCs) and their progenitors. The cellular profile of these niches has been explored through studies conducted in transgenic and xenograft mouse models that employed the use of sequencing and imaging platforms to map the transcriptome, secretome and spatial distribution of niche elements in both normal and malignant phenotypes. Modifications to this framework as we age have been implicated in several myeloid malignancies. This review will provide a brief insight into key

Abbreviations: HSC, haematopoietic stem cell; ROS, reactive oxygen species.

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micro-environment interactions in adulthood and how this alters with age and in relation to myeloid malignancies.

2 | ADULT BONE MARROW (BM) NICHE MICRO-ENVIRONMENT

Haematopoiesis takes place in the bone marrow after cell migration from the foetal liver following remodelling of the portal vessels and brief residence in the spleen with subsequent homing in the bone marrow occurring shortly before birth (Gao et al., 2018). The bone marrow is a vascularized tissue found in the medullary regions of bones with the axial skeleton reported to be the major site of haematopoiesis in humans (Pinho & Frenette, 2019). Initial cell migration from the foetal liver into the developing bone marrow is influenced by an increasing gradient of **C-X-C motif chemokine ligand 12 (CXCL12)** that acts as a chemoattractant and potentiates the activity of cell adhesion molecules in HSCs (Christensen et al., 2004; Greenbaum et al., 2013; Lewis et al., 2021). A key source of CXCL12 in the bone marrow is the perisinusoidal niche that hosts CXCL12-abundant reticular (CAR) cells (Greenbaum et al., 2013) found around sinusoidal vessels, where a large proportion of HSCs have been shown to localize as illustrated in Figure 1a (Asada et al., 2017; Baryawno et al., 2019; Comazzetto et al., 2019). **Leptin receptor** positive (LepR+) stromal cells and endothelial cells, that also closely associate with sinusoidal vessels have been suggested to serve as additional sources of CXCL12, **stem cell factor (SCF)**, **vascular endothelial growth factor (VEGF)**, **transforming growth factor β (TGF β 1)** and **pleiotrophin** (Asada et al., 2017; Baryawno et al., 2019). These factors promote HSC homing, differentiation, expansion and maintenance (Gao et al., 2018; Sands et al., 2013). Experiments by Comazzetto et al. (2019) illustrated that SCF secreted by LepR+ stroma but not endothelial cells promote the survival and proliferation of haematopoietic stem and progenitor cells (HSPCs). This was further supported by the observed reduction in the haematopoietic stem and progenitor cell population following the conditional deletion of SCF in LepR+ cells of transgenic mouse models (Comazzetto et al., 2019).

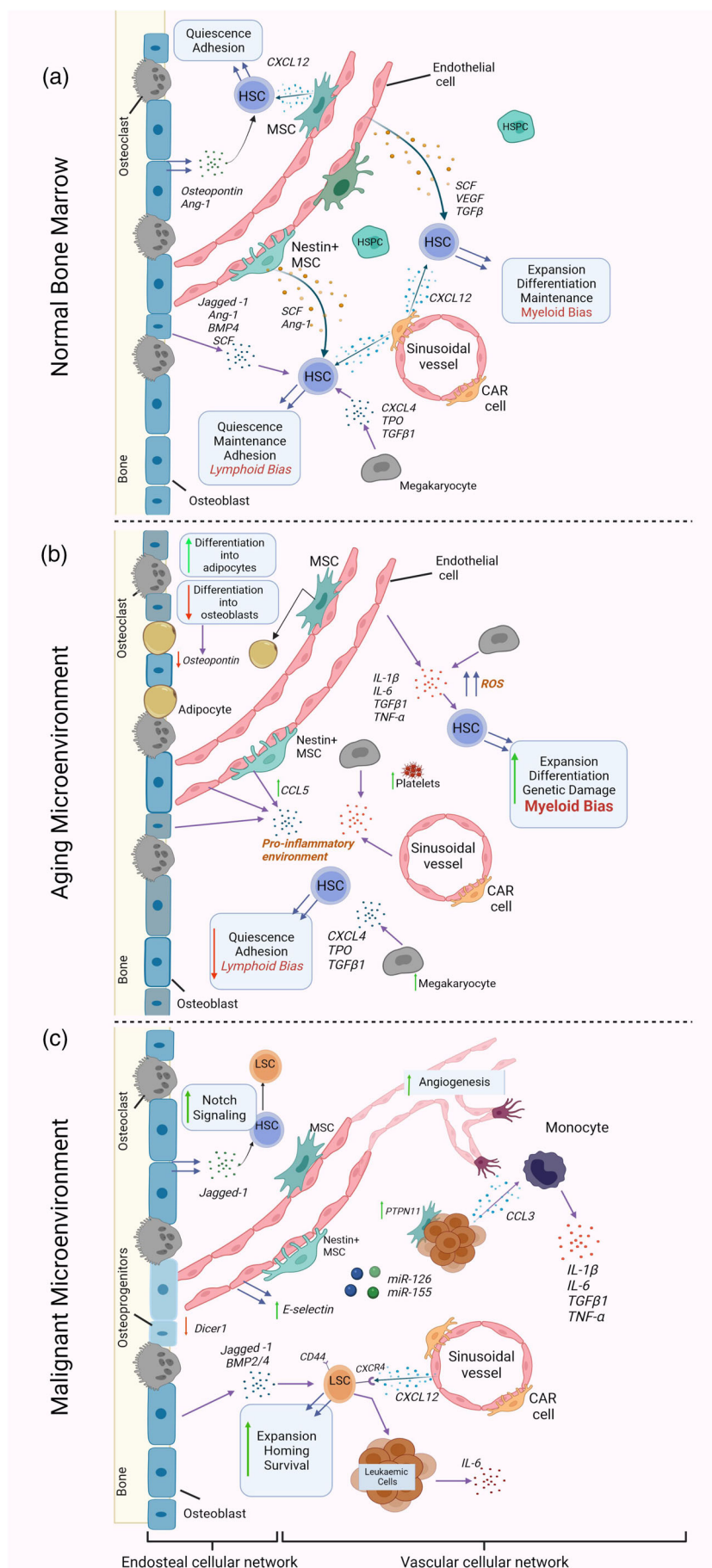
Stroma closely associating with arteriole vessels in the bone marrow have also been identified to play an important role in HSC maintenance. Investigation using Nestin-GFP+ stromal cells, which are of mesenchyme origin and possess similar tri-lineage differentiation capacity to CXCL12 abundant reticular cells (CAR) cells, indicated they express high levels of CXCL12, SCF, **angiopoietin 1 (ANG-1)**, **osteopontin**, **vascular cell adhesion molecule 1 (VCAM1)** and **interleukin-7 (IL-7)** (Baryawno et al., 2019; Passaro et al., 2021; Pinho & Frenette, 2019). Whilst there is distinction in the supportive mechanisms of the different niches, there are certain areas that overlap, which is usually determined by the spatial distribution of supporting stroma. An example of this is the suggested existence of a vascular-endosteal transition zone, where Nestin-GFP+ stromal cells can be found and is characterized by arterioles branching out adjacent to the endosteal region (Ho & Méndez-Ferrer, 2020; Itkin et al., 2016; Pinho & Frenette, 2019). Work in transgenic mice by Itkin et al. (2016) illustrated that a lower proportion of reactive oxygen species (ROS)-high expressing HSCs were found close to these arteries in

comparison with the sinusoidal regions (Itkin et al., 2016). Alterations in the level of ROS can determine HSC behaviour. Experiments by Jang and Sharkis in mouse models, showed that short-term repopulating stem cells intrinsically expressing higher levels of ROS exhibit enhanced cycling and myeloid bias, whereas those identified as ROS low are quiescent long-term repopulating stem cells (Jang & Sharkis, 2007). Based on this, it can be inferred that HSCs found adjacent to the Nestin-GFP+ stroma and arteries reflect a quiescent state that would preserve their self-renewal capacity and perturb exhaustion due to replication stress. Innervation of the bone marrow niche through sympathetic and sensory nerves fibres found on arterioles can also influence normal haematopoiesis through circadian rhythm dependent regulation of CXCL12 levels (Hanoun et al., 2014). The regulated release of **noradrenaline** and **corticosterone** from nerve fibres can create a CXCL12 gradient that can lead to retention of HSCs in the bone marrow or migration into peripheral blood (Benitah & Welz, 2020; Kollet et al., 2013).

The secretion of **CXCL4**, TGF β 1 and **thrombopoietin** from megakaryocytes that are progenitor cells located close to bone marrow sinusoids, sinusoidal and arteriole vessels can promote quiescence and mobilization of HSCs (Khatib-Massalha & Méndez-Ferrer, 2022). Concurrently, thrombopoietin is a cytokine necessary for megakaryopoiesis, with loss of signalling in knockout mice leading to a depletion of cells exhibiting a bias towards megakaryocytic differentiation (Khatib-Massalha & Méndez-Ferrer, 2022; O'Neill et al., 2021).

HSC localization and interaction with cells that form part of the endosteal cellular network, which is less vascularized, more hypoxic in nature and enriched in CXCL12 and SCF can also promote quiescence (Ho & Méndez-Ferrer, 2020; Pinho et al., 2018). N-cadherin has been identified as a marker closely linked to bone-lining stroma of mesenchymal origin that is critical for HSC homeostasis. Studies indicate that close association of these cells and long-term repopulating HSCs promoted their interaction with the endosteal surface and overall maintenance depending on external cues such as stress (Ho & Méndez-Ferrer, 2020; Zhao et al., 2019). A study by Zhao et al. (2019) in transgenic mice with conditional deletion of SCF from N-cadherin rich cells under chemotherapeutic stress resulted in a decrease in long-term repopulating HSC number, which suggested the important role they play within the endosteal niche. In contrast, deletion of SCF or CXCL12 in mouse osteoblasts showed little effect on HSC number, but early lymphoid progenitors were sensitive to deletion of CXCL12 from osteoprogenitors (Greenbaum et al., 2013; Zhao et al., 2019). Osteoblasts also secrete factors such as **bone morphogenic protein 4 (BMP-4)**, osteopontin, Jagged-1 and ANG-1 in addition to SCF and CXCL12 that contribute to HSC maintenance. Osteopontin is a glycoprotein that supports the adhesion of HSCs to the endosteal surface, whilst ANG-1 binds to the Tie2 receptor found on HSCs and facilitates their adhesion via N-Cadherin that subsequently leads to quiescence (Arai et al., 2004). Whilst the role of the BMP/SMAD (small mothers against decapentaplegic)-dependant pathway, like other self-renewal pathways, is well understood in embryonic development, the effect of the canonical pathway in adult and foetal liver haematopoiesis seems dispensable in favour of alternative pathways. Recent work in xenograft mice has however suggested that a deficiency in **bone**

FIGURE 1 Microenvironmental interactions pre- and post-malignancy. Intrinsic and extrinsic cues from the surrounding micro-environment drive myeloid skewing and migration away from the endosteal cellular network in the aged niche, with additional secretion of pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α altering haematopoietic stem cell (HSC) functionality and expansion. The malignant micro-environment is characterized by a prevalence of leukaemic stem cells (LSCs) and leukaemic cells with enhanced survival potential due to angiocrine factors (CXCL12, Jagged-1, BMP2/4 and micro RNAs [miR-126/miR-155]) secreted by surrounding stroma. Remodelling of the bone marrow (BM) is partly driven by leukaemic cell production of inflammatory cytokines (CCL3 and IL-6) and adhesion molecules like CD44 and CXCR4. Elevated levels of VEGF stimulate angiogenesis and subsequent disease progression.



morphogenic protein receptor 2 (BMPR-2) reduces the reconstitutive capacity of long-term repopulating HSCs following transplantation with *Bmpr1l*^{-/-} bone marrow cells suggesting an important role of *Bmpr1l* in HSC self-renewal (Singbrant et al., 2010; Warsi et al., 2021). The bone marrow micro-environment has also been shown to influence HSC heterogeneity based on location within cellular networks. As illustrated in Figure 1a, lymphoid biased HSCs associate close to megakaryocytes and endosteal cellular networks, whilst myeloid biased HSCs are localized within the more vascularized regions (Ding & Morrison, 2013; Ho & Méndez-Ferrer, 2020; Maryanovich et al., 2018).

3 | AGEING, CHRONIC INFLAMMATION, AGE-RELATED CLONAL HAEMATOPOIESIS AND PRE-LEUKAEMIA DEVELOPMENT

3.1 | Ageing

Studies indicate that as we age the behaviour of HSCs in the bone marrow niche changes, they locate further away from the endosteal cellular network and lose some of their regenerative capacity. They also display increased mobilization (Xing et al., 2006) and migration in response to cytokine/chemokine stimulation and start to lose their balanced differentiation potential, skewing more towards a myeloid lineage (Geiger et al., 2013; Ho et al., 2019; Ho & Méndez-Ferrer, 2020; Liang et al., 2005; Xing et al., 2006). Aged HSCs also show a reduced capacity to home in the bone marrow in transplant studies (Liang et al., 2005). Studies indicate these changes may originate from cell-intrinsic changes (Dykstra et al., 2011), such as epigenetic deregulation (Chambers et al., 2007), replication stress (Flach et al., 2014) and deficiencies in DNA repair. The decrease in HSC regenerative potential may also be through altered metabolism, deregulated autophagy and protein homeostasis occurring as they age (Ho & Méndez-Ferrer, 2020). However, current studies are revealing that the bone marrow micro-environment may also contribute to HSC ageing. Key changes that occur in the bone marrow micro-environment with age include changes in stromal cell differentiation, resulting in decreased osteogenesis potential. This subsequently leads to a reduction in osteoblasts and an increase in adipocytes. Lower osteopontin secretion to the extracellular matrix (ECM) has been implicated as an important negative regulator of HSC proliferation, which may contribute to the loss in regenerative potential over time (Stier et al., 2005). These changes in differentiation also result in multipotent mesenchymal stromal cells (MSCs) losing their capacity to produce factors such as CXCL12, **insulin-like growth factor 1 (IGF-1)** and SCF, which are essential for normal haematopoietic processes (Ho & Méndez-Ferrer, 2020; Tuljapurkar et al., 2011). The composition of bone marrow stroma also shifts leading to altered ECM protein secretion and changes in the stiffness and architecture of the micro-environment. Functionally, old bone marrow MSCs exhibit reduced colony-forming capacity, decreased expression of HSC niche factors and display higher levels of

senescence (Ergen et al., 2012; Ho & Méndez-Ferrer, 2020). This reduction of the endosteal niche and simultaneous expansion within the central bone marrow niches is thought to help facilitates age-associated myeloid skewing (Geiger et al., 2013).

3.2 | Chronic inflammation

It is still unclear what impact age related chronic inflammatory conditions such as autoimmune diseases, chronic infection, kidney disease and metabolic diseases like atherosclerosis and type II diabetes have on the haematopoietic system. Weight gain is also commonly associated with ageing, with obesity known to promote MSC differentiation into adipocytes within the bone marrow. Adipocytes secrete mediators such as **tumour necrosis factor (TNF)-α** known to suppress haematopoiesis whilst promoting a pro-inflammatory environment (Witkowski et al., 2020). Chronic inflammation results in the release of pro-inflammatory cytokines such as **interferon (IFN)α**, **IFNγ**, **IL-1β**, **IL-6** and **TNF-α**. The pro-inflammatory cytokine, **CC-chemokine ligand 5 (CCL5)**, involved in bone remodelling has also been reported to increase with age. CCL5 can directly contribute to myeloid-biased differentiation at the cost of T cells, suggesting that CCL5 is important for ageing of the haematopoietic system and the micro-environment (Ergen et al., 2012). These changes can affect the mechanical stability of bone structure and normal haematopoiesis due to changes in regulatory cytokines (Tuljapurkar et al., 2011). Changes in the secretome can activate HSCs to proliferate, altering their functionality, as well as initiating changes in the bone marrow micro-environment that promote myeloid skewing, clonal haematopoiesis and potentially the initiation steps towards myeloid malignancies.

The changes in HSC cell fate observed during ageing, results in promotion of the myeloid/megakaryocyte compartment at the expense of the lymphoid compartment. Whether these changes are initiated by intrinsic abnormalities occurring as HSCs age or through bone marrow micro-environment alterations specifically influencing HSCs and directing their cell fate is yet to be fully understood. However, research suggests that they are self-perpetuating with one linked to the other. Skewing towards myeloid cell production means the bone marrow micro-environment becomes more pro-inflammatory, as mature myeloid/megakaryocytic cells are a major source of inflammatory cytokines as illustrated in Figure 1b (Ho & Méndez-Ferrer, 2020). This can result in higher intrinsic expression of ROS in HSCs that consequently affects HSC homeostasis and can lead to genetic damage. Increased ROS can cause DNA damage through double strand breaks and altered repair, resulting in genomic instability and acquisition of genomic changes. This could ultimately result in the development of a haematological disorder/malignancy (Geiger et al., 2013).

3.3 | Age-related clonal haematopoiesis

Age related clonal haematopoiesis (ARCH) could also contribute to the changes observed in HSCs and the bone marrow micro-

environment with age. ARCH results in the clonal expansion of haematopoietic stem and progenitor cells over time. These haematopoietic stem and progenitor cells carry specific somatic mutations, identified in individuals without a clear diagnosis of a haematological malignancy. The inflammatory milieu associated with ageing may favour expansion of haematopoietic stem and progenitor cells with ARCH (Jaiswal et al., 2017; Pardali et al., 2020). ARCH has been associated with 32 different somatic mutations and these are acquired with age and are most prevalent in the over 70 age group. They are associated with increased risk of cardiovascular disease and other diseases including myeloproliferative disorders, as some of the mutations can result in a myeloid cell skewing and a pro-inflammatory phenotype in the body. Over time, this can cause changes to the bone marrow micro-environment and to blood vessels and cardiac function (Pardali et al., 2020). Next-generation sequencing analysis in several large cohorts of individuals, identified mutations in *DNMT3A*, *TET2* (ten-eleven translocation methylcytosine dioxygenase 2), *ASXL1* (putative polycomb group protein) and *Janus kinase 2 (JAK2)* as being associated with an increased risk of coronary heart disease, stroke and increased mortality. Importantly, the prevalence of clonal haematopoiesis (CH) was higher in individuals with early onset of myocardial infarction (Jaiswal et al., 2014, 2017). Xenograft mouse models have confirmed that clonal haematopoiesis driver genes such as *Tet2* and *Dnmt3a* loss alter the behaviour of myeloid cells, leading to increased expression of IL-6 and IL-1 β that promotes atherosclerotic plaque formation (Fuster et al., 2017; Jaiswal et al., 2014, 2017; Sano, Oshima, Wang, Katanasaka, et al., 2018; Sano, Oshima, Wang, MacLauchlan, et al., 2018). The meta-analysis performed by Jaiswal et al. (2017) in the three patient cohorts highlighted that the hazard ratio for CH-associated genes (*DNMT3A*, *TET2* and *ASXL1*) with very distinct modes of action were similar (1.7, 1.9 and 2.0, respectively) for coronary disease. This was an interesting finding that led to recent work investigating whether atherosclerosis promotes clonal haematopoiesis through chronic proliferation of HSCs. Results using atherosclerotic mouse model *Apoe*^{-/-} fed with an atherogenic diet for 10 weeks showed an expansion of the lineage^{-/low} Sca-1⁺ c-kit⁺ haematopoietic stem and progenitor cell population, when compared with mice fed a normal diet. In addition, bone marrow from patients with atherosclerosis also revealed higher numbers of Ki67 cycling HSCs when compared with healthy matched controls. Using mathematical modelling and *Tet2*^{-/-} competitively transplanted mouse models to validate their predictions, this study demonstrated that increased stem cell proliferation expedites somatic evolution and clonal haematopoiesis (Heyde et al., 2021).

There is also a higher frequency of mutations in genes such as *DNMT3A*, *JAK2*, *TET2*, *ASXL1*, *TP53*, *SF3B1* (splicing factor 3B subunit 1, gene), *PPM1D*, *GNAS* and *BCORL1*, associated with myeloproliferative neoplasms (MPNs), myelodysplastic syndrome (MDS), chronic lymphocytic leukaemia (CLL) and acute myeloid leukaemia (AML) (Pardali et al., 2020; Xie et al., 2014). A high proportion of individuals (>90%) with clonal haematopoiesis will not develop a haematological disorder. However, in a small minority, there is an increased risk of additional mutations either occurring, through spontaneous evolution or when

patients undergo chemotherapy for non-myeloid cancers, resulting in the development and progression to a myeloid malignancy. Expansion of DNA-resistant clones are thought to contribute to treatment related myeloid malignancies, with patients more commonly having recurrent mutations in epigenetic modifiers (*DNMT3A*, *TET2* and *ASXL1*) or genes involved in response to DNA damage *TP53*, *PPM1D*, *ATM* and *CHEK2* (reviewed by Bowman et al., 2018). A large study investigating the link between clonal haematopoiesis and myeloid malignancy following cancer treatment, utilized prospective targeted sequencing data (MSK-IMPACT) from 24,146 cancer patients representing 56 different solid primary tumour types identified 30% of patients harboured clonal haematopoiesis mutations. Analysis of paired sample data taken at diagnosis and following treatment provided direct evidence that clonal haematopoiesis mutations lead to a higher incidence of transformation to a myeloid malignancy. The strongest associations were observed for mutations in *TP53* and for mutations in spliceosome genes (*SRSF2* [splicing factor, arginine/serine-rich 2 gene], *U2AF1* [splicing factor U2AF 35 kDa subunit] and *SF3B1* [splicing factor 3B subunit 1]) (Bolton et al., 2020; Bowman et al., 2018).

4 | MYELOID MALIGNANCIES AND IMPACT ON MICRO-ENVIRONMENT INTERACTIONS

There is significant interest in studying the link between chronic inflammation, ageing, clonal haematopoiesis and development of a haematological malignancy. Especially in relation to how the pro-inflammatory phenotype associated with ageing is linked to changes in bone marrow homeostasis and ultimately the predisposition to develop a haematological disease. Changes in HSCs and subsequent remodelling of the bone marrow micro-environment can result in the occurrence of myeloid malignancies, which result in the abnormal expansion of cells associated with the myeloid lineage. Affected cells can also interact with niche cells, remodelling conditions within the bone marrow micro-environment to favour their maintenance and disease progression via fibrosis, increased vascularization and a pro-inflammatory micro-environment at the expense of normal haematopoiesis (summarized in Figure 1c). In some cases, the disease develops as a consequence of remodelling of the bone marrow micro-environment. Conditions that are found within the framework of myeloid malignancies include myeloproliferative neoplasms, myelodysplastic syndrome and acute myeloid leukaemia. This section will present a brief overview of micro-environment interactions within the context of myeloid malignancies.

4.1 | Myeloproliferative neoplasms

The molecular pathogenesis of myeloproliferative neoplasms is based on the occurrence of genetic mutations in HSCs and/or their progenitors. They are further classified into chronic myeloid leukaemia (CML), characterized by expansion of granulocytic cells; polycythaemia vera

(PV), identified by increased erythrocytes and erythroid expansion; essential thrombocythemia (ET) recognized by megakaryocyte proliferation and thrombocytosis; and primary myelofibrosis (PMF) distinguished by bone marrow fibrosis and increased dysplastic megakaryocytes and granulocyte progenitors (Koschmieder et al., 2016; Vainchenker & Kralovics, 2017). PV, ET and PMF are characterized by hyperactivation of janus kinase 2 (JAK2)-signalling as a result of driver mutations in three specific genes: - JAK2, calreticulin gene (CALR) and myeloproliferative leukaemia virus gene oncogene (MPL). With the JAK2V617F mutation being the most common, present in more than 95% of PV patients and 50%–60% of ET and PMF patients. CALR gene mutations are also found in ET and PMF, with MPL mutations occurring in PMF. A less common mutation in JAK2 exon 12 is also found in PV. In addition, 35%–40% of myeloproliferative neoplasms harbour mutations in epigenetic regulators such as TET2, DNMT3A, IDH1/IDH2, EZH2 and ASXL1 (Lundberg et al., 2014). CML originates from the t(9;22) chromosomal translocation leading to the expression of the BCR-ABL oncogene in haematopoietic stem and progenitor cells resulting in a heterogeneous pool of cells consisting of leukaemic stem cells (LSCs) and malignant progenitor cells with enhanced survival and differentiation potential.

A pro-inflammatory micro-environment is a common characteristic of bone marrow remodelling in myeloproliferative neoplasms and shared across other myeloid malignancies (Reviewed in Craver et al., 2018). In myeloproliferative neoplasms the inflammatory cytokines lipocalin-2 and TNF- α are elevated in the plasma of patients with PV, ET and PMF (Fleischman et al., 2011; Lu et al., 2015). Lipocalin-2 produced by myeloid cell stimulates the proliferation of bone marrow stromal cells and subsequent secretion of VEGF, TGF- β 1, BMP2, RUNX2 (runt-related transcription factor 2), osteoprotegerin and collagen type I. This results in increased osteoblastic differentiation and fibrosis over time (Lu et al., 2015). Inflammatory cytokine levels are elevated in both JAK2V617F and MPLW515L-induced mouse models as well as patients with myeloproliferative neoplasms. IL-6 being produced by malignant cells, TNF- α , CCL2 and granulocyte-colony stimulating factor (G-CSF) by both malignant and non-malignant cells, whereas IL-10, IL-12, CXCL9 and CXCL10 are produced by cells within the bone marrow micro-environment (Kleppe et al., 2015; Koschmieder et al., 2016). JAK2V617F mutated haematopoietic progenitors in myeloproliferative neoplasm patients also exhibit augmented release of IL-1 β , which initiates depletion of Nestin⁺ MSCs subsequently reducing the expression of CXCL12 (Arranz et al., 2014). This consequently leads to expansion of erythroid, megakaryocytic and myeloid progenitors as a result of reduced HSC homing that is influenced by CXCL12 expression. Patients with myeloproliferative neoplasms also have increased platelet production and activation leading to the release of pro-inflammatory chemokines such as CCL5, CXCL4 and IL-8 from the activated platelets (Repsold & Joubert, 2021).

Increased cytokine secretion of IL-1 β and IL-6 in CML by leukaemic cells creates a paracrine feedback loop that results in increased myeloid cell expansion and occasional depletion of nestin⁺ stroma at the expense of normal HSCs (Forte et al., 2020; Vainchenker &

Kralovics, 2017). Release of these cytokines has also been suggested to drive osteoblastic differentiation however, conditional deletion of IL-6 genes in a *Scl-tTA::TRE-BCR/ABL* inducible transgenic mouse model had little impact on osteoblast expansion (Schepers et al., 2013). Overproduction of the ligand CCL3 from leukaemic cells and bone marrow MSCs with deregulated expression of PTPN11 (tyrosine-protein phosphatase non-receptor type 11) can also initiate pro-inflammatory cytokine release from monocytes, which further drive myeloproliferative neoplasm disease progression (Baba et al., 2013; Schepers et al., 2013). Myeloproliferative neoplasm patients also have elevated serum levels of IL-6 with studies indicating that overexpression of IL-6 by leukaemic cells can add to the pro-inflammatory signature in normal cells, thus disrupting their homeostatic pattern in maintaining blood cell production (Čokić et al., 2015; Mitroulis et al., 2020; Panteli et al., 2005; Reynaud et al., 2011). *In vivo* (murine models) and *in vitro* (human models) work conducted by Zhang et al. (2012) also showed that CML cells have increased expression of G-CSF compared with normal cells (Zhang et al., 2012). This has been linked to the depletion of CXCL12 that reduces LSC homing and retention in the bone marrow, promoting migration to the spleen in the case of BCR-ABL induced mice and development of extramedullary disease. Osteoblastogenesis in the malignant micro-environment is also induced culminating in a higher proportion of compromised osteoblasts, which secrete IL-1 β , TNF- α and TGF- β . The expansion of osteoblasts has been linked to the occurrence of inflammatory myelofibrotic cells that are predisposed to increased collagen production and down regulation of retention factors such as CXCL12, ANG-1 and SCF (Leimkühler & Schneider, 2019). Development of fibrosis in the micro-environment impairs normal haematopoietic processes and subsequent leads to cell migration to extramedullary sites such as the liver and spleen.

Upregulation of notch ligands like Jagged 1 in osteoblasts and the consequent activation of the Notch signalling pathway drives leukaemogenesis. On the other hand, inhibition of the same pathway through deletion of mind bomb 1 an E3 ubiquitin ligase that facilitates endocytosis of notch ligands can lead to the development of a reversible BCR-ABL⁺ myeloproliferative neoplasm-like disease in mice (Korn & Méndez-Ferrer, 2017). In addition, MSCs and their progenitors release increased levels of BMP ligands BMP-2 and BMP-4 in response to prolonged exposure to tyrosine kinase inhibitor treatment in the case of CML. Overexpression of bone morphogenetic protein receptor type IB (BMPRII) in surrounding leukaemic stem cells makes them more responsive to the upregulated BMP ligands leading to increased expansion (Grockowiak et al., 2017; Toofan et al., 2018). *In-vitro* culture of LSCs in the presence of BMP-2 and 4 resulted in sustained long-term colony forming potential inferring that the BMP pathway preserves the self-renewal capabilities of the leukaemic stem cells, thus driving disease progression.

MicroRNAs (miRNAs) have been observed to modulate the development of myeloproliferative neoplasms and behaviour of CML LSCs. There is evidence showing that loss of Notch signalling in endothelial cells of myelofibrosis patients leads to activation of miR-155 that results in myeloid expansion as a consequence of overexpression of

G-CSF and TNF- α following de-repression of nuclear factor κ B (NF κ B) (Mosteo et al., 2021). MiR-155 can also be upregulated in CML LSCs with downstream effects showing a blockade of TGF β and BMP pathway signals giving the affected cells a survival advantage over normal cells (Anelli et al., 2021; Korn & Méndez-Ferrer, 2017). Exosome-mediated exchange of miR-126 between leukaemic cell and endothelial cells downregulates key adhesion and migration factors in the niche possibly driving the potential of extramedullary haematopoiesis in secondary organs rich in the depleted chemoattractants. Increasing levels of proangiogenic factors including VEGF, **fibroblast growth factor 2 (FGF-2)** and **hepatocyte growth factor (HGF)** have also been observed in myeloid malignancies. In the context of myeloproliferative neoplasms, vascularization of the bone marrow is a common key feature with deregulation of the VEGF signalling pathway promoting vascular density.

4.2 | Acute myeloid leukaemia

Remodelling of the bone marrow micro-environment is a key feature in the initiation and progression of acute myeloid leukaemia that is characterized by the expansion of immature myeloid precursors (Mosteo et al., 2021). Increase of disease burden in the bone marrow of xenograft mice following transplantation of acute myeloid leukaemia cells has been correlated with remodelling suggesting that there is transition towards a leukaemic-supportive niche at the expense of normal HSCs (Duarte et al., 2018; Hanoun et al., 2014). Specifically, there is depletion of vessels and stroma in the vascular-endosteal transition zone during early engraftment by leukaemic cells. As engraftment by leukaemic cells increased, the osteoblast population significantly decreased and this has been suggested to be a consequence depletion of the endothelial cells that play a role in osteoblastogenesis (Duarte et al., 2018; Witkowski et al., 2020). These observations were consistent with single cell RNA-sequencing data of stroma from acute myeloid leukaemia xenografts (Baryawno et al., 2019) that showed a reduction in expression of genes associated with osteoblast maturation.

A major hurdle constantly encountered in the treatment of AML leading to poor disease prognosis is resistance conferred by leukaemic blast interaction with the bone marrow micro-environment. Increased surface expression of the chemokine CXCR4 in acute myeloid leukaemia leukaemic stem cells has been accredited with deregulating essential pro-survival and proliferation pathways such as the PI3K/Akt and ERK1/2 pathways (Passaro et al., 2021; Ramakrishnan et al., 2020). As highlighted earlier, bone marrow stroma serve as a key source of CXCL12 the ligand for CXCR4, which creates a gradient against that leukaemic cells are drawn into more protective areas of the micro-environment. **E-selectin (CD62E)** a vascular adhesion molecule found on endothelial cells has also been shown to desensitize leukaemic cells to chemotherapeutic targeting (Barbier et al., 2020). AML cells have elevated secretion of pro-inflammatory cytokines like TNF- α that induces E-selectin overexpression and adhesion to endothelial cells via CD44 and other adhesion molecules. Such activity dysregulates Akt, NF- κ B and mTOR signalling in leukaemic cells that are known to

play a role in chemoresistance (Barbier et al., 2020). CD44 has also been noted to induce proliferation and differentiation in leukaemic cells with subsequent overexpression of transcriptional target survivin known to act as an inhibitor of apoptosis being observed in acute myeloid leukaemia. Recent work also suggests that the inhibition of CD44 in AML cells provides an avenue to possibly circumvent CXCL12 driven resistance to **Bcl-2** inhibitor **venetoclax** as CD44 acts as a co-receptor for CXCR4 (Yu et al., 2021).

As in the case of the other myeloid malignancies, a pro-inflammatory micro-environment plays a crucial role in AML development and progression. *In vitro* co-culture of AML blasts with endothelial cells has led to leukaemic cell expansion as a direct consequence of key pro-inflammatory cytokine release by the former. Elevation of IL-6 amongst other growth factors in the plasma of AML patients has been associated with a poorer disease prognosis compared with those with lower levels, with research suggesting that it infers anti-apoptotic chemoresistance in AML blasts (Stevens et al., 2017; Zhang et al., 2020).

Bone marrow angiogenesis owing to increased secretion of key proangiogenic factors like VEGF drives disease progression. The VEGF signalling pathway is upregulated during foetal development and subsequently decreases after birth therefore elevated levels of its associated factors has been proposed to serve as a biomarker for disease initiation and progression. AML patients have been identified to harbour high levels of VEGF that induces angiogenesis and prevents apoptosis of leukaemic cells (Duarte et al., 2018; Méndez-Ferrer et al., 2020). In addition, increased vascularization provides another source of nutrients, which further potentiate survival and proliferation of malignant cells. Production of angiocrine factors such as **granulocyte-macrophage colony stimulating factor (GM-CSF)**, **G-CSF** and IL-6, which drive proliferation are initiated by VEGF stimulation of endothelial cells and associated with a more aggressive form of disease (Duarte et al., 2018; Passaro et al., 2017).

Mitochondrial transfer between bone marrow stroma and AML cells via nanotubules has been suggested to confer a metabolic and survival advantage to the latter when stress like chemotherapeutic targeting is induced. Studies comparing mitochondrial uptake between AML CD34⁺ and normal CD34⁺ cells following *in vitro* co-culture on stromal derived cell lines showed increased mitochondrial probe uptake in the leukaemic cells and improved resistance to chemotherapeutic agents (Moschoi et al., 2016). Another study by Forte et al. (2020) suggested that Nestin⁺ bone marrow MSCs serve as crucial source of mitochondria with their subsequent deletion leading to reduced disease burden in mice (Forte et al., 2020). This contrasts with the established depletion of osteoprogenitor cells, which leads to a decrease in bone-lining formation and more aggressive disease phenotype (Duarte et al., 2018).

5 | IN VITRO MODELLING OF THE BONE MARROW MICRO-ENVIRONMENT

In vitro modelling of the bone marrow micro-environment is emerging to be an important concept in biomedical research. It has been observed that recapitulation of the bone marrow micro-environment

properties through 3D *in vitro* systems can better inform our understanding of the role played by the bone marrow in steady state haematopoiesis, malignancy development and subsequent therapeutic targeting. Current *in vivo* systems involve the use of mouse models, which are modified to express different disease phenotypes through retroviral insertion (transgenic) or transplantation of human haematopoietic cells in immunocompromised mice (xenografts). Whilst these are valuable models for research, they do not fully recapitulate the architecture of the human bone marrow micro-environment and impose a substantial cost in the research and drug screening process (Kotha et al., 2018; Rosalem et al., 2020). This has put forward the need to develop humanized models that incorporate elements that best mimic certain attributes of the micro-environment including cell-to-cell interactions, ECM composition and availability of extrinsic molecular cues from growth factors and cytokines.

Co-culture of malignant cells with bone marrow stroma and cells in conventional 2D culture involves sustaining cell growth in a monolayer on a polystyrene surface. This facilitates investigating single micro-environment cell contributions to HSC or LSC maintenance through cell-to-cell interactions and provision of extrinsic molecular cues however research has shown that stromal cells lose their defining properties (quiescence and differentiation potential) in monolayer (Rosalem et al., 2020). The use of spheroids offers an avenue for 3D cell culture with stromal cells being exposed to conditions that promote their conformation into aggregates that exhibit enhanced quiescence, stemness and expression of VEGF, HGF and CXCL12 amongst other factors that sustain HSCs and play an important role in myeloid disease progression (Ho & Méndez-Ferrer, 2020; Sieber et al., 2018).

Methods currently used for spheroid formation include:- hanging drop method, this relies on gravitational force applied to a drop of cells inverted in a culture vessel; magnetic levitation where cells take up magnetic iron oxide nanoparticles via endocytosis followed by exposure to an external magnetic field; use of low attachment plates that owing to unique surface coatings, force cells to remain in suspension followed by their forming similar sized spheroids if microwells are present and microfluidic devices that provide a high throughput method of making spheroids. However, if no scaffold is provided for their growth, the spheroids are forced to produce their own ECM, containing collagen, hyaluronan and fibronectins (Krause et al., 2013; Rosalem et al., 2020). The ECM is an essential element of the bone marrow micro-environment that modulates extrinsic cues by acting as a reservoir for growth factors and has been noted to impact response to chemotherapeutic targeting. Embedding spheroids into natural or synthetic scaffolds like hydrogel, matrigel or alginate (suggested to best mimic bone marrow stiffness and architecture (Rosalem et al., 2020) supplemented with the necessary growth factors have been used to mimic this aspect of the bone marrow micro-environment. The use of microfluidic devices with in-built scaffolds is an approach that can also be adapted into a perfused system by introduction of pumps and channels that draw fluid through the system allowing for the replenishment of nutrients or growth factors and consequently long-term *in vitro* culture (Sui et al., 2022). Alternative approaches to modelling of the bone marrow micro-environment involving use of 3D printing to recreate the bone architecture like that of trabecular bone, further support long term culture and differential potential of HSCs *in vitro* (Sieber et al., 2018). In addition, this

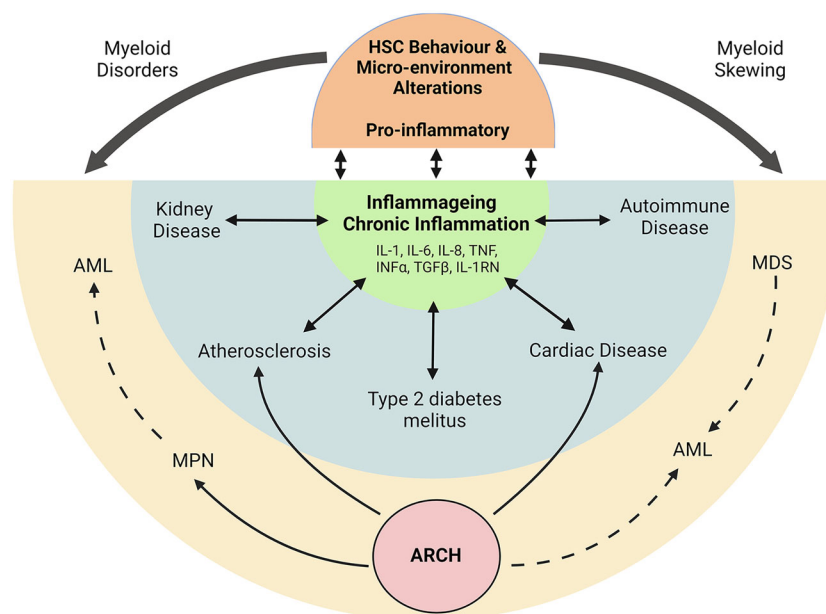


FIGURE 2 Proposed relationship between inflammaging. Haematopoietic stem cell (HSC) behaviour and micro-environment alterations. Autoimmune disease, kidney disease, type 2 diabetes, cardiac disease and atherosclerosis are examples of chronic inflammatory disorders that can occur following sustained pro-inflammatory cytokine release as a consequence of age. On the other hand, it is possible that the intrinsic changes in HSCs that are more prevalent with age can kick-start pro-inflammatory cytokine release creating a pro-inflammatory micro-environment. Development of age-related clonal haematopoiesis (ARCH), and inflammation are associated with an increased risk of developing myeloid disorders and malignancies such as myeloproliferative neoplasm (MPN), myelodysplastic syndrome (MDS) and leukaemic cells to (AML). The occurrence of ARCH can also predispose affected individuals to chronic inflammatory disorders like atherosclerosis and cardiac disease.

creates an avenue for the incorporation of more than one supportive cell type, which would better depict multi-cellular contributions to HSC/LSC maintenance.

6 | FUTURE DIRECTION

The prevalence of ARCH, myeloid disorders and myeloid malignancies all significantly rise with age. There is compelling evidence to support a pro-inflammatory environment as having a strong impact on HSC and MSC function leading to bone marrow remodelling and myeloid skewing. Most elderly individuals develop ‘inflammageing’, a strong risk factor for developing several of the chronic inflammatory conditions that are highly prevalent as we age. Inflammageing is characterized by high circulating levels of **IL-1**, **interleukin 1 receptor, type II (IL-1RN)**, IL-6, IL-8, **IL-13**, **IL-18**, C-reactive protein (CRP), **serum amyloid A**, IFN α , IFN β , TGF β , TNF and its soluble receptors **TNFR1A**, **TNFR1B** (Ferrucci & Fabbri, 2018). Whether the release of this pro-inflammatory milieu is responsible for initiating HSC and bone marrow alterations that lead to myeloid skewing and a pre-disposition to develop age-related clonal haematopoiesis and ultimately myeloid disorders over time remains to be elucidated. Figure 2 shows the proposed relationship between inflammageing, HSC behaviour and micro-environment alterations. Longitudinal studies are therefore required to ascertain whether there is a link between inflammageing and changes in the haematopoietic system. Development of advanced 3D models of the bone marrow micro-environment will help facilitate studies to elucidate the impact of inflammageing on the haematopoietic system. Studies incorporating young and old HSCs within the niche will aid our understanding of how the pro-inflammatory milieu associated with inflammageing alters HSCs and mesenchymal stromal cells behaviour, their regenerative capacity, migration and differentiation potential. This is fundamental to our understanding of how the haematopoietic system alters with age and the initiating stages that lead to myeloid malignancies.

6.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY <http://www.guidetopharmacology.org> and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos, et al., 2021; Alexander, Fabbro, et al., 2021a,b).

AUTHOR CONTRIBUTIONS

K.N. prepared the figures and wrote the manuscript. H.W. wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests associated with the manuscript.

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