Towards personalised medicine for cancer
From initial therapy to follow-up
Molenaar, R.J.

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Chapter 16: Haematopoietic stem cell niches, leukaemic cells and propagation of therapy-related myeloid neoplasms


Introduction
Leukaemias are haematologic malignancies that are characterised by an overgrowth of white blood cells and are caused by increased monoclonal cellular proliferation in the bone marrow, resulting from (epi)genetic changes in either haematopoietic stem cells (HSC), lymphoid or myeloid progenitor cells. HSCs are at the top of the haematological hierarchy as multipotent stem cells with self-renewal capacity that give rise to various types of progenitor cells and ultimately the production of mature erythrocytes, megakaryocytes, myeloid cells, and lymphocytes. Since mature blood cells are short-lived, HSCs are required throughout life to replenish progenitor and precursor cells.

The stem cell niche is a specialised microenvironment that helps to maintain stem cell characteristics. In the bone marrow, HSCs reside in bone marrow niches, which play an important role in regulating the behaviour of HSCs with respect to homeostasis and stress responses. The proliferation and differentiation of haematopoietic progenitor cells and their daughter cells are sufficient to maintain the homeostatic haematopoiesis under normal conditions, consisting of the production of one trillion (10^12) cells per day in healthy human adult red bone marrow. In such circumstances, HSCs are in a dormant quiescent state to prevent stem cell exhaustion. Blood is a tissue with one of the highest regenerative capacities and the prevention of HSC exhaustion is extremely important considering the necessity to upregulate haematopoiesis in case of blood loss due to tissue damage or haematopoietic stress. In these contexts, HSCs are forced to leave the niches to differentiate and proliferate in order to maintain haematopoiesis.

Bone marrow niches
HSCs are currently considered to reside in one of three types of bone marrow niches: endosteal, reticular or perivascular niches. Some cell types, proteins and factors are shared between the three types of niches, others are considered to be unique for a specific niche type. A common factor of all types of niches is that they tightly regulate whether HSCs migrate into niches, are kept inside the niches or migrate out of the niches. This is crucial, because HSC stemness and quiescence are promoted in the niches, whereas migration out of the niches enables HSC differentiation and proliferation.

The endosteal niche. The endosteum is the interface between bone and bone marrow which mainly consists of osteoblasts and, to a lesser extent, osteoclasts. Mature osteoblasts produce extracellular matrix (ECM) and are responsible for bone formation whereas osteoclasts resorb bone and thus function in bone remodelling. The endosteal niche is associated with the endosteum and facilitates interactions between osteoblasts and HSCs, which keeps HSCs quiescent. The main cell types of the endosteal niche that maintain stemness and quiescence of HSCs and affect their homing and mobilisation are osteoblasts, osteoclasts and osteomacs. Osteopontin (OPN) is a matrix glycoprotein with cytokine and chemokine properties which is secreted by osteoblasts and binds to
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HSCs via CD44 or integrins containing a β1 subunit. This results in homing of HSCs in the endosteal niche. Furthermore, the chemoattractant SDF1α (CXCL12) is produced by osteoblasts under hypoxic conditions and interacts with its receptor, CXCR4, which is expressed on HSCs, resulting in the retention of HSCs in the endosteal niche.

The reticular niche. The name of reticular niches is derived from reticular cells, which are stromal cells that produce large amounts of SDF-1α (CXCL12) and are therefore called CXCL12-abundant reticular (CAR) cells. CAR cells are scattered throughout the bone marrow and have long processes that create a network, making contact with 97% of HSCs in perivascular niches and 100% of HSCs in endosteal niches. The production of stem cell factor (SCF) and SDF-1α are crucial to keep HSCs in an undifferentiated state. When production of SDF1α and SCF by CAR cells is downregulated, which occurs during haematopoietic stress, HSCs migrate out of the niche.

The perivascular niche. HSCs and endothelial cells are derived from the same embryonic progenitor cells, called haemangioblasts. Vasculogenesis and haematopoiesis occur concurrently during embryonal development. After embryonic development, HSCs are attached to the endothelium of sinusoids in the central part of perivascular HSC niches. Endothelial cells express VCAM-1, which interacts with the integrin very late antigen-4 (VLA-4) on the membrane of HSCs, and this ligand-receptor interaction facilitates retention of HSCs in perivascular niches.

Leukaemic cells hijack bone marrow niches

Leukaemias are haematologic malignancies that are classified as either lymphoid or myeloid and can be chronic or acute. AML is a heterogeneous disease that is characterised by expansion of HSCs and progenitor cells with subclonal genotypes. The subclonal evolution results in refractory clones that are able to resist chemotherapy, survive and repopulate. Although various types of chemotherapy are available for treatment of AML, therapy resistance is a major problem. Complete remission is achieved in 70-80% of patients younger than 60 years, but ultimately relapse occurs in the majority of patients and the overall 5-year survival is 40-45%. The prognosis of patients who are 60 years or older is worse with a median survival of less than one year.

There is a complex bidirectional crosstalk between bone marrow niches and AML cells. After invasion of bone marrow niches by LSCs, a decrease in the production of HSC-supportive factors has been noted, while the production of LSC-supportive factors arose in bone marrow niches. This led us to propose a concept wherein AML cells hijack bone marrow niches which leads to the acquisition of stem cell characteristics, such as quiescence and resistance to chemotherapy. After this transformation of AML cells into LSCs, bone marrow niches protect LSCs from cell-cycle targeting chemotherapeutic agents, such as Ara-C, azacitidine and decitabine. Altogether, AML cells are able to hijack, occupy and manipulate bone marrow niches and outcompete normal HSCs, protecting LSCs while worsening patient survival. Promising therapeutic approaches to eradicate LSCs include strategies to disrupt interactions between LSCs and HSC niches, to prevent dedifferentiation of AML cells into LSCs and to induce differentiation and proliferation of LSCs and sensitise AML cells to chemotherapy. These novel therapeutic strategies have been reviewed elsewhere in detail.

There is also extensive crosstalk between bone marrow niches (e.g. HSC niches and mesenchymal stem cell [MSC] niches) and cells of a disease called myelodysplastic syndrome (MDS), the benign lesion that can precede AML. MDS is characterised by ineffective haematopoiesis, progressive bone marrow failure and variable risk of progression to AML. Bone marrow niches in MDS harbour many aberrations, because MSCs that are isolated from human MDS proliferate at a reduced rate, have increased senescence, a decreased efficiency to undergo osteogenic differentiation, an impaired ability to support normal HSCs in long-term in vitro culture, and perturbed cytokine secretion. MSCs give rise to endothelial cells, smooth muscle cells and other supportive cells of the bone marrow vasculature and MSC niches are present in the adventitia of arterioles. Furthermore, the successful
Engraftment of human MDS HSCs in xenografts depends on co-engraftment of MSCs from the same
MDS patients, which indicates that bone marrow niche-MDS interactions are essential for MDS
graftment and that may contribute to disease progression.\textsuperscript{538} Another argument is presented by
gene-targeting studies that generated a severe MDS-like syndrome in mice after osteolineage cell-
specific deletion of Dicer 1, an endonuclease that is essential for correct processing of microRNAs and
the function of osteolineage cells.\textsuperscript{541}

**Therapy-related myeloid neoplasms as a distinct disease entity**

Second cancers occurring in patients treated for first cancers is a rare but devastating complication.
Whether these second cancers can be attributed to exposure to radiation and or cytotoxic
chemotherapy during treatment of the first cancer is an area of intense debate.\textsuperscript{542} Until now,
differences in prognosis have not been found in solid cancers occurring as first or second cancers, but
for haematologic malignancies this determination has significant prognostic as well as treatment
implications.\textsuperscript{542-544} Particularly, for patients with myeloid malignancies, and notably AML and MDS
that occur after treatment for an unrelated primary cancer, there is sufficient epidemiologic evidence
supported by emerging biological data to characterise these myeloid malignancies as treatment-
related.

Because of this association with prior therapies, these malignancies are recognised as a distinct
disease entity, therapy-related myeloid neoplasms (t-MN), in the World Health Organization
omenclature of haematological neoplasms.\textsuperscript{543,544} t-MN carry the worst prognosis of all types of
leukaemias. On the other hand, second cancer risk assessment studies that focused on the sites in the
body of different primary cancers have failed to show a consistent association between lymphoid
malignancies or multiple myeloma and prior treatments. Epidemiologic studies also suggest that
there is a differential risk profile for developing second haematological cancers depending on the
primary treatment modality received. Exposure to ionizing radiation causes a roughly similar
increase in the risk of AML, MDS and chronic myeloid leukaemia. However, when compared to
chemotherapy the risk for t-AML and t-MDS from prior radiation is substantially lower, whereas the
risk to develop CML following exposure to chemotherapy is clinically insignificant.

The precise aetiology of t-MN has not been completely elucidated yet. It seems logical that DNA-
damaging agents induce mutations in HSCs and progenitor cells that may result in a proliferative
advantage of these now-clonal haematopoietic cells, which can ultimately lead to malignant disease.
In that case, the question is why specifically t-MN occur after cytotoxic treatment and why therapy-
related malignancies of other rapidly dividing cells, such as those in the skin or the gut, are rare. An
alternative explanation to this conundrum is that the unique complex interactions between the bone
marrow niche and HSCs can be disturbed by cytotoxic agents. Functional defects of the bone marrow
niche induced by cytotoxic agents can contribute to the development of t-MN via this mechanism.

**The bone marrow niche in the formation of therapy-related myeloid neoplasms**

Cytotoxic therapy has been shown to cause a pro-inflammatory response and the release of
inflammatory cytokines in the bone marrow microenvironment. These effects include the release of,
among others, tumour necrosis factor (TNF), transforming growth factor β (TGF-β), and interleukin-
6 (IL-6) and are accompanied by the increased release of reactive oxygen species (ROS) by MSCs. This
results in genotoxic damage to HSCs and remodelling of the bone marrow niche.\textsuperscript{542} Investigations on
the role of the bone marrow niche in the initiation of t-MN have been performed in mouse models
with HSCs that were Trp53-/- (the mouse analogue of the human \textit{TP53} gene) and haploinsufficient for
chromosome arm 5q, which is frequently deleted in t-MN. In this model, a high frequency of t-MN was
observed after treatment of both HSCs and the bone marrow stroma with an DNA-alkylating agent,
but not after treatment of either HSCs or the bone marrow stroma alone. The necessity of concurrent
cytotoxic treatment of the bone marrow stroma together with HSCs to propagate t-MN suggests a role
for bidirectional interactions between HSCs and MSCs in myeloid diseases.\textsuperscript{545}
Cytotoxic stress induced by cytotoxic therapy, toxins, infection and/or inflammation reduce the size of the HSC pool, selects for mutant HSCs clones and contributes to subsequent disease development.\textsuperscript{542} However, it has been proposed that the aforementioned stress inducers also impact bone marrow niches in order to create an environment that exerts a selective pressure for pre-existing mutant HSC clones at the expense of normal, healthy HSCs.\textsuperscript{546} In mouse bone marrow chimaeras, DNA damage-inducing agents led to a selective clonal advantage of HSCs and progenitor cells that were deficient in Trp53 activity as a result of growth arrest of cells with normal p53.\textsuperscript{547} Critically, the expression of proteins that are involved in interactions between HSCs and bone marrow niches (\textit{e.g.} adhesion and migration) was altered in Trp53\textsuperscript{−/−} HSCs with a clonal selective advantage. This suggests a role for Trp53 levels in the regulation of gene expression in HSCs that can affect interactions with the bone marrow niche. This is supported by sequencing data from patients with t-AML, in which \textit{TP53}-mutated preleukaemic clones were often already present during chemotherapy or radiotherapy for the first, unrelated, cancer.\textsuperscript{496} Indeed, somatic \textit{TP53} mutations are a hallmark of preleukaemic stem cells in AML.\textsuperscript{548,549}

**Conclusions**

In summary, studies in mouse models and in patients have provided evidence for a model where clonal selection of pre-existing mutant HSC clones is induced by treatment with cytotoxic agents and that this selective pressure is affected by the intricate bidirectional crosstalk between healthy HSCs, mutant HSCs and the bone marrow niche. This suggests that mutations in HSCs, induced by cytotoxic agents used in the treatment for an unrelated other cancer, are not the only driver of t-MN development. Specifically, changes in bone marrow niches that form niches for malignant, leukaemic cells contribute directly to the development of t-MN.