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The effects of leukemia on niche – Hematopoietic Stem Cell interactions.

Bachelor thesis, Oncology research

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08-07-2016

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Hematopoietic stem cells (HSCs) are localised in a bone marrow niche, nearby the trabecular bone or perivascular areas like blood vessels. Important characteristics of HSCs like quiescence, self-renewal and differentiation are maintained by the diverse cells associated with the niche. The most important niche cells are osteolineage cells, mesenchymal stem cells and endothelial cells, which can regulate HSCs via secreted factors or via direct interactions with plasma membrane proteins expressed on HSCs. Furthermore also the nervous system in the niche has recently been shown to be important for maintenance of HSCs.

Leukemia is a disease in which hematopoiesis, the formation of blood, is severely impaired. Leukemia can be caused by a number of genetic mutations that accumulate in the progenitor cell or HSC to form a leukemic stem cell. Besides intracellular changes in LSCs that impair normal hematopoiesis, the LSC is also able to impact on and even change the niche. This can for instance occur by secreting factors such as inflammatory cytokines, or via upregulation of CXCL12, a molecule that affects the localisation of HSCs. Ultimately, these changes in the niche might favor leukemogenesis over normal hematopoiesis.

INHOUDSOPGAVE

THE EFFECT OF LEUKEMIA ON NICHE – HEMATOPOIETIC STEM CELL INTERACTIONS.

ABBVREVIATIONS

HSC	Hematopoietic stem cell
MSC	Mesenchymal stem cell, mesenchymal stromal cell
LSC	Leukemia stem cell
OBL	Osteoblastic lineage cell
SDF	Stromal derived factor (CXCL12)
SCF	Stem cell factor
TPO	Trombopoietin
Angpt	Angiopoietin

INTRODUCTION:

The hematopoietic stem cell (HSC) is a multipotent stem cell of the blood system, which means that it can give rise to all types of blood cells (figure 1). The HSC is also able to self-renew, a process in which a new identical HSC will be formed (Alberts, 2015), which is a unique characteristic for HSCs. Cell cycle quiescence is a third hallmark for these type of cells. HSCs are thought to divide only once a month or even less, and this is a key behaviour of stem cells most likely protects them from cellular stresses and exhaustion (Kunisaki, 2014). Appropriate control over self-renewal and differentiation is critically important to maintain normal homeostasis of blood cell formation, loss of this control might lead to cancer.

To regulate the growth of different blood cells of the hematopoietic system accurately, this system contains complex control mechanisms. The control of HSCs is regulated within the bone marrow, specifically the niche. About 1 in 50.000/100.000 cells in the bone marrow is an HSC. HSCs are dependent on signals coming from niche cells to maintain their stem cell character. The various signalling molecules in the niche probably influence the mature cell type into which the HSC will develop (Alberts, 2015).

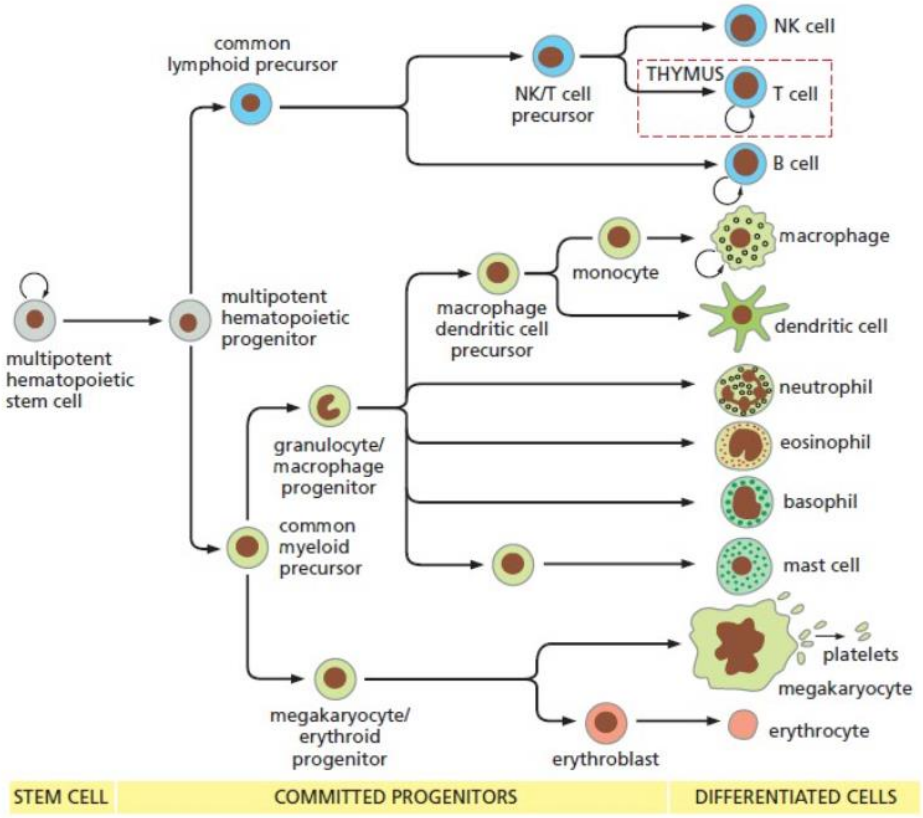

FIGURE 1 - HEMATOPOIESIS OF HSCS (DOMEN, 2011)

The hematopoietic stem cell niche is composed of many different cell types, which all work together to maintain the tissue integrity and thereby control HSC self-renewal, quiescence and differentiation (Hoggatt, 2016). The effects of the niche on HSCs and which exact molecules and cell types are involved, is a hot topic in research at the moment. The effect of leukemic cells on the bone marrow niche has been studied also in the last couple of years. The current paper discussed our current understanding of the most important components of the hematopoietic stem cell niche, and how the niche regulates normal hematopoiesis and leukemogenesis.

There is a big debate ongoing in which the relative contribution to leukemia of intrinsic alterations (e.g. genetic mutations and/or epigenetic alterations within LSCs) as well as extrinsic factors (the niche) are discussed. Mutations in the stem cells, forming LSCs, obviously impact on the biology of the LSC itself, but can also induce expression of other proteins that impact on other cells within the niche. In this paper the interactions between the niche and the stem cell and the effect of these interactions on the stem cells will be discussed, in normal compared to leukemic situations. First the normal niche will be described: the cells in this niche and their expression of different factors which all have a diverse effects on stem cells. Secondly, the process of leukemia in the niche will be defined. The changes of the niche in a disease environment will be discussed and finally the effect of leukemia stem cells on the niche will be summarized as well.

WHAT DOES A NORMAL NICHE LOOK LIKE?

In this chapter the normal niche will be described. At the moment, we assume the presence of two types of niches, the endosteal and the vascular niche. The localisation of these niches will be defined in the first paragraph. Next, the presence of different types of cells in the niche, which are all important

for maintaining different characteristics of stem cells, will be summarized. The last section will review the factors, like chemokines and cytokines, which are expressed by the specific niche cells.

TWO TYPES OF NICHES

The hematopoietic niche is a local tissue microenvironment that regulates, directly maintains and controls processes like differentiation, migration, survival and apoptosis of the HSC or progenitor cell. The niche located near trabecular bone is called the endosteal niche. Different types of cells, like endothelial and mesenchymal stromal cells create the niche (Morrison and Scadden, 2015), these will be discussed later.

The exact location of stem cells and determination which cells are located next to the HSCs and thereby regulate HSC maintenance and other HSC processes have been investigated by the Morrison and Scadden labs. Transplanted HSCs migrated to nearby the endosteum in the bone marrow, but did not migrate adjacent to osteolineage cells. HSCs migrated within 5 cell diameter of a sinusoid. This was indicated by use of positive and negative staining for CD150, CD48 and CD41. Even the most quiescent HSCs could be localised by use of this method (Morrison and Scadden, 2015). Other cells, expressing signalling lymphocyte activation molecules, were localised adjacent to bone marrow sinusoidal blood vessels and these cells also showed nearby interactions with transplanted HSCs. These experiments suggest the presence of a vascular niche through a frequent localisation of HSCs adjacent to blood vessels (Hoggatt, 2016). The existence of a perivascular niche is supported by the fact that endothelial cells are able to regulate the process of hematopoiesis. These endothelial cells are localised adjacent to the blood vessels. The known influence of endothelial cells on hematopoiesis results in the assumption that the HSCs are localised near these endothelial cells, as it may be in an perivascular niche.

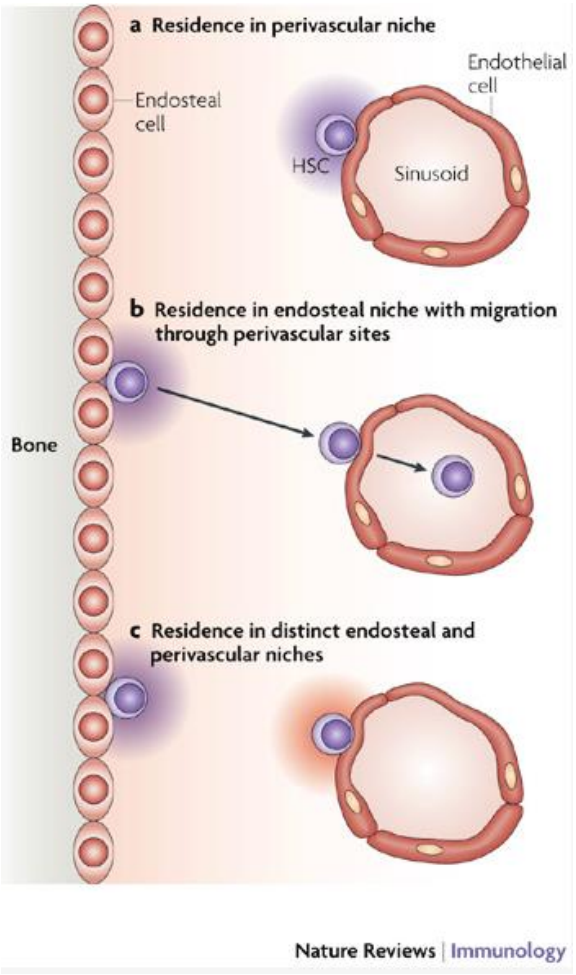

FIGURE 2 – LOCALISATION OF HSCS IN THE BONE MARROW, ENDOSTEAL OR PERIVASCULAR NICHE (KIEL AND MORRISON, 2008).

Figure 2 reviews possible reasons for the observation of HSCs in the perivascular site. *A.* When the localisation of HSCs is examined using validated markers, many HSCs are found adjacent to sinusoids. Thus, HSCs may reside perivascular niches. *B.* HSCs may reside in endosteal niches but are often observed in perivascular sites through their frequent migration through circulation. *C.* HSCs may reside in endosteal and perivascular niches. Both niches may have different roles in the regulation of HSCs (Kiel and Morrison).

A last option for HSC maintenance in the perivascular niche is a possible overlapping region between the endosteal and vascular niche. High vascularisation of the endosteal region and close association of HSCs with blood vessels supports the presence of an overlapping region (Ellis, 2011). The existence of

an endosteal and perivascular niche is supported by these theories but the exact effect of the different niches on HSCs maintenance is still unclear.

CELLS IN THE NICHE

Different types of cells are involved in the maintenance and quiescence of HSCs in the niche. Mature descendants of the stem cells are able to regulate the niche (Sato, 2011). These cell types include osteolineage cells, macrophages, stromal mesenchymal stem- and endothelial cells and will be discussed in detail here (figure 3).

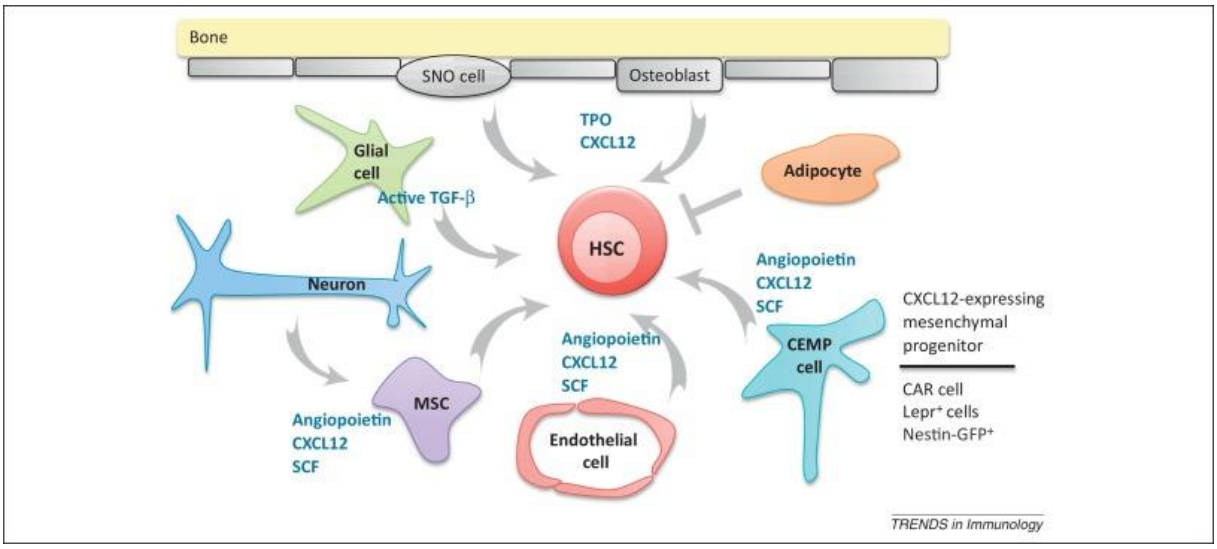

FIGURE 3 - TYPES OF CELLS WHICH AFFECT HSCS IN THE BONE MARROW NICHE (ANTHONY, 2014)

The first type of cells known to have an influence on HSCs and progenitor cells are the osteolineage cells (Morrison and Scadden, 2016). Osteolineage cells involve osteoblasts and osteoclast cells. These types of cells are involved in the regulation of bone formation and resorption within the endosteal bone marrow niche. Besides that, osteoblast and osteoclast cells are important for the endosteal niche in the regulation of HSCs characteristics (Schepers and Campbell, 2015).

Osteoblastic lineage cells (OBL) express some markers, for example CD51 and N-cadherin, an activated leukocyte cell adhesion molecule (Schepers and Campbell, 2015). Nowadays, studies try to clarify if these osteolineage cells do have a direct or indirect effect on HSCs. Imaging studies, using validated markers of stem cells, found few HSCs in contact with osteolineage cells and a deletion for osteoblastic cells resulted in no effect on the frequency of HSC occurrence (Morrison and Scadden, 2015). Meanwhile, a study by Mansour et al showed that a knockout (oc/oc mice) for osteoclast activity results in an increased proportion of mesenchymal progenitors and a defective HSC niche association, but reduced osteoblastic differentiation (Mansour, 2012). This knock-out model has been obtained by crossing heterozygous oc/+ mice. These oc/+ mice do have osteolineage cells but these are ineffective. The mutations are autosomal recessive, consisting of a 1.6 kb deletion in the Tvirg1 gene encoding for the vacuolar proton pomp. In oc/oc mice this pomp is present in endosomal vesicles of the osteolineage cells, but absent at the cell surface (Blin-Wakkach, 2004). The defect in the niche lead to damaged HSC homing (Mansour 2012), indicating that the absence of osteoclasts results in a defective niche. Second, a depletion of OBLs resulted in a reduction in HSC numbers, and an increase of OBL was related to an increase of the HSC pool size in the bone marrow. The effects of OBLs were partly caused by direct cell-cell interactions with stem cells. Different factors are expressed during this adherence and the OBL can thereby affect the HSC (Mansour, 2012). A study by Taichman and colleagues demonstrated that osteolineage cells produce many supportive growth factors, like G-CSF,

IL-1, Il-6 and TGF-β (Taichman, 1994), indicating that osteoblastic cells directly participate in the hematopoiesis. The results of the first and second mentioned experiments are somewhat contradictory. Further research in the effect of OBL in the niche should make a clear image of the role of OBL in HSC maintenance.

Stromal mesenchymal stem cells (MSCs) reside perivascular around arteriole and sinusoid vessels. These types of cells are in direct contact with endothelial cells. Migration of these MSCs to the endosteal surface will be followed by differentiation into osteoblasts (Morrison and Scadden, 2015). The undifferentiated MSCs, organised around the blood vessels, promote HSC maintenance by expressing different types of factors, like CXCL12 and SCF, which are important for controlling HSC proliferation (Schepers and Campbell, 2015). So, MSCs have a specialised function in controlling HSC cell cycle and migration activity. A deletion of this type of cell will cause loss of all HSC in the adult bone marrow niche (Zhou, 2015), due to a lack of expression of the different factors. A part of the MSCs are Nestin+ MSCs, which are strictly perivascular and are localised in the central areas of the bone marrow. Interaction between cells in the niche can result in an increased effect on HSC regulation. These Nestin+ MSCs are associated with the sympathetic nervous system resulting in higher levels of HSC maintenance factor transcripts, like CXCL12, SCF, ANGpt-1 and VCAM-1 compared to other stromal cells (Ehninger, 2011).

Endothelial cells are known to express many HSC supportive factors, like CXCL12, SCF, E-selectin and other cell-bound molecules that promote HSC proliferation. These factors will be discussed in detail later in this report. Endothelial cells are directly involved in maintaining HSCs in the bone marrow niche. This has been demonstrated by studies investigating the effect of deletion of SCF and CXCL12 expression (Schepers and Campbell, 2016). In this study SCF and CXCL12 were deleted from endothelial and hematopoietic cells by use of TIE2-Cre recombinase, but no deletion in mesenchymal cells has been made. CXCL12 has been deleted in stromal cells using Lepr-cre. These mice had normal bone marrow, blood cell counts and normal lineage composition. Prxl-cre has been used for deletion in HSCs. In normal conditions, hematopoietic cell do not express SCF so a deletion of this gene did not affect the HSC frequency. An effect on the frequency of HSCs should thus be a result of the deletion of the SCF gene in the endothelial cells. Eight week old Tie2-Cre mice showed significantly reduced amounts of HSCs in the bone marrow compared to normal mice. Therefore we can conclude that endothelial cells are an important source of SCF in the HSC maintenance (Ding and Morrison, 2012).

Megakaryocytes are descendants of the stem cells with both indirect and direct effects within the endosteal niche. An increased number of megakaryocytes have a positive effect on the number of osteolineage cells, which will multiply in an environment with an increased number of megakaryocytes. For this process, direct cell adhesion of megakaryocytes and osteolineage cells is essential (Kacena, 2004). The megakaryocytes also produce a factor that has an indirect effect on the HSCs, named OPN (Storan, 2015). Megakaryocytes cleave and activate OPN and regulate thus the activation and function of a key HSC niche molecule. Megakaryocytes are also able to regulate HSC quiescence. This effect is probably indirect, but the definite factor that is responsible for the quiescence, produced by megakaryocytes, has not been identified (Hoggatt, 2016).

The bone marrow is highly innervated with sympathetic nerves (Kunisaki and Bruns, 2013). Via circadian rhythms the nervous system is capable to regulate and influence the HSC niche, by signalling through the β3-adrenergic receptors (Lucas, 2008). Different factors, like CXCR4 expression (receptor for CXCXL12) and SDF-1α production, stimulated by the adrenergic receptor, have indirect effects on the HSCs and the niche. The direct effects of the nervous system are caused by the release of neurotransmitters, like norepinephrine and dopamine which serve as a direct chemoattractant for HSCs on $CD34^{+}$ hematopoietic cells. These cells contain dopamine- and β2-adrenergic receptors

(Spiegel, 2007). Signalling through the β2 receptor also causes an upregulation of a vitamin D receptor, on osteolineage cells. Vitamin D is involved in the formation of bone by osteolineage cells (Kawamori, 2010). The nerves in the bone marrow are covered by nonmyelinating schwann cells. These cells are able to activate latent TGF-β, produced by a variety of cells. HSC dormancy, a period in which growth, development and proliferation are stopped, is maintained by Schwann cells by regulating the activity of TGF-β (Yamazaki, 2011). The effect of TGF-β will be discussed in the next paragraph.

The last cell known to be important in the bone marrow niche that will be discussed in this paper is the macrophage . Macrophages line next to the endosteal surfaces of bone and presumably have an effect on the osteolineage cells. A reduction in number of macrophages has a negative effect on the regulation of osteolineage cells due to a decreased expression of CXCL12 and IL-7 (Winkler, 2010). Therefore, specialized bone-associated macrophages are controlling HSC differentiation potential and migration activity by indirect modulation of other niche cells (Schepers and Campbell, 2015). The factor that supports these niche cells has not been identified yet (Christopher, 2011).

An overview of the mentioned cells in the niche and their effects on HSCs is shown in table 1. More cells are involved in the regulation of the niche, like regulatory T-cells, but these are not reviewed in this paper.

TABLE 1 - DIFFERENT TYPES OF NICHE CELLS AND THEIR EFFECTS ON HSCS

Type of cell	Direct/indirect	Effect
Osteolineage cells	Cell-cell contact	Important for cell survival for HSCs and progenitor cells Absence results in a defective niche. Produce supportive growth factors for hematopoiesis
Mesenchymal stem cells	Indirect	Promote HSC maintenance and controlling HSC cell cycle and migration by expression of different factors.
Endothelial cells	Unknown	Maintenance and quiescence of HSCs. Production of SCF.
Megakaryocytes	Both	Indirect effect on niche: multiply number of osteolineage cells (adhesion essential). Effect on quiescent state of HSCs by production OPN
Nervous system	Both	Direct: release of neurotransmitters Indirect: stimulation release of chemokines Upregulation Vit. D receptor
Nonmyelinating Schwann cells	Indirect	Regulation of activation of TGF-β, thereby maintenance of quiescence.
Macrophages	Indirect	Effect on number of osteolineage cells and promote HSC quiescence.

FACTORS WITH A DIRECT/INDIRECT EFFECT ON HSCS IN THE NICHE.

Multiple factors have effects on different aspects of the HSC. The mobilisation and localisation of the HSC depends on direct cell-cell adhesion, but this is also affected by diverse chemokines. Secondly, the quiescence and differentiation of the HSC could be influenced by indirect or direct factors in the niche. A summary of the most important factos, their receptors and effects, are shown in table 2.

LOCALISATION OF HSCS

The localisation of HSCs depends on the concentration of multiple factors in the niche (Figure 4). Transplantation of labelled stem cells has been a great tool that allowed imaging of HSCs in mice to identify their exact locations in the niche. CXCL12 and E-selectin are examples of factors which control the localisation of HSCs. CXCL12 is a chemokine, also named stromal derived factor 1 (SDF-1), and is the main chemo attractant for HSCs in the adult bone marrow (Hanoun, 2013). CXCL12 is expressed by many cells involved in the bone marrow niche, e.g. leptin receptor^{+} perivascular stromal cells express high levels of CXCL12. The receptor for CXCL12 is the specific CXCR4 receptor, expressed on the membrane of HSCs (Haggott, 2016). Loss of function of these cells leads to loss of all quiescent HSCs localised in the bone marrow (Zhou, 2015). E-selectin is a second ligand that effects the homing of the stem cells. The only cells in the vascular bone marrow niche which are capable of E-selectin production are the endothelial cells. HSCs and progenitor cells are able to adhere to this ligand (Winkler and Barbier, 2012). Besides CXCL12 and E-selectin are diverse types of integrins important as adhesion molecules in the niche to play a role in tethering HSCs (Haggott, 2016).

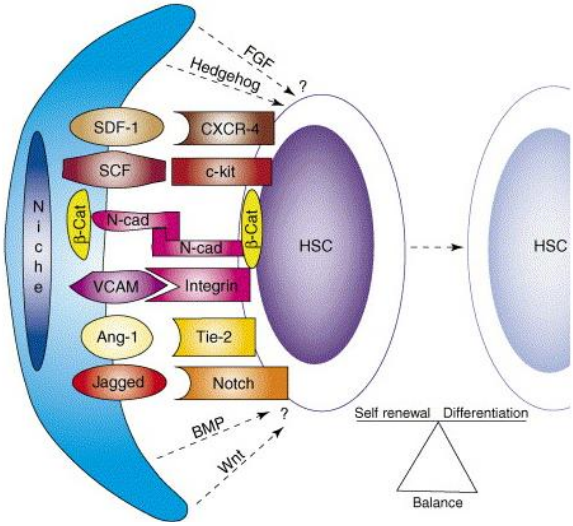

FIGURE 4 - MOLECULES RESPONSIBLE IN ADHERENCE OF HSCS (ZHIXING, 2006)

A second chemokine important for the localisation of HSCs is Stem cell Factor (SCF). SCF appears in a membrane-bound and soluble form. Membrane-bound SCF is necessary for HSC maintenance in the niche and facilitates cell-cell contact between HSCs. The cells that synthesize SCF are MSCs and OBL cells, which are essential for HSC maintenance (Morrison and Scadden, 2015). SCF binds to its receptor tyrosine kinase, KIT. Migration of HSCs will be guided by SCF concentrations along the pathway of migration. Stimulation of migration out of the bone marrow is also regulated by G-CSF. G-CSF mobilizes stem- and progenitor cells and stimulate these to traffic out of the bone marrow, towards the peripheral blood (Haggott, 2016). Active migration in and out the bone marrow of HSCs appears in oscillating cycles. These circulating HSCs can enter peripheral organs for proliferation and differentiation before infiltration of the lymphoid organs (Isern, 2011). Mobilisation of HSCs is clinically routinely used in hematopoietic stem cell transplantation protocols.

CELL QUIESCENCE

TGF-β, CXCL4, Angpoietin-1 and TPO are enforcers of HSC quiescence. The quiescence of stem cells is essential to protect the stem cell pool for exhaustion during stress conditions (Arai, 2008). Quiescence in HSCs is regulated by cdkn genes, like p27, which encodes for enzyme inhibitors. These proteins bind to an prevent the activation of cyclins. Thereby cell cycle progression to G1 is stopped and the cell cycle can thus be regulated (Polyak, 1994).

Angpt-1 is one of the factors responsible for cell quiescence and is expressed by osteoblasts, HSCs and perivascular stromal cells in the bone marrow. Release of this chemokine will induct maintenance of quiescent HSC in an endosteal niche, angiogenesis also depends on Angpt-1. The receptor for Angpt-1 is a TIE2 receptor, which is mainly expressed by endothelial cells but also by HSCs (Zhou, 2015). A second signalling chemokine for HSCs that maintains the quiescent state of the cell is TGF-β. Reduction

of TGF β will be followed by reduction of HSC amounts in the niche (Yamazaki, 2011). TGF-β negatively regulates HSC and hematopoietic progenitor cell proliferation. Secretion of latent TGF-B by different types of cells and activation by nonmyelinating Schwann cells results in support of the quiescence of the stem cell. TGF-β is able to induce this quiescence through inhibition of lipid raft clustering that assembles growth factor signalling microdomains (Kunisaki and Frenette, 2014). E-selectin also has an influence on HSCs. Deficiency of E-selectin results in more quiescent HSCs. E-selectin has been suggested to be exclusively expressed by endothelial cells in the bone marrow (Morrison and Scadden, 2015.

The environment of HSCs is partly determined by increasing concentrations of HIF-1α. Areas in the niche with low concentrations of oxygen will have increased activity of HIF-1α. HSCs residing in areas with higher concentrations of HIF-1α will maintain their fitness and stemness. HIF-1α is a transcriptional regulator of numerous hematopoiesis regulatory genes, like VEGF and groups for cell proliferation and survival. The production of SDF-1α and expression of its receptor will be increased by HIF-1α (Lin, 2006). HIF-1α knock out mouse models show loss of quiescence and a reduced amount of HSCs (Takubo, 2010). Stem cells in an area with reduced concentrations of oxygen show greater hematopoietic repopulating than the stem cells in more perfused areas (Winkler and Barbier, 2010).

Notch ligand receptor, expressed by HSCs, regulates cell fate decisions. Self-renewal will be caused by presence of notch ligand Jagged 1, expressed by osteolineage cells, binding to the notch ligand receptor on HSCs. Amounts of HSCs thereby increase without differentiation (Haggott, 2016), so expansion of the HSC pool is regulated by signalling through Jagged-1.

All chemokines and molecules mentioned above are signals for HSCs for the short-range. There are also signals circulating through the blood vessels that regulate the HSC state and niche function. For example hormones or hematopoietic cytokines, like Thrombopoietin, are required for HSC maintenance. Thrombopoietin is mostly synthesised in the liver and kidney. The importance of this factor is not investigated yet (Morrison and Scadden, 2015).

TABLE 2 - EFFECT OF PARTICLES IN THE BONE MARROW NICHE ON HSCS

Factor	Receptor	Effect
CXCL12	CXCR4	Main chemo attractant for HSC in the niche
E-selectin	-	Influence on localisation of HSCs
SCF	Tyrosine kinase receptor: c-kit	Membrane form essential for HSC maintenance
OPN	-	
VCAM	integrins	Adhesion
G-CSF	-	Mobilisation of stem and progenitor cells, stimulation of migration to peripheral blood
HIF-1α	-	HSCs in high HIF-1α maintain fitness and stemness. Upregulation of SDF1α and its receptor Influence on quiescence
OPN	-	Inhibition of HSC proliferation, promotes HSC apoptosis
Angiopoietin-1	TIE2	Maintenance quiescent state HSC
TGF-β	TGF-β receptor	Maintenance quiescent state HSC
Jagged 1 (Notch)	Notch receptor	Self-renewal
Thrombopoietin	-	Effect on HSC state and niche function

WHAT IS LEUKEMIA?

Myeloproliferation, abnormalities in the differentiation of the hematopoietic system, caused by mutations can for example cause leukemia. In leukemia, the normal HSCs or progenitor cells are mutated and no longer able to give rise to the normal types of cells. The abnormalities in the HSCs will be discussed in this chapter. Next, the differences in the niche after development of leukemia will be explained. Last, the effect of LSCs on the normal niche and HSCs will be discussed.

Leukemia is a disease with a abberant hematopoiesis that results in a differentiation block that can occur at several stages of development.. There are 4 subtypes of leukemia called acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia and chronic lymphoid leukemia, all with specific mutations which cause a specific type of disease. In this paper only AML will be discussed. Development of leukemia is a stepwise process in which a number of accumulating somatic mutations give rise to an increasing and altered population of immature blast-like cells (Passegué and Jamieson, 2003). Not all types of leukemia display the same number and type of mutations. For example AML genomes have fewer mutations than most other adult cancers, with an average of 13 mutations (Ley, 2013). The number of different genes mutated in leukemia make it more difficult to find a good target for therapy. The mutated genes that occur with the highest frequencies in AML are shown in figure 5. The most relevant mutations for pathogenesis of leukemia include fusions of transcriptional factors, tumor suppressor genes, myeloid transcription factor genes and copy number alterations but many more types of genes can be mutated and essential for leukemia (Ley, 2013).

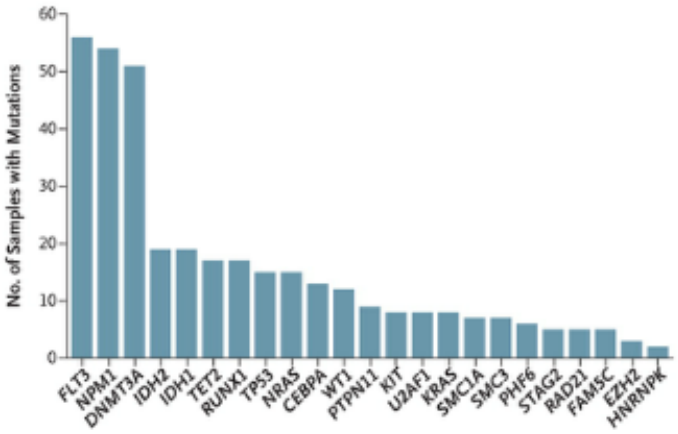

FIGURE 5 - SIGNIFICANTLY MUTATED GENES IN AML (LEY, 2013)

Like the normal hematopoietic system, leukemia is organized as a hierarchy, with leukemic stem cells (LSCs) residing at the top (Bonnet and Dick, 1997). LSCs can self-renew to give rise to new LSCs as well as to fast dividing leukemic progenitor cells that lack self-renewal properties (Passegué and Jamieson, 2003). Accumulation of a large number of abnormal leukemic progenitor cells that fail to differentiate into functional blood cells is a typical characteristic of leukemia. Whether normal HSCs or progenitor cells act as the "cell of origin" is still under debate (schematically depicted in figure 6). The most likely scenario appears to be that HSCs are the first cells in which mutations occur, resulting in the formation of LSCs. Alternatively, it might also be possible that progenitor cells are the first cells in which mutations arise. On the one hand, this might be more likely since these cells proliferate much faster than HSCs

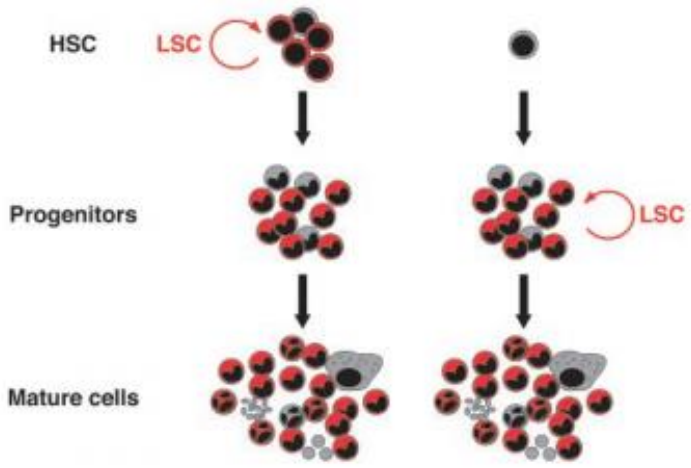

FIGURE 6 - ORIGIN OF LSC (PASSEGUÉ AND JAMIESON, 2003)

thereby increasing the chance of accumulating mutations. On the other hand, in this scenario the mutations must be able to re-install self-renewal properties, and most oncogene overexpression models suggest that this is challenging. Regardless of the cell of origin, the LSC is equally quiescent as normal HSCs thereby rendering it relatively insensitive to chemotherapy that is currently being used to treat leukemia patients.

CHANGES IN THE NICHE DURING LEUKEMIA

While it is clear that genetic mutations in stem/progenitor cells play an important role in the development of leukemia, the bone marrow niche might contribute to the process of leukemic transformation as well. For instance, via secretion of various factors such as inflammatory cytokines leukemic cells might change the niche such that it becomes an excellent place for leukemogenesis but at the same time inhibits normal hematopoiesis. In this scenario, the leukemic niche would facilitate the process of leukemic transformation. However, there is also recent data that suggests that the niche can also act as a direct initiator of disease. These aspects will be discussed below.

HSCs: INITIATORS OF DISEASE

The dependence of normal HSCs on their niche in maintenance of stem cell characteristics makes it conceivable that LSCs have the same dependency on the niche as HSCs (Raaijmakers and Scadden, 2008). Mutations in the HSCs leading to leukemia can alter the normal interactions with the niche. Chronic myelogenous leukemia cells produce less CXCL12 than healthy HSCs do, this therefore results in an altered localisation of HSCs. The niche will in respond upregulate other molecules, like cytokines, which are supportive for the malignant cells. Examples for these upregulated cytokines are IL-6 and PlGF. Higher concentrations of PlGF can be related with angiogenesis stimulation and CML cell growth (Haggott, 2016). IL-6 signalling blocks the by mutation driven pro B cell and upgrades the myeloid proliferation, so promoting the CML development (Reynaud, 2012). This indicates that LSCs can contribute to stimulate their own tumor growth by initiating upregulation of cytokines by the niche.

Multiple types of integrins are involved in the adherence of the stem cells in the niche. An increased number of these adhesion molecules, for example VLA-4 and LFA-1, correlate with an increased amount of leukemic cells in the bone marrow (Vila, 1995). Second, these molecules are involved in the development of resistance against chemotherapy. Administration of antibodies, which can neutralise the adhesion molecules and thus reduce the increased number of adhesion molecules, can eliminate this resistance (Doan, 2012).

THE NICHE: INITIATOR OF DISEASE.

Differences in the expression of molecules by the niche are, in some cases, able to cause a disease phenotype. Multiple studies made knock out mouse models for chemokine expression, specific for the niche, which resulted in development of leukemia in these mice. Deregulation of the HSC activity and the bone marrow environment are important factors in the development of malignancies. Changes in signalling activity can alter HSC numbers and thereby cause defects in blood production (Schepers, 2013).

The effect of a mutated niche in development of leukemia has been investigated by Raaijmakers and Scadden labs. A deletion of the dicer1 gene in osteoprogenitor cells in mice disrupts normal hematopoiesis. Dicer1 is a RNase endonuclease, essential for microRNA biogenesis and RNA processing. In normal conditions, miRNAs regulate the hematopoietic cell fate. Downregulation of these miRNA by a Dicer1 deletion promoted tumorigenesis. As a result, osteoblastic numbers were decreased whereas osteoclast numbers and function were not affected. The deletion of the dicer1 gene resulted in aberrant numbers of all different cell types. Deletion of the dicer1 gene in

osteoprogenitor cells thus indicates disrupted functions in regulating hematopoiesis (Raaijmakers and Scadden, 2010).

The effect of the niche on leukemia increased Notch signalling in mutated osteolineage cells results in AML development after an essential activation of the Wnt-signaling pathway (Kode, 2014). Myeloproliferation disease can be simulated by utilization of two independent Cre-systems in which E3-ubiquitine is knocked out. E3-ubiquitine is necessary for processing Notch and a defective Notch activation leads to the development of disease (Haggott, 2016). Expansion of osteolineage cells can be caused by malignant cells. These osteolineage cells are preferentially supportive for malignant cells and discriminate healthy HSC hematopoiesis. Cell-cell contact of the mutated and expanded osteolineage cells with malignant cells, but also the production of some soluble factors results in a significant expansion of the bone marrow cells (Schepers, 2013).

Development of myeloid proliferation could be a result of genetic alterations in the bone marrow. Two different studies have investigated the effect of genetic alterations and the development of leukemia. One study made a knock out model of the retinoic acid receptor-gamma, leading to development of a leukemia disease phenotype. Results showed significantly reduced numbers of HSCs accompanied by an increased amount of immature progenitor cells in their bone marrow after eight weeks. The immature progenitor cells are not able to grow up as in normal hematopoiesis. Significantly reduced cellularity in the thymus is a result of a growth deficiency (Walkley and Olsen, 2007). The second study made mice deficient for the retinoblastoma gene in hematopoietic cells by using the interferon inducible Mx-Cre transgene and pRb animals. The retinoblastoma gene encodes for a protein which is a key regulator of the cell cycle. The knock out mice showed significant increased amount of stem and progenitor cells in the bone marrow so the hematopoiesis has been disturbed. This indicates that Rb extrinsically regulates HSCs by maintaining the competence of the adult bone marrow to support HSCs (Walkley and Shea, 2007).

Finally, the innervation of sympathetic neurons play a role in the regulation of the niche and HSC function. Decreased levels of Angpt-1, CXCL12, SCF and VCAM-1 are seen in patients with AML. These changes in the niche result in a reduced ability to repopulate and less migration to the peripheral blood of normal HSCs. These changes are an indirect benefit for the leukemia cells. The progression of the acute myeloid leukemia cells could be slowed by a β-2 agonist, like Clenbuterol (Haggott, 2016).

DOES THE LEUKEMIC STEM CELL AFFECT THE NORMAL NICHE?

LSCs may be able to alter the environment in the niche in their favour whereby healthy HSCs will be impaired (Zhang, 2012). The conditions in a niche can determine which cell will win the competition for the same localisation in the niche (Boyd, 2014). Which cells will be discriminated in a leukemia environment and how LSCs influence the normal healthy niche will be discussed in this paragraph.

The function of the bone marrow microenvironment can be affected by malignant myeloid cells (Schepers, 2012). Abnormal secretion of pro-inflammatory cytokines by leukemic cells results in a feedforward loop that stimulates myeloid differentiation and leukemia cell expansion (Reynaud, 2012). Leukemic cells are capable of remodelling the endosteal bone marrow niche into a leukemic niche that reduces normal hematopoiesis (Schepers, 2012).

Loss of normal hematopoiesis occurs during development of most types of leukemias. Leukemic hematopoiesis turns the endosteal bone marrow niche into a niche which promotes LSC function and reduces numbers of normal HSCs. Expression of many factors essential for the HSC niche, like CXCL12, SCF, Angpt-1 and TGF-β, are downregulated by the new niche. Factors that promote the myeloid differentiation are upregulated. The loss, or decreased CXCL12 expression is suspected to be particularly essential for the loss of normal HSCs. LSCs do not suffer under these conditions due to their

specificity for different types of adhesion molecules for the localisation in the bone marrow (Schepers, 2012).

CONCLUSION

The hematopoietic niche regulates, via direct and indirect factors, the maintenance, quiescence, proliferation and differentiation of hematopoietic stem cells. The niche contains multiple cell types, like osteolineage cells, macrophages, mesenchymal-, endothelial cells, megakaryocytes and regulatory T cells. Innervation of the bone marrow with sympathetic nerves and release of transmitters are essential for the stem cells, especially the β-2 receptor is necessary.

The state of the stem cell is regulated by expression of numerous factors by previous mentioned cells. CXCL12, TGF-β and Angpt-1 are the most relevant factors in the localisation and quiescence of HSCs. These factors do also play an important role in the development of diseases, like leukemia. Upregulation or different expression of the ligands/receptors of the niche are able to cause a type of leukemia. This can be a result of differences caused by the niche, or mutations in the stem cells. The (indirect) alterations in the niche may have a negative effect on the normal stem cells and the normal process of hematopoiesis will be inhibited.

The effects of leukemia cells on the niche, and the cause of leukemia are important aspects of the disease to investigate in the future. A well-known genotype and phenotype of leukemia could be helpful for finding new targets for specific therapy. Targeting essential niche factors for leukemia stem cells could result in no adherence of LSCs in the niche and could thereby be useful in healing patients.

Further research should make a clear and full list of differences between a normal and leukemic niche. All factors which are involved in the normal interactions of the niche should be known before we are able to understand more about the in leukemia process. Schepers and Passegué have done research in the interactions of the leukemic stem cell and the normal niche. More research should make a clear overview of involved molecules and factors and the differences in the leukemic niche compared to the normal niche. When more is known about the differences in the niche and HSC during leukemia more specific therapies can be developed and leukemia will hopefully be less lethal in the future.

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