

Extramedullary hematopoiesis: Elucidating the function of the hematopoietic stem cell niche (Review)

KOUHEI YAMAMOTO¹, YUKAKO MIWA², SHIHO ABE-SUZUKI¹, SHINYA ABE¹,
SUSUMU KIRIMURA¹, IICHIROH ONISHI¹, MASANOBU KITAGAWA¹ and MORITO KURATA¹

¹Department of Comprehensive Pathology, Graduate School of Medical and Dental Sciences,
Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8519; ²Department of Pathology,
Kanazawa Medical University Hospital, Kanazawa, Ishikawa 920-0293, Japan

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Abstract. Extramedullary hematopoiesis (EMH) occurs under various circumstances, including during embryonic/developmental periods, pathological status secondary to insufficient bone marrow function or ineffective hematopoiesis, in hematological disorders, for example malignancies, as well as stromal disorders of the bone. EMH is characterized by hematopoietic cell accumulations in multiple body locations. Common EMH locations observed in clinical and pathological practice include the spleen, liver, lymph nodes and para-vertebral regions. Among the various organs associated with EMH, the spleen offers a unique site for evaluation of hematopoietic stem cell (HSC)/niche interactions, as this organ is one of the most common sites of EMH. However, the spleen does not have a major role in embryonic/developmental hematopoiesis. A recent study by our group revealed that circulating HSCs may be trapped by chemokine (C-X-C motif) ligand 12 (CXCL12)-positive cells at the margin of sinuses near CXCL12-positive endothelial cells, resulting in the initiation of the first step of EMH, which is a similar mechanism to bone marrow hematopoiesis. The present review briefly discusses the environment of EMH in extramedullary spaces in order to investigate the mechanisms underlying HSC maintenance, and aid the elucidation of the niche-stem cell interactions that occur in the bone marrow.

Contents

1. Introduction
2. Sites of EMH
3. Embryonic/developmental EMH
4. EMH associated with hematological disorders
5. EMH associated with stromal abnormalities
6. Microenvironment of EMH
7. Conclusion

1. Introduction

Extramedullary hematopoiesis (EMH) is characterized by the formation and development of blood cells, external to the bone marrow medullary spaces. However, this condition has not been identified physiologically in adults. Therefore, the pathophysiological variations in hematopoietic stem cells (HSCs) and their microenvironment may be a component of the homing, proliferation and maturation processes associated with hematopoietic cells in the extramedullary organs of adults; although, there is currently a dearth of studies addressing the prevalence and fundamental processes of EMH (1). This may be explained by the fact that EMH is generally identified as a secondary or accessory event to another disorder, including benign hematologic disorder (2) or cancer (3), in certain cases developing independently or lacking apparent trigger and without clinical or diagnostic implications (4,5).

In bone marrow, the HSC niche, a distinct microenvironment, maintains the characteristics of HSCs (6). It was recently demonstrated that niche-constituting cells within human bone marrow include critical elements, for example mesenchymal stromal cells (MSCs), blood vessels, perivascular cells, macrophages and endosteal cells (7). Investigations into the various mechanisms underlying the interaction between these elements and HSCs have also been conducted. HSCs occur in the vicinity of, but not necessarily in direct contact with, osteoblastic bone-lining cells that constitute the endosteal niche regions. However, the sinusoidal endothelium also interacts with HSCs, and form the vascular niche (6). Initially it was suggested that the endosteal and vascular niches may control distinct HSC populations; however, more recent data

Correspondence to: Dr Morito Kurata or Professor Masanobu Kitagawa, Department of Comprehensive Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
E-mail: kurata.pth2@tmd.ac.jp
E-mail: masa.pth2@tmd.ac.jp

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suggests that their effects may be more complicated due to functional crosstalk between cells within the two regions (8,9). Furthermore, the vascular niche has been suggested to regulate HSCs in a distinct mechanism or potentially harbor HSCs with various self-renewable properties (10-12).

Certain mesenchymal cells, including chemokine (C-X-C motif) ligand 12 (CXCL12)-abundant reticular cells (CAR cells) (13), nestin-expressing cells (14) and non-myelinating Schwann cells derived from the neural crest (15), have been reported to function as bone marrow niches. Of particular significance in localizing and retaining HSCs in the vicinity of niches are the interactions between CXCR4 and HSCs or CXCL12 (SDF-1). In addition, upregulation of vascular cell adhesion molecule-1 (VCAM-1) and very late antigen-4 (VLA-4) expression (16) may be induced by chemokine interactions through CXCL12. Furthermore, CXCL12-deficient embryos exhibited a marked decrease in HSC populations and hematopoietic function was inhibited; therefore indicating that CXCL12 has a critical role during the early stages of bone marrow hematopoietic cell colonization (17).

CXCL12 may potentially be involved, not only in hematopoietic cell colonization, but also in the migration and maintenance of HSCs during EMH. For example, it was demonstrated that stromal cells express CXCL12 and induce B-cell migration in the spleen (18). Recently, in a study by our group, whether CXCL12-positive cells were able to be identified and if they had a role in hematopoietic regions of the spleen during EMH was determined by investigating CXCL12 messenger (m)RNA expression levels and localization of CXCL12-positive cells in the spleen (19). A previous study suggested that hematopoiesis in bone marrow cannot be maintained by a vascular niche alone, as indicated by osteoblast deficient mice (20). Since splenic EMH exhibits neither osteoblastic nor endosteal niches, elucidation of the details of crosstalk between HSCs and the niche and/or microenvironments in splenic EMH may be significant in understanding the overall biology of hematopoiesis in terms of niche.

2. Sites of EMH

Spleen. The spleen is a frequent site of EMH during the postnatal period; although, it is only a minor factor in the embryonic stages of hematopoiesis. Despite the fact that the splenic microenvironment is characterized by hypoxic/acidic conditions and numerous macrophages that are harsh and inhospitable for HSCs, EMH usually occurs within the red pulp (21). The mechanisms underlying the interaction between HSCs and the stromal cells of the spleen during EMH will be discussed subsequently.

Liver. Hepatic EMH is normal in infants up to ~5 weeks of age (22). Conversely, hepatic EMH in adults is associated with various pathological states, including hepatic disorders, sepsis, transplantation, hepatic tumors, for example hepatoblastoma; adenomas and hepatocellular carcinomas (2,23,24). EMH is most often observed within the hepatic sinusoids.

Lymph nodes. As with the spleen or liver, EMH may occasionally occur in the lymph nodes and is frequently associated with underlying hematopoietic neoplasms, for example

myeloproliferative neoplasms (MPNs) (25). Therefore, in adults and infants; a hematologic evaluation, including blood cell counts, peripheral blood smear and potentially a bone marrow aspiration/biopsy should be performed if EMH is identified in the lymph nodes.

Other sites. Other tissues that may be associated with EMH include the heart, fatty tissue, adrenal glands, kidney, periosteum, pleural cavity, para-vertebral regions, intra-spinal tissue, pre-sacral region, nasopharyngeal region, para-nasal sinuses (5) and numerous types of benign/malignant neoplasm (26).

3. Embryonic/developmental EMH

The high clinical significance of bone marrow transplantation has attracted researchers to focus on the developmental origin of HSCs and niches (27). The yolk sac, in the initial stages of embryonic development, is the initial site of primordial development in the human hematopoietic system, and occurs in the third week of development (28). Vascular elements are initially formed within the yolk sac, as the mesodermal cells begin to accumulate (29). Subsequently, clusters of primitive mesodermal cells emerge in close association with these vascular structures, termed blood islands, and the mesenchymal cells in these blood islands develop into hematopoietic cells and begin to circulate on approximately day 20 of development (30). The development of the rudimentary liver occurs simultaneously to these events (29). HSCs are not generated directly, but instead circulating hematopoietic precursor cells populate the fetal liver (29). These cells proliferate and differentiate in the hepatic parenchyma, forming the primary site of hematopoiesis on approximately day 30 (29).

The aorta-gonads-mesonephros region also produces HSCs. Bone marrow hematopoietic elements commence formation at approximately eight weeks of development, which is relatively late in embryogenesis (30). By week 11, HSCs and the bone marrow niche environment are established in the marrow cavity (30). By contrast, the spleen performs a minor function as a site of erythropoiesis (26). At each stage of development, stromal cell/HSC interactions via cytokines, chemokines and growth factors have significant roles in the maintenance of normal hematopoiesis (31).

4. EMH associated with hematological disorders

Expansion of the hematopoietic space outside of the bone marrow has been observed in numerous benign hematological disorders, including thalassemia, sickle cell anemia, hereditary spherocytosis, congenital dyserythroblastic anemia and idiopathic thrombocytopenic purpura (26).

In response to reduced red blood cell (RBC) numbers, a homeostatic mechanism induces an increase in the synthesis of RBCs, typically via the production of erythropoietin (26). If the reduction in RBCs is sufficiently severe, hematopoiesis will occur in the extramedullary spaces (26). If granulocyte or platelet production is also insufficient, a similar state occurs (26).

Not only benign conditions, but also neoplastic disorders, have been described by the term EMH (32-34). Neoplastic myeloid

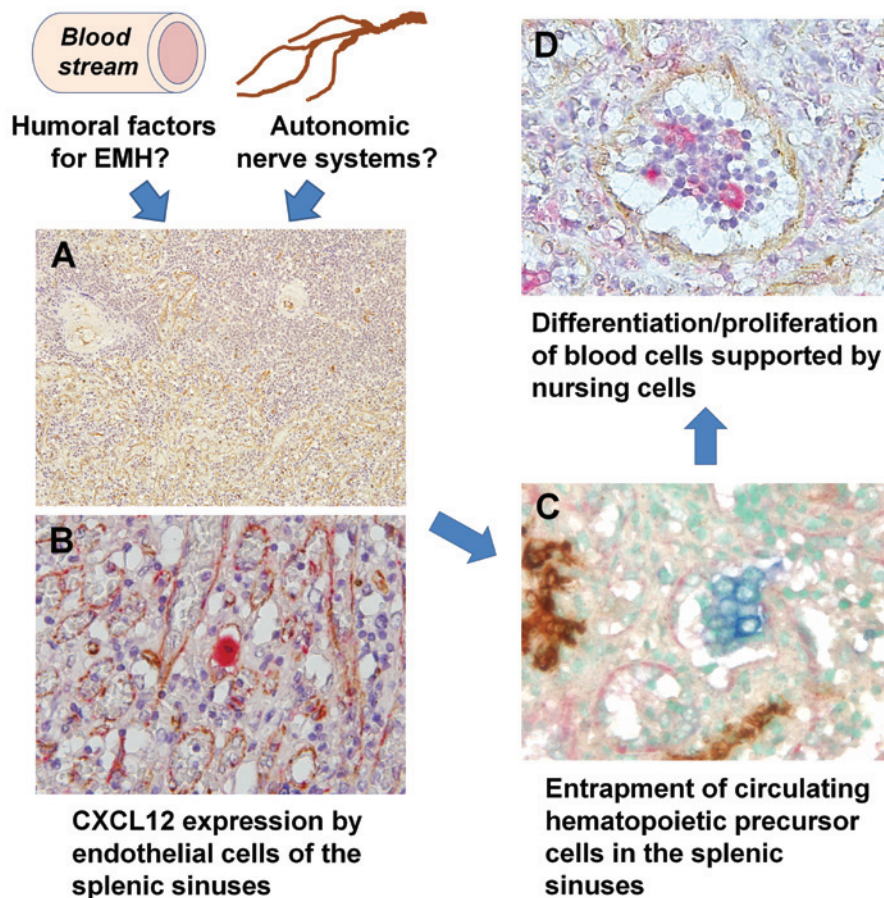


Figure 1. Summary of the distribution of EMH-associated molecules in the spleen. Humoral factors and/or autonomic nerve systems may induce the expression of CXCL12 in splenic sinus endothelial cells. These cells may maintain HSCs, and certain HSCs are able to differentiate into various lineage cells, which are subsequently maintained by macrophages in the sinuses. (A) Localization of CXCL12 (brown) in EMH-positive spleens (single immunohistochemistry; magnification, x50). Note the positive reaction in the margin of the sinuses of the red pulp and arterioles of the white pulp. (B) CXCL12-positive (brown) cells were also positive for factor VIII (red), which was used as a sinus endothelial cell marker, indicating that these cells were sinus endothelial cells (double immunostaining; magnification, x200). (C) c-kit-positive (blue) and CD34-negative (brown) hematopoietic precursor cells were observed adjacent to the CXCL12-positive (red) sinus endothelial cells (triple immunostaining; magnification, x200). (D) Erythroblasts and CD68-positive (red) macrophage-lineage cells were identified in the sinuses of EMH-positive spleens. CXCL12-positive endothelial cells (brown) existed separately from the focus of erythroblastic accumulation (double immunostaining; magnification, x200). CXCL12, chemokine (C-X-C motif) ligand 12; EMH, extramedullary hematopoiesis; HSCs, hematopoietic stem cells.

proliferation in the extramedullary spaces has been identified in association with MPNs, myelodysplastic syndromes, granulocytic sarcoma and other myeloid neoplasms (32-34). Cellular proliferation may consist of trilineage marrow elements similar to those of benign EMH (32-34). The most common sites of EMH associated with neoplastic disorder are the spleen, lymph nodes, skin, bone, small intestine, orbit, breast, cervix, nasal sinus, mediastinum and brain (26,28,35).

5. EMH associated with stromal abnormalities

In the infrequent case of stromal disorders of the bone, irregularities in the bone marrow environment trigger EMH (1). Osteopetrosis is an example of such a case, where the marrow cavity is compromised due to excess bone formation and proliferation, resulting in insufficient space for the required hematopoietic element (36). In severe renal osteodystrophy and Paget's disease similar transformations have also been observed (26).

Additionally, EMH is prompted by the replacement of bone marrow cavity spaces with non-hematopoietic and non-osteoid

tissues (35). For example, EMH occasionally occurs as a result of extensive involvement of infectious diseases, marrow fibrosis following inflammation, storage diseases including Gaucher and Niemann-Pick diseases or tumor metastasis (37).

6. Microenvironment of EMH

Of the various organs associated with EMH, the spleen offers a unique site for evaluating HSC/niche interactions as it is one of the most common sites of EMH, but does not have a major role in embryonic/developmental hematopoiesis. In the human spleen, it was verified that the expression levels of CXCL12, one of the candidate markers of bone marrow niche-constituting cells, were significantly higher in EMH-positive cases, compared with those in EMH-negative cases (Fig. 1) (19). Furthermore, CXCL12 was demonstrated to be localized to the sinus endothelial cells of the red pulp in EMH-positive spleens; whereas, CXCL12 was expressed throughout the vascular endothelial cells of the white pulp in spleens of EMH-negative and -positive cases (19). EMH frequently occurs in the red pulp, and the data presented in the current

review suggest that splenic sinus endothelial cells expressing CXCL12 may contribute to the attachment and recruitment of circulating hematopoietic precursor cells, forming bone marrow niche-like regions of EMH in the human spleen (19).

Splenic EMH, in particular erythroid EMH, is suggested to occur within the red pulp of the spleen (39). A study confirmed that erythroblasts, myeloid cells and megakaryocytes were identifiable within the splenic cords and intrasinusoidal spaces in the red pulp of the spleen in EMH-positive cases (21). The identification of multiple stages of differentiation and mitotic erythroblast figures further supported the hypothesis that erythroid EMH occurred in the red pulp (21).

Erythroblastic islands of bone marrow constitute the basic structure required for erythropoiesis, and include central macrophages that form the microenvironment required for erythroblast development and enucleation, as well as phagocytosis of extruded nuclei (40,41). Following the release of HSCs from bone marrow niches, erythroid cell maturation is facilitated and the resulting erythroid colony-stimulating units may adhere to the central macrophages forming distinct erythroblastic islands that are separate from the existing niches (41). This hypothesis was found to be applicable to EMH-positive spleens, where CXCL12-positive signals on endothelial cells were identified distant from clusters of mature erythroblasts (21).

A small population of c-kit-positive/CD34-negative cells have been identified adjacent to CXCL12-positive sinus endothelial cells in the EMH-positive spleen (21). The CD34 antigen has previously been used to identify immature HSCs; however, c-kit-positive/CD34-negative cells have also been shown to comprise a fraction of HSCs (42) or hematopoietic precursor cells, including erythroid progenitors (43). For this reason, c-kit-positive/CD34-negative cells in the EMH-positive spleen may potentially be a type of hematopoietic precursor cell. Bone marrow-derived HSCs in the arterial blood should enter into an open blood system comprised of the red pulp of the splenic cords, so that maturing cells may infiltrate into the venous sinuses (26,44-48). Previous studies have reported the presence of bone marrow-derived HSCs within the sinuses and adjacent to endothelial cells of the spleen (5,49). These results indicate that circulating HSCs may be trapped by CXCL12-positive cells at the sinus margins proximal to CXCL12-positive endothelial cells, in order to initiate the first stage of EMH.

In order for EMH to develop, a niche environment must be activated and expanded by multiple factors, including bone marrow failure, excessive hematopoietic stimulation and tissue inflammation, injury and repair, as well as abnormal cytokine production (7,26). It has been demonstrated that treatment with granulocyte-colony stimulating factor and granulocyte macrophage colony-stimulating factor induces an increase in spleen size associated with EMH (50,51). The underlying causes of EMH in the spleen were not able to be distinctly classified into the categories, described above; however, further study is required in order to elucidate the mechanisms underlying the upregulation of CXCL12 expression in the spleen.

In addition, distinct tissue niches may induce the development of various subsets of hematopoietic cells from HSCs. A study has suggested that splenic niches may support the development of certain distinct, dendritic-like antigen-presenting

cells (52). Although such experiments utilizing animal models have provided useful results and hypotheses, further studies should be conducted in order to identify specific differences in the characteristics and differentiation patterns of HSCs between human bone marrow and splenic EMH.

Bone marrow-derived mesenchymal stromal/stem cells (MSCs) are non-hematopoietic cells with the ability to differentiate into various types of cell, including osteoblasts, adipocytes and chondrocytes. MSCs are known to participate in the formation of niches for HSC development in the bone marrow. Several markers, including CD106, CD166, CD105, CD73 and CD44, are known to be useful in the isolation of MSCs from various organs, including bone marrow, placenta, adipose tissue, synovial membrane, endometrium and cartilage (53). The construction of niche-like regions during EMH may be attributed to the MSC-derived cells of EMH organs, although CXCL12-positive cells were only identified in the red pulp of EMH-positive sinus endothelial cells (19).

7. Conclusion

In contrast to how endothelial and perivascular cells and autonomic nerve systems maintain HSCs in the bone marrow (11,54) and how endosteal niches may maintain lymphoid progenitor cells (55), CXCL12-positive endothelial cells in the EMH-positive spleen may only contribute to the localization of HSCs to EMH-positive organs (5,56,57). As the results of a previous study indicated, CXCL12 is expressed in the sinus endothelial cells of the EMH-positive spleen, which may function in the entrapment of circulating hematopoietic precursor cells, whereas the cluster of macrophage/differentiated hematopoietic cells is separated from the CXCL12-positive endothelial cells (19). Other types of cell, including macrophages, as well as nerve, adipose, and dendritic cells, may contribute to the maintenance and differentiation of hematopoietic cells in EMH-positive spleens. CXCL12 was therefore suggested to have a restricted role during EMH, trapping the hematopoietic precursor cells in the sinuses of the splenic red pulp. The function of CXCL12 in bone marrow niche-constituting cells, for example CAR cells, may differ from its function in the EMH-positive spleen. Further studies are required in order to elucidate the mechanisms controlling the EMH niche-like function of various cells in comparison with those of the perivascular and/or endosteal cells of bone marrow niches.

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