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J Thromb Haemost. Author manuscript; available in PMC 2013 February 1.

Published in final edited form as:
J Thromb Haemost. 2012 February ; 10(2): 177–188. doi:10.1111/j.1538-7836.2011.04571.x.

Megakaryocytes, Malignancy and Bone Marrow Vascular Niches

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Summary

Dynamic interactions between haematopoietic cells and their specialised bone marrow microenvironments, namely the vascular and osteoblastic 'niches' regulate haematopoiesis. The vascular niche is conducive for thrombopoiesis, and megakaryocytes may, in turn, regulate the vascular niche, especially in supporting vascular and haematopoietic regeneration following irradiation or chemotherapy. A role for platelets in tumour growth and metastasis is well established and, more recently, the vascular niche has also been implicated as an area for preferential homing and engraftment of malignant cells. This article aims to provide an overview of the dynamic interactions between cellular and molecular components of the bone marrow vascular niche and the potential role of megakaryocytes in bone marrow malignancy.

Bone marrow haematopoietic niches in normal physiology

Haematopoietic stem cells (HSCs) reside in complex, dynamic microenvironments, or 'niches'. These are composed of supportive cells, extracellular growth factors, metabolic constituents and matrix factors that actively regulate stem cell function (Table 1)[1–9], and enable a sustainable and responsive HSC pool[1, 10]. Two physiologically distinct HSC niches have been described in the bone marrow; the ***endosteal*** (or ***osteoblastic***) niche at the bone-marrow interface, and the ***vascular*** niche around the specialized vascular endothelium (Figure 1)[9]. Interactions between stem cells and niches are bidirectional; the niche regulates stem cell self-renewal and cell fate decisions, and stem cells in turn modulate the nurturing microenvironments in which they reside[7].

The endosteal niche: osteoblasts and osteoclasts

The majority of long-term repopulating HSCs are found near the endosteal lining of the bone where they are in close contact with the bone remodelling cells, osteoblasts and osteoclasts[11–14]. Osteoblasts secrete several growth factors required for HSC maintenance, including angiopoietin 1 (Ang-1), thrombopoietin (TPO) and stromal-derived factor-1 (SDF1) (Table 1)[15, 16]. Manipulations that increase the numbers of osteoblasts also increase HSC numbers, suggesting that the availability of osteoblastic niches may limit the HSC pool[13, 14]. Notch and N-cadherin-mediated interactions between HSCs and osteoblasts promote cellular quiescence and self-renewal[14, 17]. However, whether direct cell-cell contact between osteoblasts and HSCs is necessary has yet to be resolved[18].

Osteoclasts also contribute to the HSC niche. Calcium ions (Ca^{+}) released by bone resorption retain HSCs close to the endosteal lining[19] and osteoclast-derived proteolytic enzymes cleave anchorage proteins such as SDF1 and stem cell factor (SCF) enabling mobilization of HSCs from the endosteal region[20].

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The vascular niche

While the osteoblastic niche promotes stem cell quiescence, the perivascular region is thought to be more conducive to HSC expansion[21–24]. Early *in vivo* studies indicating that the perivascular regions within bone marrow are important for haematopoiesis described restoration of thrombopoiesis following chemokine-induced localization of megakaryocytes to the vascular niche in TPO-deficient mice[25]. Subsequently, immunofluorescence studies using antibodies to the CD150 (SLAM) family of receptors which are differentially expressed among HSCs and haematopoietic progenitor cells (HPCs) suggested that about two-thirds of HSCs in the murine bone marrow and spleen are located adjacent to blood vessels[23]. Studies by Chute *et al* and others demonstrate that endothelial cells regulate HSC self renewal and cell fate decisions[26]. The relative importance of direct cell-cell contact versus secreted factors in this process remains uncertain.

Cellular components of the vascular niche

Bone marrow vascular endothelium

The bone marrow vasculature is a dense network of smooth muscle-invested afferent arterioles and capillaries, and thin-walled, fenestrated venous sinusoids. It has been suggested that the sinusoidal endothelium is uniquely specialized to support haematopoiesis over other vessel types[27], although this has not been definitively shown and the majority of studies have used pan-endothelial markers such as PECAM (CD31), vWF, or MECA32 (the murine equivalent of CD31). Unlike other vessels, bone marrow sinusoidal endothelium is devoid of pericytes although it is invested with specialized SDF1-secreting reticular cells, as described below. It is presumed that vascular niches in bone marrow and spleen are dedicated for haematopoietic stem/progenitor cells, although non-haematopoietic stem cells such as neural stem cells are thought to reside in analogous vascular niches in other organs[28, 29].

In vitro studies have shown that endothelial cells produce factors that support HSC maintenance, and their unique expression of adhesion factors is important for trafficking and homing of HSCs to the bone marrow[22, 30–33]. Detailed investigation of the bone marrow vasculature has been hampered by the inability to distinguish between arteriole and sinusoidal vessel types. A recent study clarified the immunohistological profile of specific vessel types, reporting that sinusoids can be specifically identified by their expression of VEGFR3, while arterioles uniquely express Sca1[27]. However, the relative contribution of arterioles versus sinusoids to the haematopoietic niche remains to be clarified.

Perivascular stromal cells

Almost all HSCs (97%) are in contact with a small population of perivascular, SDF1-producing reticular cells, termed CXCL12-abundant reticular cells (CAR cells)[34]. SDF1-CXCR4 signalling is essential for HSC colonization of the bone marrow and for HSC maintenance, and while CAR cells are highly enriched for SDF1, comparatively little SDF1 production is observed at the endosteal region[34]. Although endothelial cells produce negligible SDF1, they are often seen surrounded by the cell bodies or cell processes of the CAR cells[34].

A second population of perivascular cells, identified by their expression of nestin, a filament protein typically characteristic of neuroectoderm-derived cells, has also recently been described as key components of the HSC niche[35]. $Nestin^{+}$ cells were identified as mesenchymal stem cells (MSCs), with colony-forming capacity and osteoblastic, chondrocytic and adipocytic differentiation potential [35]. Previous work revealed that release of HSCs from the bone marrow is orchestrated by circadian rhythms via signals from

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the sympathetic nervous system (SNS) that control SDF1 expression[36]. Virtually all SNS fibres in the bone marrow were found to be associated with the nestin$^+$ cells, which express high levels of SDF1 and therefore are likely to be the same, or an overlapping, population of cells as the CAR cells. Other groups have described similar subendothelial stromal cells expressing Ang-1 that are able to support HSCs in the bone marrow and that may also establish heterotopic haematopoietic microenvironments following subcutaneous transplantation[37].

Vascular and osteoblastic niches: overlapping or distinct entities?

Classically, dichotomous vascular and osteoblastic haematopoietic microenvironments have been proposed, although whether these niches are indeed anatomically distinct entities is unclear[38]. Advances in confocal and two-photon microscopy have enabled a dynamic exploration of bone marrow niches within their physiological context *in vivo*[39, 40]. These insights suggest that several cellular and molecular components are shared (Figure 1). The endosteal bone lining is heavily invested with blood vessels[23, 27, 39, 40], with 90% of osteoblasts being within 20 μm of a blood vessel[39]. Furthermore, the nestin$^+$ SDF1-secreting MSCs described by Méndez-Ferrer *et al* were observed adjacent to HSCs regardless of whether they were in osteoblastic or vascular niches, suggesting that this niche cell may also be common to both osteoblastic and vascular niches[34, 35]. It is therefore possible that the role of osteoblasts may be more indirect, influencing a subset of perivascular niches to promote stem cell quiescence and self-renewal. Although further study is warranted, given the close physical interaction between the cellular elements it seems likely the osteoblastic and vascular niches are not spatially distinct but overlapping entities. Nonetheless, specialized ‘sub-pockets’ of this haematopoietic microenvironment may exist which are more conducive for stem cell renewal *vs.* differentiation depending on the balance of osteoblastic *vs.* endothelial derived signals. Further studies are required to delineate the migration patterns of haematopoietic cells between these two areas, and to better characterize the subtypes and maturation state of cells at each site.

Megakaryocyte – niche interactions

The vascular niche supports thrombopoiesis—Mature, polyploid megakaryocytes within the bone marrow are primarily located adjacent to bone marrow vessels, where they extend transendothelial pseudopods or migrate through the endothelium to produce platelets. The processes of megakaryopoiesis and thrombopoiesis have been extensively reviewed elsewhere[41–43]. While the vascular niche supports expansion of all haematopoietic lineages, it appears especially critical for megakaryocyte function and platelet production[22, 24, 25, 44–46]. This was first suggested by *in vitro* studies demonstrating that, in the absence of exogenous cytokines, bone marrow endothelial monolayers supported differentiation of human CD34$^+$ HSCs to megakaryocyte and myeloid progenitors via their secretion of the thrombopoietic cytokines SCF and interleukin (IL)-6[21, 22]. Notably, endothelial cells derived from bone marrow supported significantly greater megakaryocyte differentiation than did human umbilical vein endothelial cells (HUVECs) or bone marrow stromal cells, suggesting that bone marrow endothelium was uniquely specialized in this regard[22]. One explanation for this is that production of haematopoietic cytokines by the other types of endothelium is not constitutive but occurs only following stimulation with IL-1 or TNF[22]. Furthermore, this study reported that CD34$^+$ cells that were cultured in direct cellular contact with endothelial cells gave rise to a larger number of megakaryocyte progenitors than did CD34$^+$ cells separated from the endothelial cells by transwell coculture chambers[22]. Further evidence that interactions between megakaryocytes and endothelium are important is that administration of SDF1 enhances platelet production only in the presence of bone marrow sinusoids[47, 48].

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Megakaryocyte-vessel interactions were subsequently explored *in vivo* in thrombocytopenic, TPO-deficient mice. Even in the absence of TPO, administration of SDF1 and fibroblast growth factor (FGF)-4 caused a three-fold increase in the platelet count as a result of enhanced interactions between the small number of megakaryocyte progenitors and the bone marrow vascular endothelium[25]. Administration of vascular disrupting agents, which inhibit bone marrow vascular regeneration after chemotherapy-induced myelosuppression, has also been shown to cause prolonged and selective thrombocytopenia which continues after restoration of other haematopoietic lineages[49].

Studies of mice deficient in PECAM-1 provided further evidence that megakaryocyte positioning at the vascular niche is important in thrombopoiesis[45]. Following antibody-induced thrombocytopenia, recovery of the peripheral platelet count was impaired in PECAM-$1^{-/-}$ mice even though megakaryocyte maturation, proplatelet formation and platelet production *in vitro* was unaffected. This finding was attributed to reduced directional migration in response to SDF1 due to defective polarization of the CXCR4 receptor in the PECAM-$1^{-/-}$ megakaryocytes[45]. Movement of PECAM-$1^{-/-}$megakaryocytes towards an SDF1 gradient was less than one third of that of wild type megakaryocytes and PECAM-1 deficiency was also associated with an increase in adhesion to the matrix protein fibronectin due to constitutive activation of one of the fibronectin-binding surface integrins, αIIbβ3[45]. These data indicate that PECAM-1 is required for migration of megakaryocytes from osteoblastic to perivascular sites by regulating their directional movement and adhesion to matrix proteins in the bone marrow microenvironment.

Although proplatelet formation (PPF) by megakaryocytes is a spontaneous process *in vitro*, *in vivo* it appears to be temporally regulated by interactions with the extracellular matrix, in that while Type I collagen strongly inhibits PPF, collagen types IV and III support PPF[50, 51]. Type I collagen is particularly abundant at the osteoblastic zone while types III and IV are found around the bone marrow vessels. It is possible that this prevents premature release of platelets in the bone marrow prior to megakaryocyte localization at the bone marrow – blood vascular interface. Similarly, the relative hypoxia at the endosteal regions also inhibits PPF[52], while adhesion to perivascular fibronectin and vitronectin enhance megakaryopoiesis and PPF (Figure 1)[53, 54].

Mechanical forces at the vascular niche are also pivotal to platelet release. Blood flow-induced hydrodynamic stress is required for platelet release from proplatelet-forming megakaryocytes, sheering off fragments of megakaryocyte protrusions and enabling release of proplatelets/platelets into the circulation[46, 55].

Reciprocity: Megakaryocytes supporting haematopoietic niches

Evidence suggests that megakaryocytes both support and regulate the bone marrow niches that they inhabit via their abundant production and delivery of growth factors and cytokines (Table 2). Genetic mouse models in which megakaryocyte numbers are increased, such as mice over-expressing TPO or GATA-1 and NF-E2 knockout strains, have an osteosclerotic phenotype[56]. In addition, both mature and immature megakaryocytes stimulate proliferation and differentiation of osteoblasts *in vitro* and inhibit osteoclast differentiation[57, 58], possibly via α3β1 (VLA4) and α5βmeditated megakaryocyte-osteoblast adhesion and activation[59]. Unlike the majority of haematopoietic cells, megakaryocytes remain functional for around 7 days following irradiation and bone marrow transplantation[60]. It has therefore been postulated that megakaryocytes play a role in the reconstitution of bone marrow niches. An investigation of the bone marrow of mice 48 hours after sub-lethal total body irradiation (TBI) revealed that megakaryocytes had trans-located from the central vascular marrow areas to the diaphysis in response to upregulation of SDF1

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in these areas[61]. Expression of platelet-derived growth factor (PDGF)-β and FGF-2, two megakaryocyte-derived factors known to stimulate osteoblast differentiation and proliferation, was significantly increased following TBI[61]. These data suggest that megakaryocytes play an important role in recovery of osteoblastic niches following bone marrow damage.

Despite extensive research into the vital contribution of platelets to angiogenesis and maintenance of blood vessel integrity, surprisingly few studies have examined the functional contribution of megakaryocytes to the regulation of bone marrow vasculature. Megakaryocytes are a major source of both proangiogenic and antiangiogenic factors in the bone marrow (Table 2), support the survival of bone marrow-derived sinusoidal endothelial cells *in vitro*[62] and *in vivo* evidence from murine studies suggest that angiogenic processes both in the bone marrow and in other tissues are regulated by the absolute number of megakaryocytes in the bone marrow and their content of angiogenic regulatory factors[63, 64]. Immunohistological staining of mouse bone marrow for thrombospondin (TSP)1, the first endogenous angiogenesis inhibitor to be recognized[65], revealed that bone marrow TSP1 expression was limited to megakaryocytes, platelets and the endosteal surface. Other groups have shown that bone marrow vascularity is increased in $TSP1^{-/-}$ mice[64]. Recovery of the bone marrow vasculature following 5-FU treatment occurred significantly faster in TSP1 and TSP2 double knock-out (TSP-DKO) mice than in wild-type mice. Furthermore, vessel sprouting in Matrigel plugs loaded with TSP-DKO megakaryocytes was nearly 2-fold higher than wild-type megakaryocytes, and revascularization of ischaemic hind limbs was also accelerated[64]. Megakaryocyte content of vascular regulatory factors may be a key determinant of bone marrow vascularity and by regulating osteoblast proliferation, megakaryocytes could influence the number and function of both osteoblastic and haematopoietic vascular niches.

Megakaryocyte and platelet intracellular cargo

Megakaryocytes and platelets store intracellular cargo in α-granules, dense bodies and lysosomes[66]. α-granules, the primary storage vesicle, are formed from vesicular budding of the Golgi apparatus, then mature into multi-vesicular bodies (MVB), which is where organization of specific proteins into distinct subclasses of α-granules is thought to take place prior to their packaging into platelets[67]. Less is known about the origin and packaging of dense bodies, which contain haemostatically active components including serotonin, adenosine 5′diphosphate (ADP), adenosine 5′triphosphate (ATP), catecholamines and calcium.

Megakaryocyte-derived soluble factors may be either synthesized intracellularly or endocytosed and/or pinocytosed. The bone marrow microenvironment may thereby modify the protein content of megakaryocytes and platelets either by altering megakaryocytic gene transcription or by increasing the availability of proteins for cellular uptake.

Regulation of granule release

It was originally assumed that megakaryocyte and platelet granular contents were universally and randomly deployed upon cellular activation. However, it was recently suggested that α-granules are heterogeneous and highly organized, and that *selective* deployment of granules containing certain protein families may occur[67].

Preliminary evidence for the notion that platelets were able to differentially secrete certain proteins over others was apparent from data showing that the relative release of SDF1 as compared to serotonin was higher by platelets from $TSP^{-/-}$ mice than by platelets from wild-type mice[64]. This observation was supported and extended by subsequent reports

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indicating that pro- and anti-angiogenic proteins are organized into distinct platelet α-granules[68]. Using double immunofluorescence labelling of human platelets and *in vitro* cultured mouse megakaryocytes it was shown that angiogenesis stimulators (VEGF, FGF2) and inhibitors (endostatin, TSP1) were segregated into distinct α-granule subpopulations[68]. Similar granule heterogeneity has been reported for the adhesive proteins vWF and fibronectin[69] and for tissue inhibitors of MMPs (TIMPs)[70]. However, a very recent study using quantitative immunofluorescence co-localization confocal microscopy of platelets suggested instead that while α-granule functionally-synergistic cargo may be clustered *within* the granule, no evidence was found for organized subsets of α-granules with distinct biological activities[71].

Given the implications for therapeutic manipulation, the hypothesis that platelets may selectively release pro-angiogenic mediators over anti-angiogenic factors and enhance tumour growth or healing is an area of intense interest. The selective release of angiogenic simulators versus inhibitors appears to be counter-regulated by specific proteinase-activated receptors (PARs)[68, 72]. Treatment of human platelets with a selective PAR-1 agonist specifically released VEGF granules, while a selective PAR-4 agonist resulted in the release of endostatin but not VEGF[68]. There is some evidence that tumours may modulate the release of angiogenic factors from platelets by the secretion of specific PAR ligands or, alternatively, by proteolytic degradation of opposing PAR receptors[72]. Many tumour cell lines express PAR-1[73] and overexpression of PAR-1 in B16 melanoma cells increases experimental pulmonary metastasis 5-fold[74]. Furthermore, *in vitro* studies have demonstrated that a breast cancer cell line MCF-7 selectively stimulates platelets to release VEGF and pro-angiogenic releasate, an effect which was inhibited by aspirin treatment[75]. Whether ligation of PAR receptors, or indeed other signalling pathways on megakaryocytes, alters the packaging of proteins into nascent platelets by megakaryocytes is not known.

Platelets in malignancy

Platelets and cancer have been associated since the observation of Trousseau in the 1860s that a migratory thrombophlebitis due to blood clotting and inflammation of the blood vessels was indicative of an occult carcinoma. He later diagnosed the same syndrome in himself and died soon afterwards of gastric carcinoma[76]. It has since been well established that advanced malignancy is associated with platelet abnormalities and hypercoagulability. Higher platelet counts correlate with poor prognosis, and depletion of platelets in animal models reduces metastases in both xenograft and syngeneic tumour models and for a wide range of cancer cell lines[77–79]. Roles for platelets in cancer progression are reviewed elsewhere[80, 81] and outlined briefly below (Table 3).

1. *Primary tumour angiogenesis*: The transition of tumours from an avascular state to the active recruitment of blood vessels into the tumour, termed the ‘angiogenic switch’, marks the onset of aggressive and invasive growth[82–85], and a dose-response relationship exists between blood platelet levels and new vessel sprouting in angiogenesis. Platelets may also mediate the recruitment and retention of bone marrow-derived endothelial progenitor cells to sites of vasculogenesis[86–88] and their subsequent maturation[89–92].

2. *Immune evasion:* It is thought that circulating tumour cells travel through the circulation coated with platelets that ‘shield’ them from immune destruction. Experimental animal models of haematogenous metastasis suggest that platelets interfere with natural killer (NK) cell anti-tumoural activity[93, 94], possibly via platelet release of TGFβ[94]. Other studies have also shown that the capability of both mouse and human tumour cell lines to activate platelets correlates with their metastatic efficiency[95, 96].

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3. *Tumour cell adhesion/invasion at metastatic sites:* Formation of platelet-tumour cell aggregates has been shown to enhance lodgement at metastatic sites via platelet expression of adhesion integrins, in particular GPIIb-IIIa (αIIbβ) and GPIb-IX [97–99]. Once bound to the endothelial cell surface, platelets provide growth factors, coagulation factors and MMPs (Table 3), which support the accumulation of more platelets, leukocytes and tumour invasion. A recent report has suggested that direct cell contacts between platelets and tumour cells during their transit through the bloodstream, and in particular platelet-derived TGFβ and NF-κB signalling, induces cancer cells to undergo an epithelial-mesenchymal-like transition thereby enhancing their metastatic and invasory phenotype[100].

Bone marrow vasculature in haematological malignancy

Tumour vascularisation is well recognized as a fundamental pathological factor in ‘solid’ tumours and there is increasing evidence that the progression of haematological malignancy also requires the induction of new blood vessels within the bone marrow. Increased bone marrow angiogenesis has been reported in acute and chronic leukaemias[101, 102], myelofibrosis and myelodysplastic syndromes[10–105] and multiple myeloma[106]. Bone marrow of acute and chronic leukaemia patients shows enhanced VEGF expression as well as increased microvessel density[101–103, 107]. Leukaemic cells may secrete VEGF and can induce upregulation of VEGF production by BM stromal cells including megakaryocytes[108]. VEGF also has autocrine effects on leukaemic cells, and both VEGF signalling and direct contact with the sinusoidal endothelium, have been shown to promote survival, proliferation, motility of human leukaemia cells and also resistance to chemotherapy[109–111]. The persistence of leukaemic stem cells following chemotherapy is thought to be responsible for disease persistence and relapse, and localization of malignant cells at the vascular niche may confer resistance to chemotherapy and radiotherapy[8, 26, 112, 113].

The bone marrow as a ‘metastatic microenvironment’

The bone marrow is a common site for metastatic disease. Steven Paget’s ‘seed and soil’ hypothesis introduced the concept that a receptive microenvironment was required for malignant cells to engraft distant tissues and form metastases[114]. This microenvironment is now known to comprise supportive (non-malignant) stromal cells, soluble factors, vascular networks, nutrients and metabolic components, and the extracellular matrix[83, 115–119]. A ‘tumour-permissive’ inflammatory microenvironment also contributes to tumour progression by downregulating anti-tumoural immunity[120].

There is evidence that tumour cells preferentially home to, and engraft, the vascular stem cell niche in preference to other areas of bone marrow[121, 122]. Studies in mice using dynamic, *intra-vital* confocal imaging indicated that fluorescently-labelled human Nalm-6 leukaemic cells injected into immunodeficient mice preferentially engraft the bone marrow vascular niche via SDF1-CXCR4 interactions, suggesting analogous dependence on niche-specific signals to those required by physiological bone marrow-derived cells[121]. Other cell lines, including murine and human leukaemias, multiple myeloma, prostate and melanoma cell lines engrafted perivascular bone marrow regions in a similar fashion[121]. It is well established from both human and animal studies that expression of the CXCR4 receptor on breast, prostate and other carcinomas increases the risk of bone marrow metastases (reviewed in Burger and Kipps, 2006 and Taichman et al., 2002).

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Megakaryocytes/platelets in bone marrow metastasis

Despite the accumulating evidence that platelets play important pathological roles in tumourigenesis, metastasis, angiogenesis and inflammation, there have been very few studies examining bone marrow histology and specifically megakaryocytes in metastatic disease. This is perhaps in part due to limited availability of clinical specimens and technical challenges associated with isolation of intact cells from the bone marrow.

An early histological study examined megakaryocyte ploidy in postmortem bone marrow aspirates from 30 patients with metastatic carcinoma (half of whom had paraneoplastic thromboembolic events) and compared these to 3 control groups: patients with localized carcinoma, patients with thromboses but no carcinoma, and healthy controls[123]. Megakaryocyte ploidy was found to be significantly higher in patients with metastatic disease, regardless of whether they had associated thromboembolic disease, than in any of the control groups[123]. The authors speculated that the increased megakaryocyte ploidy resulted in increased platelet production and altered platelet heterogeneity, although this was not specifically examined in this study.

After the bone marrow and the spleen, the next main reservoir for megakaryocytes is the lung, where they may be observed within alveolar capillaries albeit in low numbers. It has been reported that the numbers of pulmonary megakaryocytes associated with lung metastases and pulmonary tumour emboli are higher than in lungs from patients with localized carcinomas and healthy individuals [124–126]. In a study of mammary carcinoma metastasis in rats, highly metastatic cell line clones implanted in the mammary fat pad induced increased numbers of megakaryocytes in the bone marrow which was attributed to secretion of a growth factor with GM-CSF/IL-3-like effect[127]. This was not apparent in animals bearing poorly metastatic tumours.

Megakaryocyte/platelet surface integrin αIIb/β3 may be involved in tumour colonization of the bone marrow[128]. Mice deficient in β3 or mice receiving specific αIIb/β3 inhibitors are protected from spontaneous bone metastases. Notably, following direct intrafemoral tumour cell delivery, although tumour growth occurred in the bone marrow of $\beta3^{-/-}$ mice, there was no associated bone destruction apparent, suggesting that interactions between platelets/megakaryocytes and tumour cells support tumour-mediated bone destruction[128].

Concluding remarks

Many questions about the role of megakaryocytes in the vascular niche both under physiological conditions and in malignancy remain unanswered. Firstly, the functional contribution of megakaryocytes to the vascular niche and the pathways that determine the net effects of megakaryocyte-derived factors (e.g. the pro- versus anti- angiogenic stimuli) have not been fully clarified. Secondly, the pathways that mediate homing of circulating tumour cells to the bone marrow vascular niche are not fully understood. While a wealth of evidence supports an integral role for platelets in metastasis and the recruitment of bone marrow-derived haematopoietic cells to sites of vasculogenesis, the role of megakaryocytes in recruitment of malignant cells to bone marrow niches has been relatively little studied. It is possible that tumour-derived factors, either acting systemically from distant primary tumours or more directly via tumour cells resident in the bone marrow, may influence the megakaryocyte/platelet ‘phenotype’. Indeed, there are data to suggest that platelet gene expression is altered in metastatic lung cancer as compared to controls[129]. As well as altering megakaryocyte secretion of tumour-regulatory factors within the bone marrow microenvironment, an alteration in the packaging of these factors into nascent platelets would modulate the ‘pro-malignant’ platelet phenotype with widespread, systemic effects.

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Acknowledgments

B.P. received funding from a Kay Kendall Leukaemia Fund Travelling Fellowship and a Fulbright Scholarship in Cancer Research. D.L. receives support from the NCI; Children's Cancer and Blood foundation; Stavros S. Niarchos Foundation; Champalimaud Foundation; Nancy C. and Daniel P. Paduano Foundation; Hartwell Foundation; Witmer Family Foundation; Malcolm Hewitt Wiener Foundation; Mary Kay Ash Charitable Foundation; the AHEPA 5th District; National Foundation for Cancer Research and Susan G. Komen for the Cure. I.R. is funded by Leukaemia & Lymphoma Research Specialist Programme Award 08030 and the Imperial College London Comprehensive Biomedical Research Centre.

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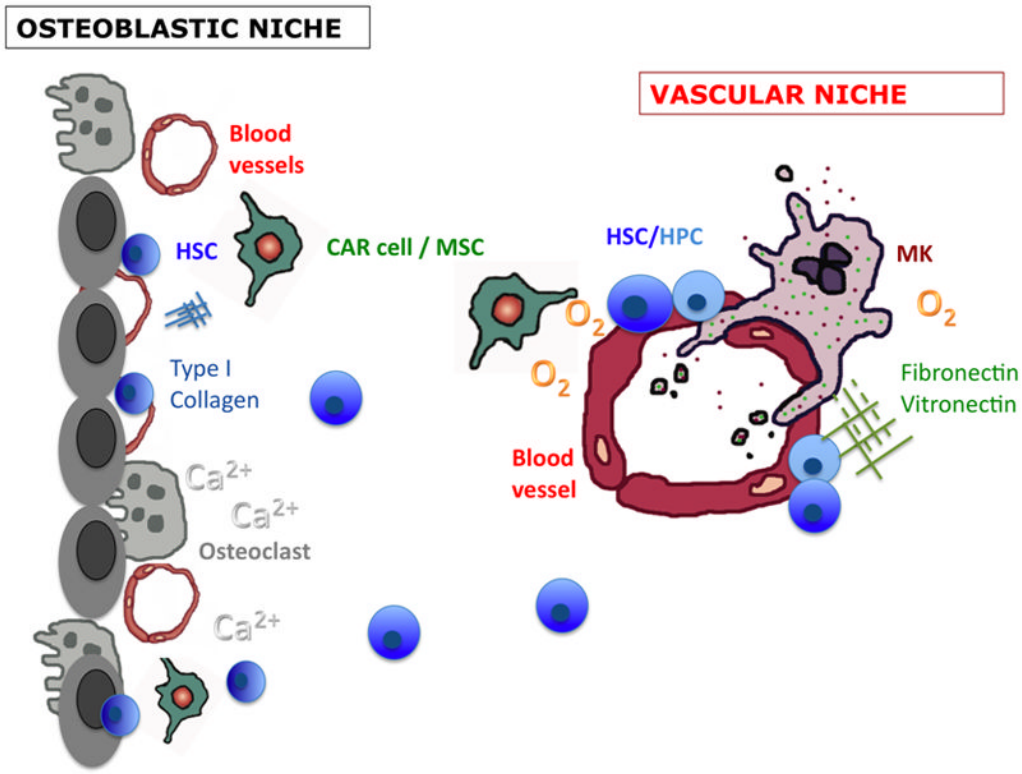

Figure 1. Cells, matrix factors and metabolic constituents contributing to osteoblastic and vascular bone marrow niches

The osteoblastic niche. Osteoblasts and osteoclasts secrete numerous factors that regulate haematopoietic stem cells (HSCs; dark blue). Bone resorption releases calcium ions (Ca^{2+}) influencing HSCs through the Ca^{2+}-sensing receptor. The endosteal surface is also heavily invested with blood vessels. Vascular and perivascular cells, such as CXCL12 (SDF1)-producing reticular cells (CAR) and mesenchymal stem cells (MSCs) are also thought to contribute to HSC niches at the endosteum. The relative hypoxia and abundance of Type I collagen inhibit proplatelet formation by megakaryocytes.

The vascular niche. Perivascular sites maintain HSCs and haematopoietic progenitor cells (HPCs; lighter blue) in the bone marrow and in extramedullary sites including spleen, liver and the placenta. A wide variety of vascular and perivascular cells are likely to contribute to vascular niches, including endothelial cells, CAR cells/MSCs and possibly megakaryocytes. Matrix factors fibronectin, vitronectin and Type III and IV collagen are found around blood vessels, which enhance megakaryocyte development and function.

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Table 1

Cell-extrinsic soluble factors essential for HSC maintenance and their source in bone marrow niches

Factor	Receptor	Role/Function	Primary Source
SCF (kit ligand)	c-Kit	HSC localization, survival and maintenance.	Bone marrow stromal cells[130]
SDF1	CXCR4	Principal axis determining HSC/HPC localization [131]. Promotes surface integrin upregulation and HSC survival [45, 132].	Mesenchymal stem cells [36] Reticular cells [34] Osteoblasts
Ang-1	Tie2	Promotes maintenance of quiescent HSCs	Osteoblasts[15], endothelial cells, pericytes[133]
TPO	TPO receptor (c-Mpl)	Regulates stem cell quiescence [16, 134]	Liver, kidney, bone marrow stroma, osteoblasts [42]
Ca^{2+} ions	G-protein coupled Ca^{2+}-sensing receptor	Maintains localization of HSCs near the endosteal bone lining[19]	Osteoclast-mediated bone degradation

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Table 2

Megakaryocyte-derived factors that may influence haematopoietic niches

Osteoblastic niche	**Osteoclastogenic promoters**	RANKL[135]
	Osteoclastogenic inhibitors	Osteoprotegerin[57, 135]
	Osteoblast promoters/ Mesenchymal growth factors	PDGF, FGF2, TGFβ, fibronectin-binding surface integrins[56, 58]
	Bone matrix proteins	Osteocalcin, osteonectin, osteopontin, bone sialoprotein, BMP-2, -4 and -6. [56]
Vascular niche	**Proangiogenic factors**	VEGF, PDGF, TGFβ IGF-2, b-FGF (FGF2), hepatocyte growth factor (HGF), epidermal growth factor (EGF), SDF1 (CXCL12), Lipid mediators e.g. sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) and MMPs[68, 136]
	Antiangiogenic factors	TSPs, Endostatin, Plasminogen activator inhibitor (PAI) type 1, TGFβ, PF4 (CXCL4)[68, 136]
	Chemokines	SDF1[64, 88] (synthesized in low amounts by megakaryocytes-largely taken up from the microenvironment), PF4[137]

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Table 3

Roles of platelet/megakaryocyte-derived proteins in carcinogenesis and metastasis

Protein	Function in carcinogenesis/metastasis
TGFβ	• Suppresses growth of early tumours but later promotes tumour invasion and migration[138]. • Inhibits NK cell-mediated tumour cell destruction[94] • Activates TGFβ/Smad and NF-κB pathways in cancer cells inducing their transition to an invasive mesenchymal-like phenotype[100]
SDF1	• Recruits $CXCR4^{+}$ tumour cells, bone marrow-derived HPCs and endothelial progenitors to sites of vasculogenesis[88].
PF4	• Inhibits growth and metastasis of murine models of melanoma and colon carcinoma[139–141]. • Regulates vasculature by inhibiting endothelial cell proliferation and migration[142, 143].
TSP1	Both pro- and anti-metastatic effects have been reported. • Inhibits endothelial cell migration, proliferation[144] and tumour angiogenesis[64, 145] • TSP1 mRNA and protein expression is higher in metastatic breast carcinoma than in patients with locally invasive, non-metastatic disease[146, 147]. • Stimulates tumour cell adhesion to vessel walls[148] • TSP1 knock-down was associated with reduced metastasis to blood and to lung in spontaneous mouse models of breast cancer metastasis, thought to be due to TSP1-induced activation of TGFβ. [149]. • Mediate haplotaxis and chemotaxis of human melanoma and carcinoma cells *in vitro*[150]
P-selectin, vWF, GPIIb/IIIa and Ib-IX	• Mediate initial rolling/tethering of circulating tumour cells to endothelium in vessels of metastatic organs[97–99].
MMP9, MMP2	• Digestion of local tissue matrix enabling invasion of local tissues and activation via cleavage of surface-bound factors[151]
Angiogenic growth factors	See Table 2.
Coagulation factors: Thrombin, tissue factor	• In tumour cells lines B16-F10 and UMCL, thrombin promotes GRO-a and Twist gene expression leading to a more malignant phenotype, enhancing cell motility and angiogenesis. • Inhibition of thrombin decreased tumour growth and metastasis in 4TI mice that develop spontaneous breast carcinoma[152–154] • Tissue factor expression mediates haematogenous metastasis of melanoma and generation of thrombin[155, 156].
Bioactive lipids: S1P and LPA	• S1P promotes vascular maturation by mediating N-cadherin function[157]. • LPA receptors are found in human breast cancer and platelet LPA stimulates osteolytic bone metastases in studies using the breast cancer cell line MDA-BO2 [158]. • LPA mediates proliferation, migration, and invasion of MDA-BO2 cells through activation of a Ga/ERK1/2-dependent signalling pathway[159]. • LPA and S1P act directly on endothelial cells to stimulate angiogenesis and also induce breast cancer cells to VEGF[159].