Megakaryocytes (MKS) are important components of the hematopoietic niche. Compared to the non-hematopoietic niche cells, MKs serving as part of the hematopoietic niche provides a mechanism for feedback regulation of hematopoietic stem cells (HSCs), in which HSC progeny (MKS) can modulate HSC adaptation to hematopoietic demands during both steady-state and stress hematopoiesis. MKs are often located adjacent to marrow sinusoids. Considering that most HSCs reside close to a marrow vascular sinusoid, as do MKs, the interactions between MKs and vascular endothelial cells are positioned to play important roles in modulating HSC function, and by extrapolation, might be dysregulated in various disease states. In this review, we discuss the interactions between MKs and the vascular niche in both normal and neoplastic hematopoiesis.

Keywords: megakaryocyte (MK), vascular niche, hematopoietic stem cell (HSC), myeloproliferative neoplasms, hematopoietic microenvironment

INTRODUCTION

Hematopoietic stem cells (HSCs) reside in a specified microenvironment, or the “stem cell niche”. Technical advancements in imaging HSCs in the marrow cavity, coupled with a series of elegant functional studies in murine models, have identified a number of HSC niche cells (e.g., perivascular stromal cells, endothelial cells, nerve cells) that provide the secreted factors and cell-surface molecules essential for HSC maintenance and function (1–5). It has become increasingly evident that the cellular niche components exist in close proximity to one another, and that the interaction between these cells contributes to the resilience and function of HSCs. Megakaryocytes (MK) are rare polyploid marrow cells that give rise to blood platelets. Recent evidence has also implicated MKs in regulating HSC quiescence and proliferation during both steady-state and stress hematopoiesis, mediated by the many cytokines and extracellular matrix components produced by these cells (6–12). In addition, MKs express many inflammatory and immunologic surface markers and signaling molecules and may participate in pathogen surveillance and immune response (13–18). MKs are often located adjacent to marrow sinusoids, a “geography” required for the cells to issue platelets directly into the sinusoidal vascular lumen (19, 20). Vascular endothelial cells (ECs) are also an essential component of the hematopoietic niche with most HSCs found adjacent to a marrow sinusoid (the “vascular niche”) (3, 21–24). Considering that MKs, sinusoids, and HSCs are closely located to each other, the interactions between MKs and ECs are positioned to play an important role in modulating HSC function. Few studies have examined the role of MKs in the regulation of stem cell vascular niche function, despite MKs representing a major source of both proangiogenic and antiangiogenic factors in the marrow. Here, we review the interactions between MKs and the vascular niche in both normal and neoplastic hematopoiesis.
MEGAKARYOCYTES ARE AN IMPORTANT COMPONENT OF THE HEMATOPOIETIC NICHE

Recent evidence has implicated MKs in regulating HSC quiescence and proliferation during both steady-state and stress hematopoiesis (6–12). For example, MKs produce TGFβ1 and CXCL4 to promote HSC quiescence in vivo (8, 9); during stress hematopoiesis (e.g., after chemotherapy), the production of TGFβ1 and CXCL4 by MKs surrounding clusters of myeloid progenitor cells may help re-establish HSC quiescence (25). MKs can also promote HSC niche remodeling (e.g., osteoblast expansion) following radiation injury (26, 27) and activate HSC proliferation under stress by synthesizing FGF1 and IGF-1 (6–8). Spatial positioning of HSCs next to MKs not only regulates HSC activity, but also affects HSC developmental potential — HSCs located adjacent to MKs are more platelet- and myeloid-biased compared to HSCs enriched in other regions of the marrow (e.g., the arteriolar niche), and MK depletion can reprogram these myeloid-biased HSCs to a lineage-balanced HSCs (28). Taken together, these studies indicate that the functional effects of MKs in the HSC niche are context-dependent and can be tailored based on demands. Compared to the non-hematopoietic niche cells (e.g., endothelial cells, mesenchymal stromal cells), the MK niche provides a mechanism for a feedback regulation of HSCs by their own progeny. The adaptation of the MK niche function to various hematopoietic demands suggests that HSC and its niche can affect each other, which is critical for the resilience and function of the hematopoietic system.

THE INTERACTIONS BETWEEN VASCULAR ENDOTHELIAL CELLS AND MEGAKARYOCYTES IN THE HEMATOPOIETIC NICHE

MKs are the largest cells in the marrow with a mean diameter of ~20–50um, with their cell sizes correlating with their degree of maturation (19, 29). MKs are mostly sessile, exhibiting minimal migration in a highly crowded environment (19, 29). ~70% of marrow MKs are intimately associated with the sinusoids, a “geography” required for the cells to issue platelets by the forces generated by flowing sinusoidal blood (19, 20, 30). Ultrastructural observations (31) and intravital imaging (19, 29) revealed that these MKs extend both perivascular pseudopodia to anchor the cells to endothelium, and long transendothelial cellular processes (or proplatelets) to release platelets into the flowing sinusoidal blood. Under inflammatory conditions or acute platelet needs, platelet release can also occur via MK rupture (32). In both cases (proplatelet formation and MK rupture), MKs must reside next to the sinusoids to release platelets into the bloodstream.

The MK-EC interactions are regulated by chemokines [e.g., CXCL12 (20, 33–36), FGF4 (20, 37, 38)], adhesion molecules [e.g., VE-cadherin (20), VCAM-1 (20, 39), PECAM-1 (40, 41)], and blood lipids (e.g., sphingosine-1-phosphate or S1P) (42), through many MK cell surface receptors (e.g., CXCR4, a4b5, VLA-4, S1P receptor) (20, 34, 43). ECs have an important role in the regulation of MK maturation and release of platelets (20, 33, 42). A functional vascular niche is critical for platelet production; disrupting the marrow vascular niche (e.g., by inhibiting VE-cadherin which supports EC integrity) impairs MK maturation and thrombopoiesis, while enhancing MK-EC interaction (e.g., by administering CXCL12 or FGF4) increases platelet production (20). (Table 1)

MKs also regulate vascular EC function, mediated by the numerous cytokines and growth factors produced by these MK cells. MKs are important sources for both the pro-angiogenic factors such as vascular endothelial growth factor (VEGF) (44, 45) and fibroblast growth factors (FGFs) (6, 48), and the anti-angiogenic factors such as thrombospondin (46) and platelet factor 4 (49). Italiano and colleagues reported that these pro- and anti-angiogenic factors are stored in different alpha-granules and can undergo selective release upon different stimulus (50), providing a mechanism by which MK can differentially stimulate or inhibit the vascular niche under different physiologic and pathologic conditions. These MK vascular regulatory factors may be a key determinant of marrow vascular niche function. However, despite recent progress in understanding the unique process of megakaryocyte lineage development (51–53) and the close interactions between MKs and vascular ECs in the marrow (19, 29) and lung (18, 54), few studies have examined the effects of MKs on vascular niche function and their contributions to normal and neoplastic hematopoiesis.

### Table 1

| Factor         | Effects on MK-EC interactions                                                                                           | References |
|----------------|-----------------------------------------------------------------------------------------------------------------------|------------|
| CXCL12         | Promotes the interactions of MKs with the BMECs and the transendothelial migration of MKs in vivo.                     | (20, 33–36) |
| FGF4           | Promotes MK maturation and the adhesion of MKs to ECs both in vitro and in vivo.                                       | (20, 37, 38) |
| VE-cadherin    | Supports the integrity and formation of BMECs. Neutralizing antibodies to VE-cadherin block FGF4-mediated MK adhesion to BMECs and CXCL12-induced MK transendothelial migration in vivo. | (20)       |
| VEGF-A         | Promotes angiogenesis and regulates vascular permeability/integrity.                                                    | (44, 45)   |
| VCAM-1         | Supports the attachment of MKs to HUVECs in vitro. Antibodies to VCAM-1 inhibit FGF4-mediated MK adhesion to BMECs and CXCL12-induced MK transendothelial migration in vivo. | (20, 39)   |
| PECAM-1        | Regulates MK migration towards the vascular niche through modulating the CXCR4 receptor and adhesion molecules (e.g., α4β1) of MKs. | (40, 41)   |
| Thrombospondin | An anti-angiogenic regulator that inhibits marrow vascular regeneration following myelosuppression and inhibits thrombopoiesis. | (46, 47)   |
ALTED MK- VASCULAR NICHE INTERACTIONS IN MYELOPROLIFERATIVE NEOPLASMS

In addition to normal cell physiologic studies, the mechanisms by which MKs regulate the vascular niche function can be better understood by assessing the dysregulated MK-EC interactions found in neoplastic hematopoiesis. The myeloproliferative neoplasms (MPNs), which include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), are stem cell disorders characterized by hematopoietic stem/progenitor cell expansion and overproduction of mature blood cells. Patients with MPNs are often characterized by increased marrow angiogenesis (55–57) and MK hyperplasia (58) when compared to normal marrow. In this section, we will discuss the dysregulated MK-EC interactions and their contributions to the neoplastic hematopoiesis in MPNs.

Megakaryocytes Are an Important Component of the Perivascular Stem Cell Niche in MPNs

MK hyperplasia is a hallmark feature of MPNs (58) and many MPN-associated genetic mutations/deregulations are preferentially enriched in MKs (59–61). The acquired kinase mutation JAK2V617F plays a central role in MPNs, having been found in virtually all patients with PV and about half of the patients with ET or PMF. To study the effects of a JAK2V617F-bearing MK niche on MPN disease development in vivo, we crossed mice that bear a Cre-inducible human JAK2V617F transgene (termed Flip-Flop, or FF1) (62) with the Pf4-cre mice [which bear a Cre recombinase driven by the MK-specific platelet factor 4 promoter (63)] to express JAK2V617F specifically in MKs (Pf4-FF1). We found that the JAK2V617F mutant MKs promote the development of an ET phenotype — at 6-mo of age, JAK2V617F transgenic mice manifest modest thrombocytosis, splenomegaly, 2-3 fold increased numbers of marrow MKs, and 2-3 fold increased numbers of HSCs compared to control mice (64). We observed a strong correlation between marrow MK numbers and HSC cell numbers in the Pf4-FF1 mice, and marrow HSCs in the JAK2V617F-bearing mice are more quiescent and display increased engraftment capacity (65). These results indicate that both HSC number and function are increased in the JAK2V617F-bearing MK niche.

We found that JAK2V617F-driven MK hyperplasia is accompanied by changes in the vascular niche: not only are marrow sinusoids more dilated and MKs are more preferentially located near the marrow sinusoids, but also there is an increased sinusoidal vascular density in the Pf4-FF1 mice compared to age-matched control mice (64). The effect of JAK2V617F MKs on vascular niche function was studied using tube formation assay (as a measure of in vitro angiogenesis) and scratch assay (as a measure of in vitro EC migration) — we found that conditioned medium from the JAK2V617F mutant MK culture significantly stimulated EC tube formation and EC migration compared to conditioned medium from wild-type MKs (64). These findings suggest that JAK2V617F mutant MKs can expand the marrow sinusoidal vascular niche, which in turn could contribute to the thrombocytosis and HSC expansion phenotype.

The JAK2V617F Mutation Alters Megakaryocyte-Endothelial Cell Interactions in the Vascular Niche

In addition to mutant blood cells (including MKs), the JAK2V617F mutation is also present in isolated liver, spleen, and marrow ECs from patients with MPNs (66–68). To study the effects of the JAK2V617F mutation on vascular niche function, we employed a murine model in which mice that bear the Cre-inducible human JAK2V617F transgene (FF1) (62) were crossed with a Tie2-Cre transgenic mouse (69) to express JAK2V617F in all hematopoietic cells (including HSCs and MKs) and ECs (Tie2-FF1) (70–72). As expected, these mice developed a robust MPN phenotype characterized by neutrophilia, thrombocytosis, splenomegaly, and hematopoietic stem/progenitor cell (HSPC) expansion within 2 months of birth. Histological examination of marrow hematoxylin/eosin sections showed that, compared to control mice, there are markedly increased numbers of MKs in the Tie2-FF1 mice and many clusters of MKs are preferentially located near marrow sinusoidal vessels (70). Ex vivo co-culture assays revealed that not only do JAK2V617F-bearing ECs directly stimulate JAK2V617F mutant MK expansion, which in turn contributes to HSPC expansion, but also that JAK2V617F mutant MK-conditioned medium significantly stimulates JAK2V617F EC migration compared to that of wild-type MK-conditioned medium. Immunofluorescence staining and deep confocal imaging revealed that MK-EC contact is significantly increased in the Tie2-FF1 mice compared to WT control mice (Figure 1). These data indicate that the JAK2V617F mutation can alter the MK-EC interactions in the vascular niche to promote the neoplastic hematopoiesis in MPNs.

Megakaryocyte Niche Function Evolves During Hematopoietic Aging in MPNs, and This Is Associated With Changes in its Regulation of the Vascular Niche

Aging within the HSC compartment contributes to many age-related diseases including an increased incidence of hematological malignancies in the elderly. HSC aging is characterized by an expansion of phenotypically defined HSCs with impaired function, such as reduced engraftment and self-renewal capacity, a perturbed state of quiescence, and a skewed differentiation towards the myeloid lineage (73, 74). Studies over the past decade suggest that HSC aging is driven by both cell-intrinsic alterations in the stem cells (75–79), and cell-extrinsic mediators from the aged microenvironment in which the stem cells reside (80–83). The relative contribution of intrinsic and extrinsic mechanisms to HSC aging remains debated. One key question is whether microenvironmental alterations initiate HSC aging or whether aged HSCs cause niche remodeling.

In contrast to the non-hematopoietic niche cells, niche MKs provide direct feedback to their HSC precursors, many of which
are located directly adjacent to MKs in vivo (8, 9). We investigated the effects of a JAK2V617F-bearing MK niche on HSC aging using the same Pf4+FF1+ murine model in which the JAK2V617F mutation is expressed exclusively in the MK lineage (64, 84). The mice maintain an essential thrombocythemia phenotype during a 2-yr follow-up, with no evidence of transformation to leukemia or myelofibrosis. Compared to age-matched control mice, the Pf4+FF1+ mice demonstrated an acceleration of several hallmarks of HSC aging, including an increase in the absolute numbers of HSCs with myeloid-skewed hematopoiesis (and maintenance of this myeloid skewing during marrow transplantation), a reduced engraftment and self-renewal capacity, and a reduced differentiation capacity (84). We showed that the JAK2V617F mutant MK niche can promote hematopoietic aging mediated by enhanced HSC proliferation/cycling. Both flow cytometry analysis and confocal whole-mount imaging revealed decreased marrow EC numbers (especially CD45 CD31+Sca1+ sinusoidal marrow ECs) and decreased marrow vascular areas in 2yr old Pf4+FF1+ compared to age-matched control mice (84). We noticed altered morphology of the marrow sinusoids in the aged marrow ECs when compared to marrow sinusoids in aged control mice (Figure 2) (84). Conditioned medium collected from JAK2V617F mutant MKs of aged Pf4+FF1+ mice significantly inhibited EC tube formation in vitro (84), which differs from what we have observed in the young (6-mo old) Pf4+FF1+ mice (64). While the pro-inflammatory factors [IL-6 (85–87), IL-12 (88), MIP-1a (89)] and anti-angiogenic factors [Fas ligand, IL-10 (90, 91)] are upregulated in old JAK2V617F mutant MKs compared to young mutant MKs, their levels are downregulated in aged control MKs compared to young control MKs. These data suggest that the JAK2V617F-bearing MKs inhibit/disrupt the vascular niche during aging, which in turn can promote HSC aging.

Despite recent technical breakthroughs in both imaging and functional studies of HSCs in the marrow, we know very little about how the niche changes with age. Our studies showed that the effect of JAK2V617F mutant MKs on HSC function changes during aging in a murine model of MPN: in young mice, mutant MKs expand the marrow sinusoidal vascular niche, induce HSC quiescence with increased repopulating capacity (64, 65); in aged mice, mutant MKs inhibit/disrupt the vascular niche, promote HSC proliferation with a reduced engraftment and self-renewal capacity (84). Despite these changes in MK niche function during aging, how MKs regulate vascular niche function appears to correlate with how MKs regulate HSC function.

CONCLUDING REMARKS

Further work is needed to functionally test the importance of MK-EC interaction in niche/HSC function and to understand the molecular mechanisms that mediate these effects. Murine...
models remain an irreplaceable approach to investigating MK-EC interaction in vivo in both normal and neoplastic hematopoiesis. Patient-derived induced pluripotent stem cells (iPS) provide an important alternative to model human disease in vitro. The capability to differentiate these iPS cell lines towards a specific cell lineage (e.g., MK (92, 93), EC (94)) is a powerful tool to model human diseases with acquired genetic alterations such as the JAK2V617F mutation. It is hoped that such studies will lead to novel therapeutic strategies to target the HSC niche-forming MKs and their interactions with the vascular niche, and to provide better treatments for patients with various aging and neoplastic conditions.

**REFERENCES**

1. Schepers K, Campbell TB, Passegue E. Normal and Leukemic Stem Cell Niches: Insights and Therapeutic Opportunities. Cell Stem Cell (2015) 16 (3):254–67. doi: 10.1016/j.stem.2015.02.014

2. Yu VW, Scadden DT. Hematopoietic Stem Cell and Its Bone Marrow Niche. Curt Top Dev Biol (2016) 118:21–44. doi: 10.1016/fs.cdb.2016.01.009

3. Crane GM, Jeffery E, Morrison SJ. Adult Haematopoietic Stem Cell Niches. Nat Rev Immunol (2017). doi: 10.1038/nri.2017.53

4. Pinho S, Frenette PS. Haematopoietic Stem Cell Activity and Interactions With the Niche. Nat Rev Mol Cell Biol (2019) 20(5):303–20. doi: 10.1038/s41580-019-0103-9

5. Mendez-Ferrer S, Bonnet D, Steensma DP, Hasserjian RP, Ghobrial IM, Gribben JG, et al. Bone Marrow Niches in Haematological Malignancies. Nat Rev Cancer (2020) 20(5):285–98. doi: 10.1038/s41568-020-0245-2

6. Zhao M, Ross JT, Ikin T, Perry JM, Venkatraman A, Haug JS, et al. FGF Signaling Facilitates Postinjury Recovery of Mouse Hematopoietic System. Blood (2012) 120(9):1831–42. doi: 10.1182/blood-2011-11-393991

7. Heazlewood SY, Neaves RJ, Williams B, Haylock DN, Adams TE, Nilsson SK. CXCL4 Secretion. J Biol Chem (2014) 20(11):1315–20. doi: 10.1038/nm.3707

8. Zhao M, Perry JM, Marshall H, Venkataraman A, Qian P, He XC, et al. Megakaryocytes Maintain Homeostatic Quiescence and Promote Post-Injury Regeneration of Hematopoietic Stem Cells. Nat Med (2014) 20(11):1321–6. doi: 10.1038/nm.3706

9. Bruns I, Lucas D, Pinho S, Ahmed J, Lambert MP, Kunisaki Y, et al. Megakaryocytes Regulate Hematopoietic Stem Cell Quiescence Through CXCL4 Secretion. Nat Med (2014) 20(11):1315–20. doi: 10.1038/nm.3707

10. Malara A, Currao M, Gruppi C, Celesti G, Viarengo G, Buracchi C, et al. Megakaryocytes Contribute to the Bone Marrow-Matrix Environment by Expressing Fibronectin, Type IV Collagen, and Laminin. Stem Cells (2014) 32 (4):926–37. doi: 10.1002/stem.1626

11. Nakamura-Ishizu A, Takubo K, Kojima M, Suda T. Megakaryocytes are Essential for HSC Quiescence Through the Production of Thrombopoietin. Biochem Biophys Res Commun (2014) 454(2):353–7. doi: 10.1016/j.bbrc.2014.10.095

12. Nakamura-Ishizu A, Takubo K, Kobayashi H, Suzuki-Inoue K, Suda T. CLEC-2 in Megakaryocytes is Critical for Maintenance of Hematopoietic Stem Cells in the Bone Marrow. J Exp Med (2015) 212(12):2133–46. doi: 10.1084/jem.20150057

13. Shiraki R, Inoue N, Kawasaki S, Takei A, Kadotani M, Ohnishi Y, et al. Expression of Toll-Like Receptors on Human Platelets. Thromb Res (2004) 113(6):379–85. doi: 10.1016/j.thromres.2004.03.023

14. Beaulieu LM, Lin E, Morin KM, Tannahervi K, Freedman JE. Regulatory Effects of TLIR2 on Megakaryocytic Cell Function. Blood (2011) 117(22):5963–74. doi: 10.1182/blood-2010-09-304949

15. Crist SA, Elzeby BD, Ahmann MT, Ratliff TL. Early Growth Response-1 (EGR-1) and Nuclear Factor of Activated T Cells (NFAT) Cooperate to Mediate CD40L Expression in Megakaryocytes and Platelets. J Biol Chem (2013) 288 (47):33985–96. doi: 10.1074/jbc.M113.511881

**AUTHOR CONTRIBUTIONS**

HZ wrote the manuscript; KK reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

**FUNDING**

This research was supported by the National Heart, Lung, and Blood Institute grant NIH R01 HL134970 (HZ) and VA Merit Award BX003947 (HZ).
31. Ishibata S, Nagaoaki M, Kunishima S, Sawaguchi A, Sakata A, Sakaguchi H, et al. IL-1α Induces Thrombopoiesis Through Megakaryocyte Rupture in Response to Acute Platelet Needs. J Cell Biol (2015) 209(3):453–66. doi: 10.1083/jcb.201410052

32. Hamada T, Mohle R, Hesselgesser J, Hoxie J, Nachman RL, Moore MA, et al. Transendothelial Migration of Megakaryocytes in Response to Stromal Cell-Derived Factor 1 (SDF-1) Enhances Platelet Formation. J Exp Med (1998) 188(5):539–48. doi: 10.1084/jem.188.3.5339

33. Wang JF, Liu ZY, Groopman JE. The Alpha-Chemokine Receptor CXCR4 is Expressed on the Megakaryocytic Lineage From Progenitor to Platelets and Modulates Migration and Adhesion. Blood (1998) 92(3):756–64. doi: 10.1182/blood.V92.3.756.

34. Lane WJ, Dias S, Hattori K, Heissig B, Choy M, Rabbany SY, et al. Stromal-Derived Factor 1-Induced Megakaryocyte Migration and Platelet Production is Dependent on Matrix Metalloproteinases. Blood (2000) 96(13):4152–9. doi: 10.1182/blood.V96.13.4152

35. Niswander LM, Fegan KH, Kingsley PD, McGrath KE, Palis J. SDF-1 Dynamically Mediates Megakaryocyte Niche Occupancy and Thrombopoiesis at Steady State and Following Radiation Injury. Blood (2014) 124(2):277–86. doi: 10.1182/blood-2014-01-457638

36. Konishi H, Ochiya T, Yasuda Y, Sakamoto H, Muto T, Sugimura T, et al. HST-1: FGF-4 Stimulates Proliferation of Megakaryocyte Progenitors Synergistically and Promotes Megakaryocyte Maturation. Oncogene (1996) 13(1):9–19.

37. Konishi H, Ochiya T, Sakamoto H, Tsukamoto M, Yasuda Y, Muto T, et al. Effective Prevention of Thrombocytopenia Using Adenosine-Mediated Transfer of HST-1 FGF-4 Gene: In Vivo and In Vitro Studies. Leukemia (1997) 11(3):530–2.

38. Avraham H, Cowley S, Chi SY, Jiang S, Groopman JE. Characterization of Adhesive Interactions Between Human Endothelial Cells and Megakaryocytes. J Clin Invest (1993) 91(6):2378–84. doi: 10.1172/JCI116470

39. Dhanjal TS, Pendaries C, Ross EA, Larson MK, Protti MB, Buckley CD, et al. A Novel Role for PECAM-1 in Megakaryocytogenesis and Recovery of Platelet Counts in Thrombocytopenic Mice. Blood (2007) 109(10):4327–44. doi: 10.1182/blood-2006-10-050740

40. Wu Y, Welte T, Michaud M, Madri JA. PECAM-1: A Multifaceted Regulator of the Vascular Niche. Blood (2007) 110(3):851–9. doi: 10.1182/blood-2006-05-220878

41. Rafii S, Shapiro F, Pettengell R, Ferris B, Nachman RL, Moore MA, et al. Human Bone Marrow Microvascular Endothelial Cells Support Long-Term Proliferation and Differentiation of Myeloid and Megakaryocytic Progenitors. Blood (1995) 86(9):3353–63. doi: 10.1182/blood.V86.9.3353.bloodjournal1995132

42. Larson MK, Watson SP. Regulation of Proplatelet Formation and Platelet Release by Integrin Alphabeta3. Blood (2006) 108(5):1509–14. doi: 10.1182/blood-2005-11-011957

43. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive Production and Thrombin-Induced Release of Vascular Endothelial Growth Factor by Human Megakaryocytes and Platelets. Proc Natl Acad Sci U S A. (1997) 94(2):663–8. doi: 10.1073/pnas.94.2.663

44. Bobik R, Hong Y, Breier G, Martin JF, Erusalimsky JD. Thromboopoietin Stimulates VEGF Release From C-Mpl-Expressing Cell Lines and Haematopoietic Progenitors. FEBS Lett. (1998) 423(1):10–4. doi: 10.1016/s0014-5793(98)00056-8

45. Kopp HG, Hooper AT, Broekman MJ, Avecilla ST, Petit I, Luo M, et al. IL-1alpha Induces Thrombopoiesis Through Megakaryocyte Rupture and Responses of Platelet-Associated Female Fibroblast Growth Factor-2 (FGF-2) and Vascular Endothelial Growth Factor (VEGF). Thromb Haemost (2002) 88(5):834–42. doi: 10.1055/s-0037-1613311

46. Vannucchi AM, Rotunno G, Bartalucci N, Raugei G, Carrai V, Balliu M, et al. Clonal Analysis Unveils Self-Renewing Lineage-Restricted Progenitors Generated Directly From Hematopoietic Stem Cells. Cell (2013) 154(5):1112–26. doi: 10.1016/j.cell.2013.08.007

47. Rodriguez-Fraticelli AE, Wolok SL, Weinreb CS, Panero R, Patel SH, Jankovic M, et al. Clonal Analysis of Lineage Fate in Native Haematopoiesis. Nature (2018) 553(7667):212–6. doi: 10.1038/s41586-018-02158

48. Lane WJ, Dias S, Hattori K, Heissig B, Choy M, Rabbany SY, et al. Stromal-Derived Factor 1-Induced Megakaryocyte Migration and Platelet Production is Dependent on Matrix Metalloproteinases. Blood (2000) 96(13):4152–9. doi: 10.1182/blood.V96.13.4152

49. Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, et al. Inhibition of Angiogenesis by Recombinant Human Platelet Factor-4 and Related Peptides. Science (1997) 274(5297):77–9. doi: 10.1126/science.988470

50. Italiano JE Jr., Richardson JL, Patel-Hett S, Battinelli E, Zaslawsky A, Short S, et al. Angiogenesis is Regulated by a Novel Mechanism: Pro- and Antiangiogenic Proteins are Organized Into Separate Platelet Alpha Granules and Differentially Released. Blood (2008) 111(3):1227–33. doi: 10.1182/blood-2007-09-113837

51. Sanjuan-Pla A, Macaulay IC, Jensen CT, Woll PS, Luis TC, Mead A, et al. Platelet-Biased Stem Cells Reside at the Apex of the Haematopoietic Stem-Cell Hierarchy. Nature (2013) 502(7470):232–6. doi: 10.1038/nature12495

52. Tiedt R, Schomer T, Hao-Shen H, Skoda RC. Pf4-Cre Transgenic Mice Allow the Generation of Lineage-Restricted Gene Knockouts for Studying Megakaryocyte and Platelet Function. In Vivo. Blood (2007) 109(4):1503–6. doi: 10.1182/blood-2006-04-020362

53. Tiedt R, Schomer T, Hao-Shen H, Skoda RC. Pf4-Cre Transgenic Mice Allow the Generation of Lineage-Restricted Gene Knockouts for Studying Megakaryocyte and Platelet Function. In Vivo. Blood (2007) 109(4):1503–6. doi: 10.1182/blood-2006-04-020362

54. Zhan H, Ma Y, Lin CH, Kaushansky K. JAK2V617F-Mutant Megakaryocytes Contribute to Hematopoietic Stem/Progenitor Cell Expansion in a Model of Murine Myeloproliferation. Leukemia (2016) 30(12):2352–41. doi: 10.1038/leu.2016.114

55. Zhang Y, Lin CH, Kaushansky K, Zhan H. JAK2V617F Megakaryocytes Promote Hematopoietic Stem/Progenitor Cell Expansion in Mice Through Thrombopoietin/MPL Signaling. Stem Cells (2018) 36(11):1676–84. doi: 10.1002/stem.2888

56. Sozer S, Fiel MI, Schiano T, Xu M, Mascarenhas J, Hoffman R. The Presence of JAK2V617F Mutation in the Liver Endothelial Cells of Patients With Budd-
ChiarI Syndrome. *Blood* (2009) 113(23):3246–9. doi: 10.1182/blood-2008-11-191544

67. Rosti V, Villani L, Riboni R, Poletto V, Bonetti E, Tozzi L, et al. Spleen Endothelial Cells From Patients With Myelofibrosis Harbor the JAK2V617F Mutation. *Blood* (2013) 121(2):360–8. doi: 10.1182/blood-2012-01-404889

68. Helman R, Pereira WO, Marti LC, Campregher PV, Puga RD, Hamerschlag N, et al. Granulocyte Whole Exome Sequencing and Endothelial JAK2V617F in Patients With JAK2V617F Positive Budd-Chiari Syndrome Without Myeloproliferative Neoplasm. *Br J Haematol* (2018) 180(3):443–5. doi: 10.1111/bjh.14327

69. Constien R, Forde A, Lilienstein B, Gronre H, Nawroth P, Hammerling G, et al. Characterization of a Novel EGFP Reporter Mouse to Monitor Cre Recombination as Demonstrated by a Tie2 Cre Mouse Line. *Genes* (2001) 30(1):36–44. doi: 10.1002/gen.1030

70. Zhan H, Lin CHS, Segal Y, Kaushansky K. The JAK2V617F-Bearing Vascular Niche Promotes Clonal Expansion in Myeloproliferative Neoplasms. *Leukemia* (2018) 32(2):462–9. doi: 10.1038/leu.2017.233

71. Lin CHS, Zhang Y, Kaushansky K, Zhan H. JAK2V617F-Bearing Vascular Niche Enhances Malignant Hematopoietic Regeneration Following Radiation Injury. *Haematologica* (2018) 103(7):1160–8. doi: 10.3324/haematol.2017.185736

72. Zhang H, Yeware A, Lee S, Zhan H. A Murine Model With JAK2V617F Expression in Both Hematopoietic Cells and Vascular Endothelial Cells Recapitulates the Key Features of Human Myeloproliferative Neoplasm. *Front Oncol* (2021). doi: 10.1016/j.2021.08.24.457585

73. de Haan G, Lazare SS. Aging of Hematopoietic Stem Cells. *Trends Mol Med* (2019) 25(6):494–515. doi: 10.1016/j.trendsmolmed.2019.04.006

74. Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, et al. Cell Autonomous Expression of JAK2V617F Recapitulates the Key Features of Human Myeloproliferative Neoplasm. *Nat Med* (2018) 24(6):782. doi: 10.1038/s41591-018-0030-x

75. Rosti V, Villani L, Riboni R, Poletto V, Bonetti E, Tozzi L, et al. Endothelial Transplantation Rejuvenates Aged Hematopoietic Stem Cells. *STEM CELLS* (2019) 25(3):407–18.e6. doi: 10.1002/stem.2019.06.0007

76. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, et al. Requirement of MIP-1 Alpha for an Inflammatory Response to Viral Infection. *Science* (1995) 269(5230):1583–5. doi: 10.1126/science.7667639

77. Silvestre JS, Mallar Z, Duriez M, Tamarat R, Bureau MF, Scherman D, et al. Antiangiogenic Effect of Interleukin-10 in Ischemia-Induced Angiogenesis in Mice Hindlimb. *Circ Res* (2000) 87(6):448–52. doi: 10.1161/01.ress.87.6.448

78. Kohno T, Mizukami H, Suzuki M, Saga Y, Takei Y, Shimpo M, et al. Interleukin-10-Mediated Inhibition of Angiogenesis and Tumor Growth in Mice Bearing VEGF-Producing Ovarian Cancer. *Cancer Res* (2003) 63(16):5091–4.

79. Liu Y, Wang Y, Gao Y, Forbes JA, Qayyum R, Becker L, et al. Efficient Generation of Megakaryocytes From Human Induced Pluripotent Stem Cells Using Food and Drug Administration-Approved Pharmacological Reagents. *Stem Cells Trans Med* (2015) 4(4):309–19. doi: 10.5966/stcm.2014-0183

80. Li Y, Jin C, Bai H, Gao Y, Sun S, Chen L, et al. Human NOTCH4 is a Key Regulator of the Vascular Niche as Regulators of the Vascular Niche. *Nat Commun* (2020) 11(1):4075. doi: 10.1038/s41467-020-17942-7

81. O’Hagan-Wong K, Nadeau S, Carrier-Lecer F, Apablaza F, Hamdy R, Shum-Tim D, et al. Increased IL-6 Secretion by Aged Human Mesenchymal Stromal Cells Disrupts Hematopoietic Stem and Progenitor Cells’ Homeostasis. *Oncotarget* (2016) 7(12):13285–96. doi: 10.18621/oncotarget.6790

82. Trinchieri G. Interleukin-12: A Proinflammatory Cytokine With Immunoregulatory Functions That Bridge Innate Resistance and Antigen-Specific Adaptive Immunity. *Annu Rev Immunol* (1995) 13:251–76. doi: 10.1146/annurev.immunol.13.040195.001343

83. Guadall A, Lesteven E, Letort G, Awan Toor S, Delord M, Pognant D, et al. JAK2V617F Mutant Megakaryocytes Contribute to Hematopoietic Aging in a Murine Model of Myeloproliferative Neoplasm. *Stem Cells* (2022). doi: 10.1002/stem.2020.08.0005

84. Lee S, Song H, Castiglione M, Murphy M, Kaushansky K, Zhan H. JAK2V617F Mutant Megakaryocytes Contribute to Hematopoietic Aging in a Murine Model of Myeloproliferative Neoplasm. *Stem Cells* (2022). doi: 10.1002/stem.2020.08.0005

85. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harb Perspect Biol* (2014) 6(10):a016295. doi: 10.1101/cshperspect.a016295

86. Valletta S, Thomas A, Meng Y, Ren X, Drissen R, Sengul H, et al. Micro-Environmental Sensing by Bone Marrow Stromal Cells Identifies IL-6 and TGFbeta1 as Regulators of Hematopoietic Ageing. *Nat Commun* (2020) 11(1):4075. doi: 10.1038/s41467-020-17942-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.