View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Varna Medical University Press: Journals

Biomedical Reviews 2003; 14: 1-8.
Founded and printed in Varna, Bulgaria
ISSN 1310-392X

CONTRIBUTION OF HEMATOPOIETIC STEM CELLS IN BLOOD VESSEL FORMATION

Rika Okamoto, Yoshihiro Yamada, Hideto Sano, Masaya Ueno, and Nobuyuki Takakura
Department of Stem Cell Biology, Cancer Research Institute of Kanazawa University, Kanazawa, Japan

Vascular development consists of vasculogenesis and angiogenesis. The system of TIE2-Angiopoietin (Ang) is involved in angiogenesis. TIE2 regulates adhesion and dissociation between endothelial cells and mural cells, and survival, apoptosis, and chemotaxis of endothelial cells. Ang-2, which is produced by endothelial cells under tissue hypoxia, has been suggested to be a key regulator for the initiation of endothelial cell sprouting from pre-existing vessels. Although Ang-2 binds to TIE2, it does not promote activation of TIE2 on endothelial cells. Ang-2 produced from endothelial cells under hypoxia inhibits the binding of Ang-1 to TIE2. On the other hand, Ang-1 promotes activation of TIE2 and adhesion between endothelial cells and mural cells. Therefore, endothelial cells dissociated from mural cells by Ang-2 are free to move to avascular area where oxygen or nutrient is needed. We recently found that hematopoietic stem cells produce Ang-1 and promote chemotaxis and network formation of TIE2-positive endothelial cells. Moreover, hematopoietic stem cells change their fate into mural cell and stabilize the vessel structure. This novel function may be applied clinically to promote neovascularization by transplanting the hematopoietic stem cells at the desired site.
Biomed Rev 2003; 14: 1-8.

INTRODUCTION

Vascular development consists of two different steps, vasculogenesis and angiogenesis. Vasculogenesis is the process by which endothelial precursors, the angioblasts, are committed from mesodermal cells and form a primitive vascular plexus and larger organized vessels in the embryo. In contrast, angiogenesis involves vascular growth and maturation by sprouting and remodeling of existing vessels (1). In both processes, bidirectional signaling between endothelial cells (ECs) and the surrounding mesenchymal cells is critical (2). Several molecules have been isolated that regulate the processes of vasculogenesis-angiogenesis, and are involved in maintaining the integrity of vessels by recruitment and formation of the periendothelial layer or by mediating interactions between arteries and veins (2-5). Among them, two receptor tyrosine kinase subfamilies are characterized by their largely endothelial specific expression. One family includes Flt-1/VEGFR1, Flk-1/KDR/VEGFR2, and Flt-4/VEGFR3, all of which are members of the vascular endothelial growth factor (VEGF) receptor family. Critical roles of Flt-1, Flk-1 and Flt-4, as well as that of VEGF, have been demonstrated for these proteins by analysis of genetically engineered mice mutants (6-9). The other family includes TIE1/TIE and TIE2/TEK. The onset of embryonic expression of these receptors seems to follow that of the VEGF receptors (VEGFRs) (10). Targeted mutation of *TIE1* or *TIE2* demonstrates that these receptors, like VEGFRs, play a critical role in embryonic vascular formation (11-13). Embryos deficient in TIE2 or TIE1 fail to develop a structural integrity of the vasculature, resulting in hemorrhage at E9.5 and 13.5, respectively. Compared with the early defect in vasculogenesis seen in *VEGF* or *VEGFR* mutant embryos, mice

Received 8 June 2003 and accepted 30 September 2003.
Correspondence and reprint requests to Dr. Nobuyuki Takakura, Department of Stem Cell Biology, Cancer Research Institute of Kanazawa University, 13-1 Takara-machi, Kanazawa, 920-0934, Japan. Tel.: 81 76 2652751, Fax: 81 76 2344513.
E-mail: ntakaku@kenroku.kanazawa-u.ac.jp

lacking *TIE1* or *TIE2* exhibit later defects in angiogenesis and vascular remodeling as well as in vascular integrity.

Blood vessels generated by vasculogenesis are immature. For the maturation of vessel structure, periendothelial cells (PECs), such as pericytes or smooth muscle cells, have to adhere to ECs. This tight adhesion between ECs and PECs is promoted by PEC-derived Ang-1, a ligand for TIE2 on ECs (13-17). Therefore, under the condition that Ang-1 is continuously produced by PECs, vessel structure is stable. However, in this situation, even if oxygen is needed in the location, new vessel cannot sprout from pre-existing vessels because ECs are not free and cannot migrate easily. When hypoxia occurs in the location, Ang-2 production is usually up-regulated in the ECs (18). Ang-2 is an Ang-1 antagonist that inhibits the binding of Ang-1 to TIE2. Therefore under conditions of tissue hypoxia, PECs dissociate from ECs and ECs migrate into the region, where new vessels are necessary (Fig. 1). Though this sequence is currently widely accepted, how ECs migrate into the restricted region where new vessels are needed and how new vessels are stabilized again have not been clarified. In this system, we have found that hematopoietic stem cells (HSCs) induce network structure of blood vessel in the location (19) and stabilize the new vessel (unpublished data). In the current paper, we would like to overview functions and contributions of HSCs in angiogenesis.

RELATIONSHIP BETWEEN HEMATOPOIESIS AND ANGIOGENESIS

Hematopoiesis is closely linked to angiogenesis, since HSCs and ECs have common ancestors, the hemangioblasts, and interact with each other (20). Definitive HSCs closely adhere to ECs at several sites in the embryo, including yolk sac (21), omphalomesenteric and vitelline arteries, and dorsal aorta (16,20-24). In addition, some stromal cell lines that are able to support hematopoiesis have been characterized as ECs (25). These observations suggest a close interaction between hematopoiesis and vascular development. However, it is difficult to observe the crosstalk between ECs and HSCs *in vivo*. To address this issue, we established a culture system supporting angiogenesis and hematopoiesis using the para-aortic splanchnopleural (P-Sp) region (pre-Aorta-Gonad-Mesonephros (AGM) region), a site where definitive HSCs are committed from hemangioblasts (16,23,24). When P-Sp explants from the E9.5 embryos were cultured on OP9 stromal cells (16), PECAM-1-positive (PECAM-1$^+$) ECs form a sheet-like structure (vascular bed) and subsequently form a network in the periphery of the endothelial sheet. We observed the development of hematopoietic cells in this culture system. HSCs, which were initially located at the peripheral edge of the vascular bed, migrated into the vascular network area and

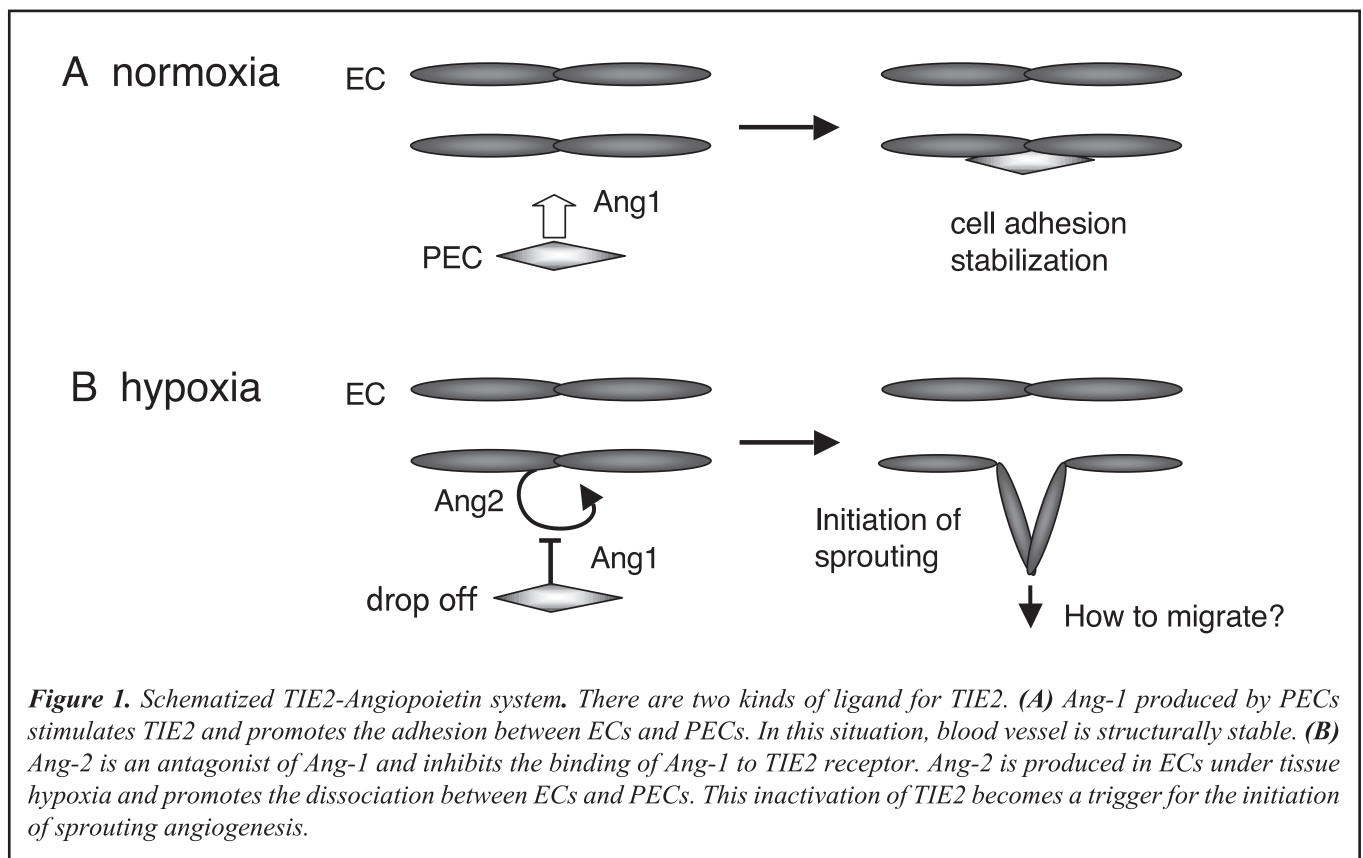

Figure 1. *Schematized TIE2-Angiopoietin system. There are two kinds of ligand for TIE2.* ***(A)*** *Ang-1 produced by PECs stimulates TIE2 and promotes the adhesion between ECs and PECs. In this situation, blood vessel is structurally stable.* ***(B)*** *Ang-2 is an antagonist of Ang-1 and inhibits the binding of Ang-1 to TIE2 receptor. Ang-2 is produced in ECs under tissue hypoxia and promotes the dissociation between ECs and PECs. This inactivation of TIE2 becomes a trigger for the initiation of sprouting angiogenesis.*

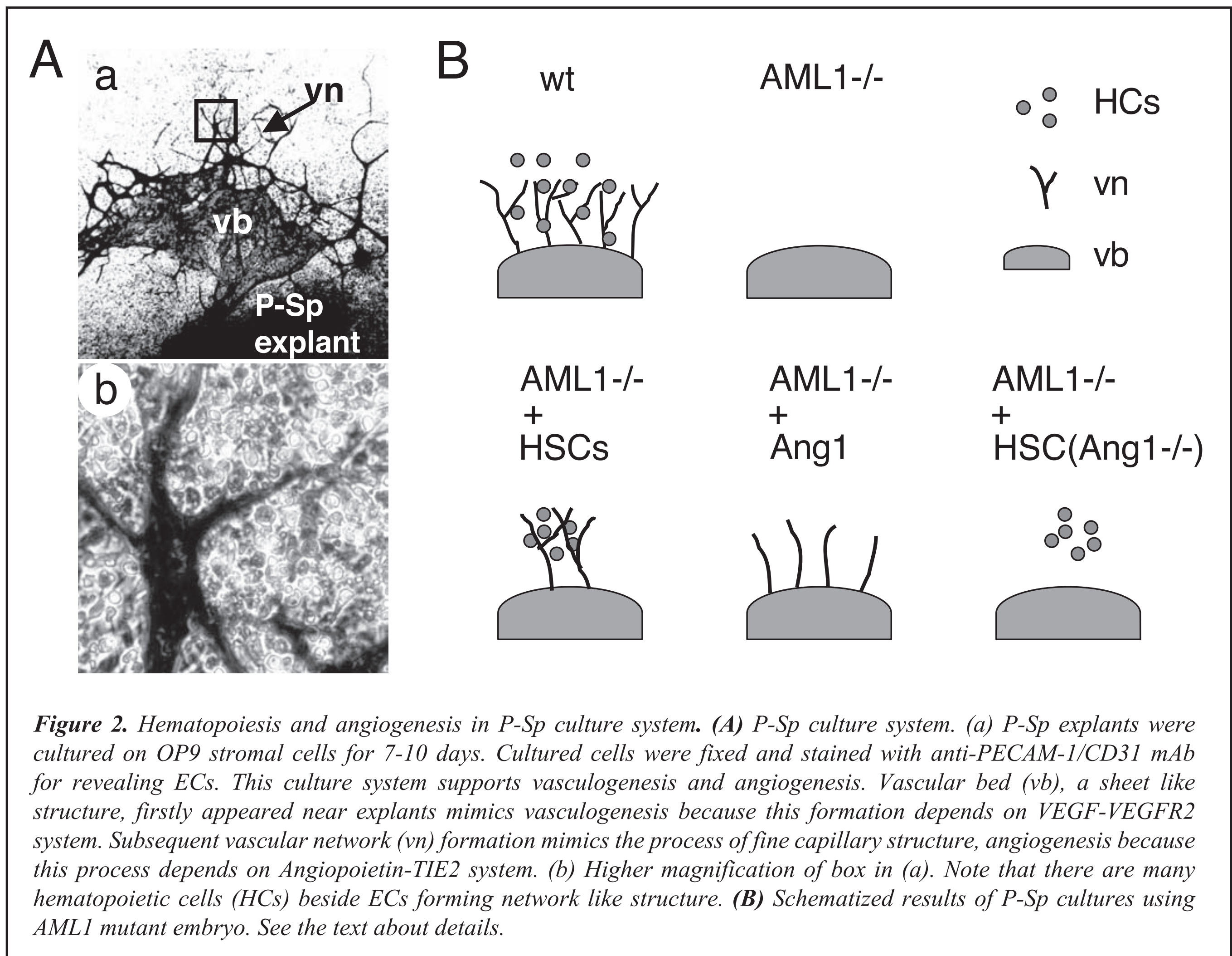

Figure 2. Hematopoiesis and angiogenesis in P-Sp culture system. ***(A)*** *P-Sp culture system. (a) P-Sp explants were cultured on OP9 stromal cells for 7-10 days. Cultured cells were fixed and stained with anti-PECAM-1/CD31 mAb for revealing ECs. This culture system supports vasculogenesis and angiogenesis. Vascular bed (vb), a sheet like structure, firstly appeared near explants mimics vasculogenesis because this formation depends on VEGF-VEGFR2 system. Subsequent vascular network (vn) formation mimics the process of fine capillary structure, angiogenesis because this process depends on Angiopoietin-TIE2 system. (b) Higher magnification of box in (a). Note that there are many hematopoietic cells (HCs) beside ECs forming network like structure.* ***(B)*** *Schematized results of P-Sp cultures using AML1 mutant embryo. See the text about details.*

proliferated (Fig. 2A). These data suggested that hematopoiesis and angiogenesis (vascular network) may occur concurrently and we hypothesized that hematopoietic cells may regulate angiogenesis.

On the other hand, we utilized mice deficient for the transcription factor AML1, which provide a tool to analyze the interaction between hematopoiesis and angiogenesis. Disruption of *AML1* gene leads to failure in the development of definitive hematopoiesis and lethality at E12.5 (26,27). Mutant embryos exhibit hemorrhages in the ventricles of the central nervous system, in the vertebral canal, within the pericardial space, and in the peritoneal cavity. AML1 deficient mice (28) showed that extensive vascular branching and remodeling into large and small vessels occurred normally in the head region at E11.5 as observed in wild type animals. However, the number of small capillaries sprouting from anterior cardinal vein in mutant embryos was less than that observed in wild type mice. In mutant embryos, less branching of capillaries was observed in vessels of the pericardium and in the vitelline artery of the yolk sac. Very strikingly, severe defective angiogenesis was observed in the fetal liver of mutant embryos (Fig. 3). In mice, liver development occurs at E10.5 and subsequently HSCs migrate into the fetal liver, where they expand and differentiate into various hematopoietic lineages. Hematopoiesis changes into definitive type in the fetal liver instead of primitive one in the yolk sac. Within this duration, very fine capillary structure that is the main environment of hematopoiesis in the fetal liver should be established. From the phenotype of AML1 deficient embryo, we concluded that hematopoietic cells are the major source for induction of very fine capillary structure in the fetal liver.

To analyze the interaction between HSCs and ECs, we observed the development of ECs in P-Sp cultures from *AML1* mutant embryos. As expected, hematopoietic cells (HCs) were not generated from P-Sp explants of *AML1* mutant embryos (Fig. 2, AML1-/-). In contrast, explants from wild type embryos developed many round hematopoietic cells adhering to the presumptive vascular network area and the wild type

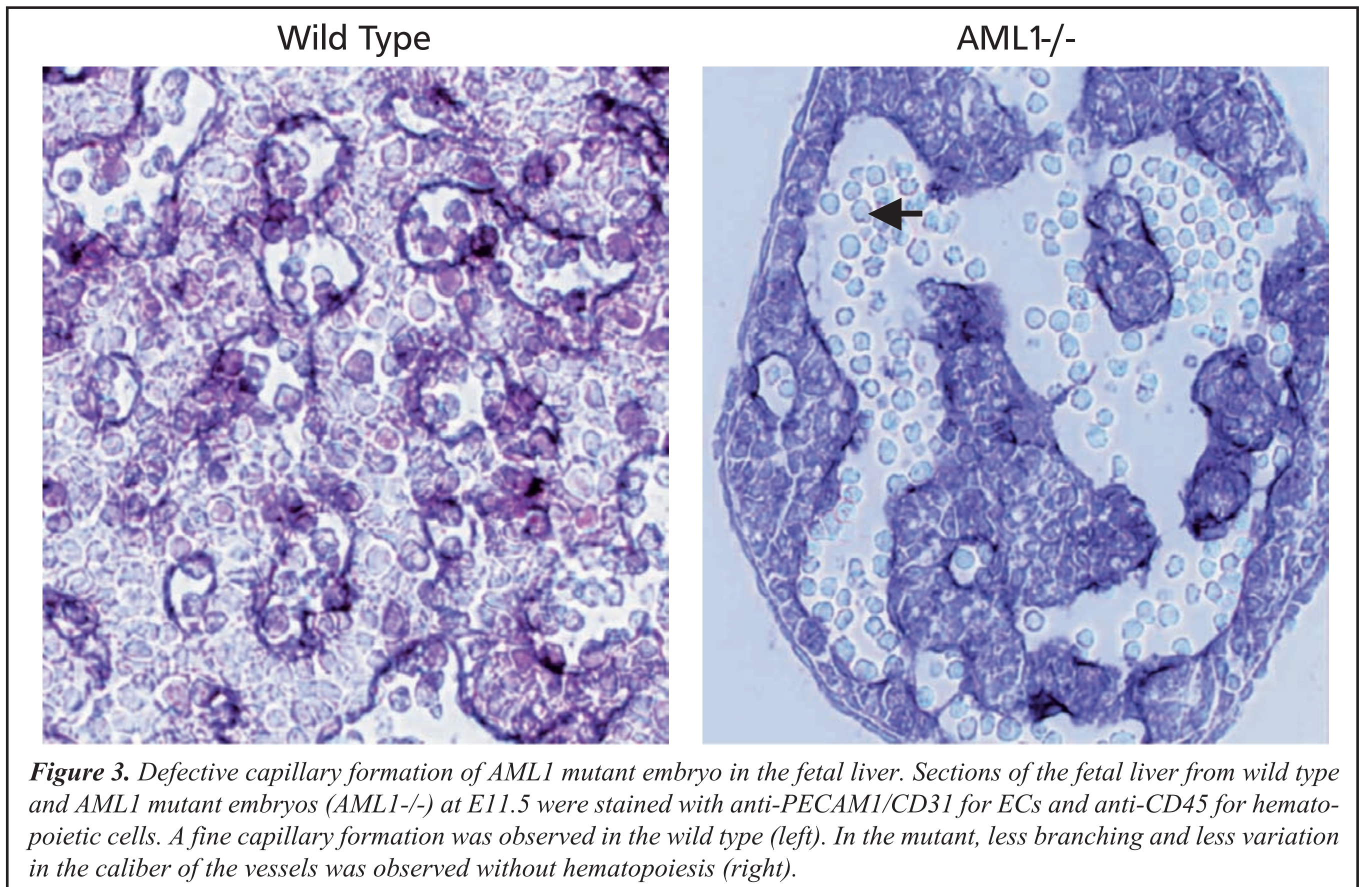

Figure 3. *Defective capillary formation of AML1 mutant embryo in the fetal liver. Sections of the fetal liver from wild type and AML1 mutant embryos (AML1-/-) at E11.5 were stained with anti-PECAM1/CD31 for ECs and anti-CD45 for hematopoietic cells. A fine capillary formation was observed in the wild type (left). In the mutant, less branching and less variation in the caliber of the vessels was observed without hematopoiesis (right).*

explants generated vascular beds and networks (Fig. 2, A and B, wt). On the other hand, poor vascular network formation was observed in cultures of *AML1* mutant explants (Fig. 2, AML1-/-). To test the hypothesis that HCs promote angiogenesis, HSC-enriched population from the bone marrow of normal mice was sorted by flow-cytometry and added to P-Sp cultures of *AML1* mutant embryos. As expected, the addition of HSCs rescued defective angiogenesis in *AML1* mutant embryos (Fig. 2, AML1-/- + HSCs).

Ang-1 PRODUCED FROM HSCs REGULATES ANGIOGENESIS

Since the results from the P-Sp culture system suggested that extrinsic signals from hematopoietic cells promote angiogenesis, we searched for factors that could mediate this process. During embryogenesis, $CD45^{+}c\text{-}Kit^{+}CD34^{+}$ cells are defined as HSCs (29). We found that these HSCs expressed Ang-1 that is essential for angiogenesis, and that mature hematopoietic cells in embryo did not express Ang-1. As mentioned above, Ang-1 produced from PECs promotes cell adhesion between ECs and PECs. It is well known that another action of Ang-1 is chemoattraction for ECs (30). Moreover, defective angiogenesis in anterior cardinal vein and pericardium observed in AML1 mutant embryos is quite similar to those observed in Ang-1 mutant embryos (data not shown). These findings suggested that Ang-1 expressed by HSC-enriched populations promotes angiogenesis by promoting chemotaxis of ECs. To test this hypothesis, we added HSCs from E10.5 Ang-1 mutants to P-Sp cultures of *AML1* mutant embryos. HSC-enriched cells from Ang-1 mutant embryos could not rescue the defective network formation of ECs of AML1 mutant culture (Fig. 2, AML1-/- + HSC(Ang-1-/-)). Furthermore, Ang-1 also rescued the network formation of ECs of AML1 mutant culture (Fig. 2, AML1-/- + Ang-1). Thus, we confirmed that Ang-1 from HSCs is important for the network formation of ECs, at least in our P-Sp culture system. To clarify how HSCs participate in angiogenesis in vivo, we examined whether they are present in the head region where severe angiogenic defects are observed in the *AML1* mutants. As expected, HSCs expressing c-Kit, a marker for HSCs located near the vessels and ECs seemed to migrate toward HSCs in the neuronal layer. These $c\text{-}Kit^{+}$ cells migrating in front of ECs also expressed CD45 and CD34, indicating that $c\text{-}Kit^{+}$ cells are in the HSC-enriched population. Based on the localization of HSCs and ECs, we hypothesized that HSCs may promote migration of ECs and network formation in the avascular area. To examine this potential, we studied the migration of $CD45^{-}PECAM\text{-}1^{+}TIE2^{+}$ ECs sorted from E10.5 embryos induced by Ang-1 or HSCs. Ang-1 led to

a dose-dependent increase in the directed migration of TIE2$^+$ ECs. As observed by Ang-1, CD45$^+$c-Kit$^+$CD34$^+$ HSCs from E10.5 embryos also promoted the migration of TIE2$^+$ ECs in a dose-dependent manner. This migration was completely suppressed by soluble TIE2 receptors. These findings indicate that the HSC-induced migration of TIE2$^+$ ECs depends on Ang-1. Therefore, we concluded that HSCs firstly migrate into avascular area, produce Ang-1 and promote migration of ECs and network like capillary formation. This scenario is not adapted to all situations in which angiogenesis is taking place, because *AML1* mutant embryos show normal angiogenesis except for anterior cardinal vein, capillaries in neuronal layer, pericardium, peritoneum, fetal liver, yolk sac, etc. However, for the migration of ECs into a proper direction, HSCs play an essential role by working as a guidepost and making a route from pre-existing vessel to ischemic region for ECs.

Although our findings indicate that ECs migrate towards Ang-1 producing HSCs, the fundamental mechanism of HSCs migration from the intraluminal cavity into parenchyma cells at a precise point of a vessel is unclear. A report that peripheral CD34$^+$ hematopoietic progenitors express high levels of matrix metalloproteinases (MMP)-2 and -9 (31,32) may shed light on this mechanism. Moreover, a recent study showed that mast cells in the skin release chymase, which activates pro-MMP-9 and is associated with stimulation of angiogenesis during squamous epithelial cell carcinogenesis (33). Our preliminary data also show that embryonic HSCs (CD45$^+$c-Kit$^+$CD34$^+$ cells) prominently express MMP-9 (data not shown). In addition, these HSCs express TIE2 and adhere to fibronectin following stimulation by Ang-1 (16). Together, these results suggest that HSCs adhere to fibronectin on ECs near the ischemic region, digest the matrix, and transmigrate through the basement membrane of capillary ECs into parenchyma cells. Therefore we hypothesize that the production of fibronectin on the intraluminal surface of ECs is the initial step in the migration of HSCs and ECs. Fibronectin is not usually observed in the intraluminal part of ECs in the adult. However, fibronectin is expressed ubiquitously on the luminal surface of vessels during embryogenesis at the time of angiogenesis. An analysis of the molecular cues promoting high fibronectin expression by ECs at the lumen may be required to better understand how vessel sprouting is initiated. Moreover, we found that there are molecules promoting migration of HSCs in the embryonic brain tissue and fetal liver (Okamoto R, unpublished data). Therefore, the mechanism how HSCs migrate into ischemic region will be clearly understood by isolation of such chemoattractive molecules in the future.

PLASTICITY OF HSCs

In the final step of angiogenesis, newly developed blood vessel should be stabilized again by the mural cell adhesion to ECs. It is widely accepted that PECs migrate from pre-existing vessels and cover the ECs of new vessels. However, it has been suggested that ECs transdifferentiate into PECs under specific conditions (34,35). This is reasonable, since ECs and PECs are major components of blood vessel and are originally developed from a common progenitor expressing Flk-1/VEGFR2 (36). However, we recently found that HSCs also have a capacity to differentiate into PECs.

Relating to such multipotentiality of HSCs for differentiation, papers showing the plasticity of HSCs have accumulated within a few years (37-40). These findings indicate that adult bone marrow HSC-enriched populations can differentiate into additional mature, nonhematopoietic cells of multiple tissues, including epithelial cells of the liver, kidney, lung, skin, gastrointestinal (GI) tract, and myocytes of heart and skeletal muscle. Although this indicates that HSCs might indeed be developmentally plastic and capable of producing both blood cells and another tissues, the mechanism underlying this phenomenon remains unclear. Two reports have raised the issue of whether fusion between donor hematopoietic cells and host hepatocytes could explain the apparent stem cell plasticity (41,42), and some researchers deny the plasticity of HSCs into multilineage descendants. Although definition of HSCs have been done by their positive expression of c-Kit/CD34/Sca-1 and negative expression of lineage specific markers (Lin) such as CD4, CD8, B220, Mac-1, Gr-1, Ter-119. These Lin$^-$c-Kit$^+$/Sca-1$^+$ or CD34$^+$ cells actually contribute to bone marrow reconstitution in the lethally irradiated recipient mice. However, it is unclear whether all HSCs isolated by means of those markers are committed to hematopoietic lineage alone. It is possible that there are several subpopulations among Lin$^-$c-Kit$^+$Sca-1$^+$ HSCs. For example, hematologists have believed that the definition of hematopoietic cells is performed by the analysis of expression of CD45, a pan-leukocyte antigen, and used CD45 as a certain hematopoietic marker. However, recently CD45 was shown to be expressed by the mesenchymal stem cell population in adult bone marrow (43). Therefore, what we call "HSCs" at present may not be a homogenous "hematopoietic" stem cell type, but rather a heterogeneous mix of stem cells.

Recent data from our lab suggests that HSCs-enriched population differentiates into PECs and promote maturation/stabilization of blood vessel (Yamada Y, Sano H, unpublished data). PECs were lost in AML1 mutant embryos that lack development of HSCs and show abnormal blood vessel structure and die by massive hemorrhage in brain, pericardium and other regions as already described. We found that PECs are located in the blood vessels of such regions in wild type embryos, and that HSC-enriched population sorted from brain of normal embryo could differentiate into PECs in a certain condition *in vitro*. Thus, HSCs population may contribute to mural cell lineage and stabilize the blood vessel structure.

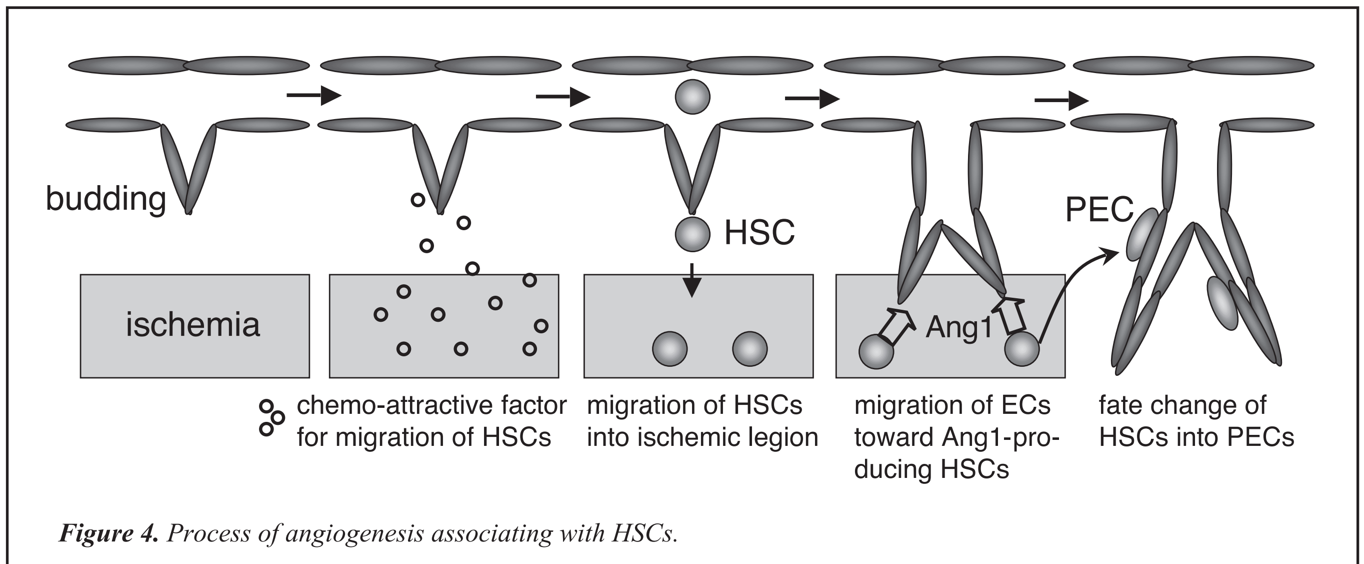

Figure 4. *Process of angiogenesis associating with HSCs.*

CONCLUSION

An important issue in vasculogenesis and angiogenesis, particularly in vessel sprouting, is the mode of ECs migration to sites where tissues require nutrients or oxygen. In this context, we propose the following scenario. As showed in Fig. 4, the blood vessel budding is firstly promoted by the overexpression of Ang-2 on ECs and subsequent dissociation between PECs and ECs. In hypoxic areas, attracting signals for the migration of HSCs are secreted by parenchymal cells. ECs follow and migrate toward HSCs as Ang-1 from HSCs promotes chemotaxis of ECs. Finally, PECs stabilize the newly developed vessel. In this case, HSCs play an important role by their differentiation into PECs.

So far, the use of Ang-1 has been considered in therapeutic angiogenesis, because Ang-1 promotes angiogenesis and inhibits the plasma leakage from tight adhesion between ECs and PECs. In our P-Sp culture system, soluble Ang-1 rescues the formation of network in *AML1* mutant mice *in vitro*. However, HSCs from embryos and adult mice could promote vascular network formation more effectively than soluble Ang-1 (as depicted in Fig. 2B). In transgenic mice expressing Ang-1 in the skin under a control of a keratinocyte-specific promoter, hypervascularity was observed in the dermis but not in another organs, indicating a localized effect of Ang-1 (44). The systemic administration of Ang-1 results in widespread stimulation of $TIE2^{+}$ ECs, while HSCs may promote localized angiogenesis. Therefore, the observations presented here may have important clinical applications. Indeed, therapeutic angiogenesis using autologous bone marrow HSCs started in patients with limb ischemia (Fig. 5).

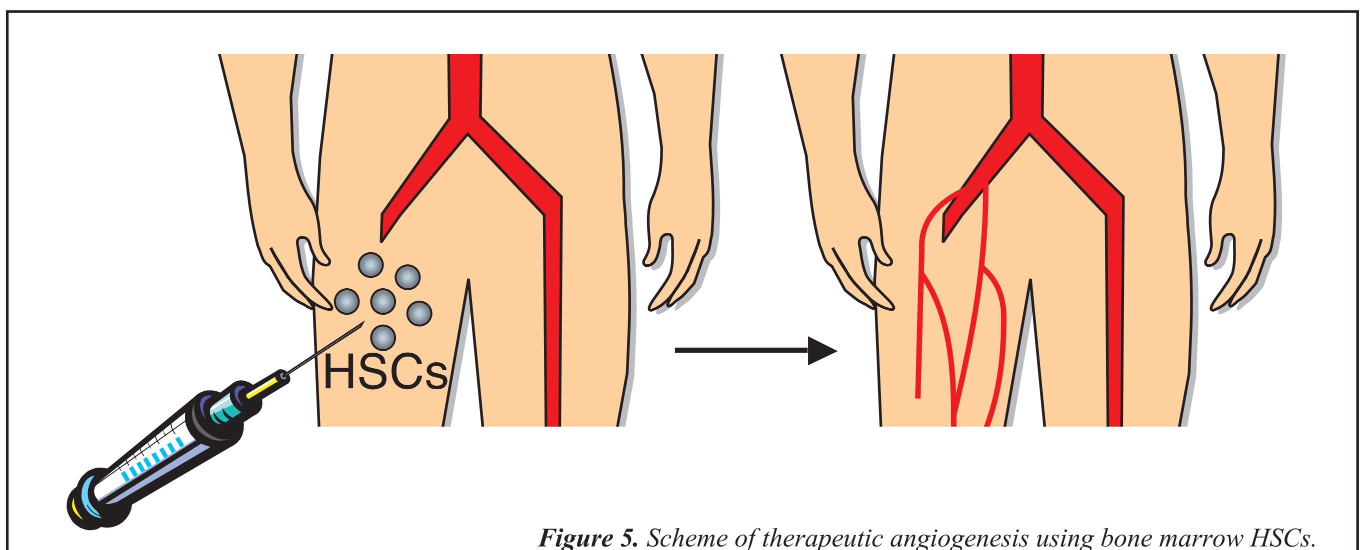

Figure 5. *Scheme of therapeutic angiogenesis using bone marrow HSCs.*

We are now testing whether or not HSCs also associate with tumor angiogenesis. Preliminary data show that HSCs often localize in the mass of tumor. When the migration of HSCs into the tumor is inhibited by an anti-c-Kit blocking antibody in a murine model, tumor angiogenesis and growth are inhibited (Okamoto R, manuscript in preparation). Therefore, we believe that the regulation of HSCs can be negatively and positively applied in therapeutic angiogenesis.

ACKNOWLEDGMENTS

We thank our collaborators Dr. G. Yancopoulos (Regeneron Pharmaceuticals, NY, USA), Dr. M. Satake and Dr. T. Watanabe (Tohoku University, Japan), and also Ms M. Sato and Ms Y. Shimizu for their technical assistance. Research was supported by grants-in-aid for scientific research (14028023, 13470207) from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. Risau W. Mechanisms of angiogenesis. *Nature* 1997; 386: 671-674.
2. Folkman J, D'Amore PA. Blood vessel formation: what is its molecular basis? *Cell* 1996; 87: 1153-1155.
3. Hanahan D. Signaling vascular morphogenesis and maintenance. *Science* 1997; 277: 48-50.
4. Wang HU, Chen Z-F, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 1998; 93: 741-753.
5. Gale NW, Yancopoulos GD. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. *Genes Dev* 1999; 13: 1055-1066.
6. Shalaby F, Rossant J, Tamaguchi TP, Gertsenstein M, Wu X-F, Breitman ML, *et al*. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995; 376: 62-66.
7. Fong G-H, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995; 376: 66-70.
8. Dumont DJ, Jussila L, Taipale J, Lymboussaki T, Mustonen T, Pajusola K, *et al*. Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. *Science* 1998; 282: 946-949.
9. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L., O'Shea KS, *et al*. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996; 380: 439-442.
10. Dumont DJ, Fong G-H, Puri MC, Gradwohl G, Alitalo K, Breitman ML. Vascularization of the mouse embryo: a study of *flk-1*, *tek*, *tie*, and vascular endothelial growth factor expression during development. *Dev Dyn* 1995; 203: 80-92.
11. Dumont DJ, Gradwohl G, Fong G-H, Puri MC, Gerstenstein M, Auerbach A, *et al*. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. *Genes Dev* 1994; 8: 1897-1909.
12. Puri MC, Rossant J, Alitalo K, Bernstein A, Partanen J. The receptor tyrosine kinase TIE is required for integrity and survival of vascular endothelial cells. *EMBO J* 1995; 14: 5884-5891.
13. Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, *et al*. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature* 1995; 376: 70-74.
14. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, *et al*. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* 1996; 87: 1161-1169.
15. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, *et al*. Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. *Science* 1997; 277: 55-61.
16. Takakura N, Huang X-L, Naruse T, Hamaguchi I, Dumont D J, Yancopoulos GD, *et al*. Critical role of the TIE2 endothelial cell receptor in the development of definitive hematopoiesis. *Immunity* 1998; 9: 677-686.
17. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, *et al*. Requisite role of angiopoietin-1, a ligand for the Tie-2 receptor, during embryonic angiogenesis. *Cell* 1996; 87: 1171-1180.
18. Oh H, Takagi H, Suzuma K, Otani A, Matsumura M, Honda Y. Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. *J Biol Chem* 1999; 274: 15732-15739.
19. Takakura N, Watanabe T, Suenobu S, Yamada Y, Noda T, Ito Y, *et al*. A role for hematopoietic stem cells in promoting angiogenesis. *Cell* 2000; 102: 199-209.
20. Eichmann A, Corbel C, Nataf V, Vaigot P, Breant C, Le Douarin NM. Ligand-dependent development of the endothelial and hemopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. *Proc Natl Acad Sci USA* 1997; 94: 5141-5146.
21. Moore MAS, Metcalf D. Ontogeny of the hematopoietic system: yolk sac of in vivo and in vitro colony forming cells in the developing mouse embryo. *Br J Haematol* 1970; 18: 279-296.
22. Dieterlen-Lièvre F, Martin C. Diffuse intraembryonic hematopoiesis in normal and chimeric avian development.

Dev Biol 1981; 88: 180-191.
23. Medvinsky A, Dierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* 1996; 86: 897-906.
24. Cumano A, Dieterlen-Lièvre F, Godin I. Lymphoid potential, probed before circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. *Cell* 1996; 86: 907-916.
25. Lu LS, Wang SJ, Auerbach R. In vitro and in vivo differentiation into B cells, T cells, and myeloid cells of primitive yolk sac hematopoietic precursor cells expanded > 100-fold by coculture with a clonal yolk sac endothelial cell line. *Proc Natl Acad Sci USA* 1996; 93: 14782-14787.
26. Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML-1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 1996; 84: 321-330.
27. Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the *Cbfa2* gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. *Proc Natl Acad Sci USA* 1996; 93: 3444-3449.
28. Okada H, Watanabe T, Niki M, Takano H, Chiba N, Yanai N, *et al*. AML1(-/-) embryos do not express certain hematopoiesis-related gene transcripts including those of the PU.1 gene. *Oncogene* 1998; 18: 2287-2293.
29. Yoder MC, Hiatt K, Dutt P, Mukherjee P, Bodine DM, Orlic D. Characterization of definitive lymphohematopoietic stem cells in the day 9 murine yolk sac. *Immunity* 1997; 7: 335-344.
30. Witzenbichler B, Maisonpierre PC, Jones P, Yancopoulos GD, Isner JM. Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. *J Biol Chem* 1998; 273: 18514-18521.
31. Janowska-Wieczorek A, Marquez LA, Nabholtz JM, Cabuhat ML, Montano J, Chang H, *et al*. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. *Blood* 1999; 93: 3379-3390.
32. Rafii S, Avecilla S, Shmelkov S, Shido K, Tejada R, Moore MA, *et al*. Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. *Ann N Y Acad Sci* 2003; 996: 49-60.
33. Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, *et al*. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999; 13: 1382-1397.
34. Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 2003; 108: 2070-2073.
35. Frid MG, Kale VA, Stenmark KR. Mature vascular endothelium can give rise to smooth muscle cells via endothelial-mesenchymal transdifferentiation: in vitro analysis. *Circ Res* 2002; 90: 1189-1196.
36. Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, Yurugi T, *et al*. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature* 2000; 408: 92-96.
37. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, *et al*. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001; 105: 369-377.
38. Orkin SH, Zon LI. Hematopoiesis and stem cells: plasticity versus developmental heterogeneity. *Nat Immunol* 2002 ;3:323-328.
39. Wagers AJ, Sherwood RI, Christensen JL, Weissman, IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 2002; 297: 2256-2259.
40. Orlic D. Adult bone marrow stem cells regenerate myocardium in ischemic heart disease. *Ann N Y Acad Sci* 2003; 996: 152-157.
41. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster, M, Al-Dhalimy M, *et al*. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003; 422: 97-901.
42. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, *et al*. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003; 425: 968-973.
43. Polesskaya A, Seale P, Rudnicki, MA. Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. *Cell* 2003; 113: 841-852.
44. Suri C, McClain J, Thurston G, McDonald DM, Zhou H, Oldmixon EH, *et al*. Increased vascularization in mice overexpressing angiopoietin-1. *Science* 1998; 282: 468-471.