Review

Roles of ZFAT in Haematopoiesis, Angiogenesis and Cancer Development

TOSHIYUKI TSUNODA[1,2] and SENJI SHIRASAWA[1,2]

[1]*Department of Cell Biology, Faculty of Medicine, and*
[2]*Central Research Institute for Advanced Molecular Medicine, Fukuoka University, Fukuoka, Japan*

Abstract. *A zinc-finger gene in autoimmune thyroid disease susceptibility region (ZFAT) was originally identified as a highly conserved immune-related transcriptional regulator containing one adenosine-thymidine (AT)-hook and 18 C_2H_2-type zinc-finger domains. Subsequently, roles of ZFAT in development, primitive haematopoiesis, angiogenesis, immune responses and several common diseases, such as multiple sclerosis, hypertension and cancer, have been demonstrated. Previously, we recorded a ZFAT protein expression in MOLT-4 human acute T-lymphoblastic leukaemia cells, while ZFAT knockdown activated caspases and induced apoptosis in these cells. Hence, the precise functions of ZFAT are of particular interest in cancer research. In this article, we have reviewed investigations on the roles of ZFAT in haematopoietic and angiogenesis, and discussed the possible involvement of ZFAT in haematopoietic malignancies.*

Recent studies have indicated a central role for the aberrant expression of transcription factors in the pathobiology of haematopoietic malignancies. Deregulated expression of these transcription factors, which are often functionally normal, leads to abnormal proliferation and differentiation arrest of lymphoid progenitors (1).

Through linkage and association analyses in a cohort of Japanese patients with autoimmune thyroid disease (AITD) (2, 3), we identified a zinc-finger gene in the AITD susceptibility region (ZFAT) (3). *ZFAT* contains 18 zinc-fingers domains and one AT-hook and is an evolutionally conserved gene from fish to human. ZFAT protein is highly expressed in T- and B-cells in the lymphoid tissues in adult mice (4) and plays critical roles in peripheral T-cell homeostasis and its receptor-mediated responses (5). We previously reported that *Zfat* deficiency ($Zfat^{-/-}$) in the mouse is embryonically lethal by embryonic day 8.5 and ZFAT is a critical transcriptional regulator for primitive haematopoiesis (6) and is functionally involved in regulation of apoptosis of mouse embryonic fibroblasts and MOLT-4 human acute T-lymphoblastic leukaemia cells (7, 8). We also found that ZFAT is expressed in human umbilical vein endothelial cells (HUVECs) and demonstrated that endothelial cell assembly and the branch point formation of capillary-like structures in HUVECs are impaired by the reduction of ZFAT expression through the use of *ZFAT* miRNA. These studies suggest that ZFAT is a key mediator of the development of specific cell lineages such as lymphocyte and endothelial cells, though the molecular and functional details in cancer development have not been determined. Here, we focus on the roles of ZFAT in the haematopoietic system and angiogenesis, and discuss the possible involvement of ZFAT in haematopoietic malignancies.

Correspondence to: Senji Shirasawa, Department of Cell Biology, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. E-mail: sshirasa@fukuoka-u.ac.jp

Key Words: ZFAT, transcription factor, haematopoietic system, angiogenesis, haematopoietic malignancies, TAL1, review.

The Role of ZFAT in the Haematopoietic System

During embryonic development, mesodermal progenitors give rise to haemangioblasts, which have differentiation potential for both endothelial and haematopoietic lineages (9-11). Haemangioblasts arise in the primitive streak and then migrate into extra-embryonic yolk sacs to form blood islands (12, 13). Blood islands are the foci of haemangioblasts, which form a luminal layer of endothelial cells that produce haematopoietic progenitor cells, and are eventually assembled into a functional vascular network that transfers nutrients from the yolk sac to the embryo proper (14, 15). Recent studies have revealed that T-cell acute lymphocytic leukemia 1 (SCL/TAL1), a basic helix–loop–helix transcription factor, is essential for differentiation of haemangioblasts into haemogenic endothelium (9, 16-20). TAL1 also plays pivotal roles in vascular and haematopoietic development when in complex with LIM domain only 2 (LMO2) and GATA-binding protein-1 (GATA1) (17, 21-25). LMO2 functions as a bridging molecule between TAL1 and GATA1 in the DNA-binding complex (22). GATA1 also functions as a key

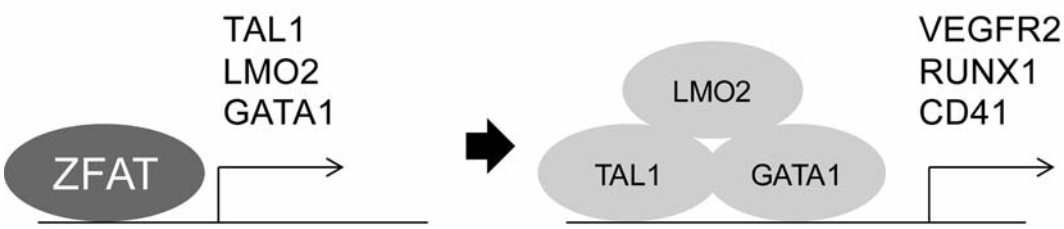

Figure 1. *Possible involvement of A zinc-finger gene in autoimmune thyroid disease susceptibility region (ZFAT) in haematopoietic system.*

molecule in the differentiation of erythroid lineages (26, 27). However, transcriptional regulation of upstream genes remains elusive. Recently, we found that *Zfat*$^{-/-}$ mice are embryonic-lethal, with impaired differentiation of haematopoietic progenitor cells in blood islands, precisely where ZFAT is highly expressed. Expression levels of *Tal1*, *Lmo2* and *Gata1* in *Zfat*$^{-/-}$ yolk sacs are greatly reduced compared with those of wild-type mice, and chromatin immunoprecipitation (ChIP)–polymerase chain reaction (PCR) analysis revealed Zfat binding to promoter regions of these genes *in vivo*. Furthermore, profound reductions in Tal1, Lmo2 and Gata1 protein expression were observed in *Zfat*$^{-/-}$ blood islands (6). We also found that vascular endothelial growth factor receptor-2 (Vegfr2), runt-related transcription factor 1 (Runx1) and integrin alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41) are directly regulated by the Zfat targets Tal1, Lmo2 and Gata1 (6) (Figure 1). These results suggest that Zfat is indispensable for mouse embryonic development, and functions as a critical transcription factor in primitive haematopoiesis. Currently, we are generating knock-in reporter mice to elucidate ZFAT expression during embryogenesis.

Role of ZFAT in Angiogenesis

ZFAT mRNA was ubiquitously expressed in tissues, but had lower expression levels in the non-immune-related tissues than in the immune-related tissues (3). This suggests that ZFAT might also play physiological roles in non-immune-related cells. Recently, genetic variants of *ZFAT* are reportedly associated with adult height in Japanese and Korean populations (28, 29), equine body size (30, 31) and with several other diseases including hypertension and cancer (32, 33). It is of great interest that a genetic variant of *ZFAT* was found to be strongly associated with interferon-β responsiveness in multiple sclerosis (34). These findings suggest that ZFAT might have critical roles in non-immune-related cells involved in human diseases or altered physiological phenotypes.

Angiogenesis is the cellular mechanism by which the primitive vasculature is remodeled into a mature vascular bed comprising arteries, capillary networks and veins (35). Vascular remodeling is an active process of structural alteration that involves changes in cellular processes, including cell growth, cell death and cell penetration into the extracellular matrix (36). The mechanisms of vascular remodeling, in which endothelial cells play pivotal roles, include penetration via the sprouting and branching of vessels into avascular regions. This is observed under physiological and pathological conditions, such as wound healing, neovascularization in tumors, inflammation, autoimmune diseases and obesity (37-40). We previously found evident expression of ZFAT in HUVECs, and evaluated the physiological roles for ZFAT in the angiogenic responses of HUVECs (41). We established HUVEC transfectants expressing *ZFAT* miRNA through the use of lentiviruses. The relative growth rates for HUVECs and HUVEC transfectants with *ZFAT* miRNA were not significantly different, indicating that the ZFAT in HUVECs is not involved in proliferation or apoptosis, which are essential components of vascular remodeling. The HUVEC transfectants with the control miRNA manifested assembly into capillary-like structures and the branch point formation of interconnected capillary-like structures, whereas the capillary-like network formations of the HUVEC transfectants with ZFAT miRNA were dramatically impaired. We further quantified the number of the segments and the segment lengths between these transfectants. The number of the segments of the HUVEC transfectants with ZFAT miRNA decreased, indicating impaired branch point formation in the capillary-like structures caused by the reduction in ZFAT expression. Furthermore, the average segment length of the capillary-like structures in the HUVEC-transfectants with *ZFAT* miRNAs was significantly higher, indicating that impaired branch point formation due to the reduction of ZFAT expression culminated in the increase in the length of each segment in the capillary-like structure (Figure 2). Indeed, Lazrak *et al*. demonstrated that ectopic expression of wild-type *TAL1* accelerated the formation of capillary-like structures *in vitro* and *in vivo* (42). These findings suggest that ZFAT may regulate TAL1 in HUVECs and play various physiological roles depending on the cell type and environmental conditions.

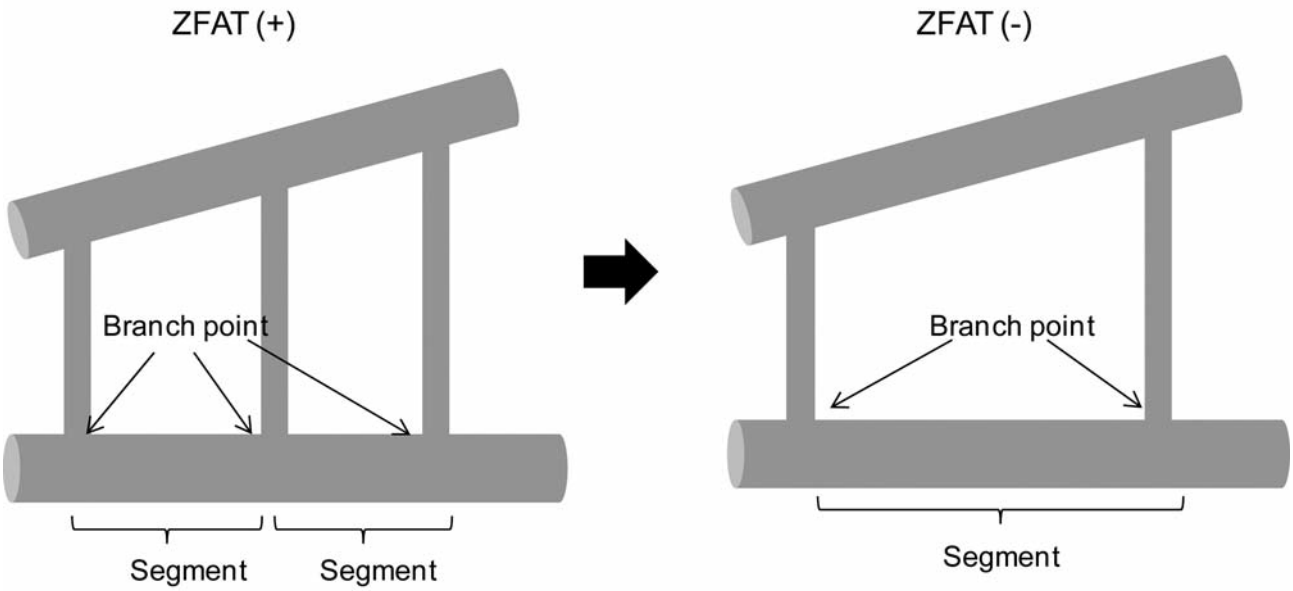

Figure 2. *Schematic illustration of capillary-like structure with (+) and without (–) A zinc-finger gene in autoimmune thyroid disease susceptibility region (ZFAT).*

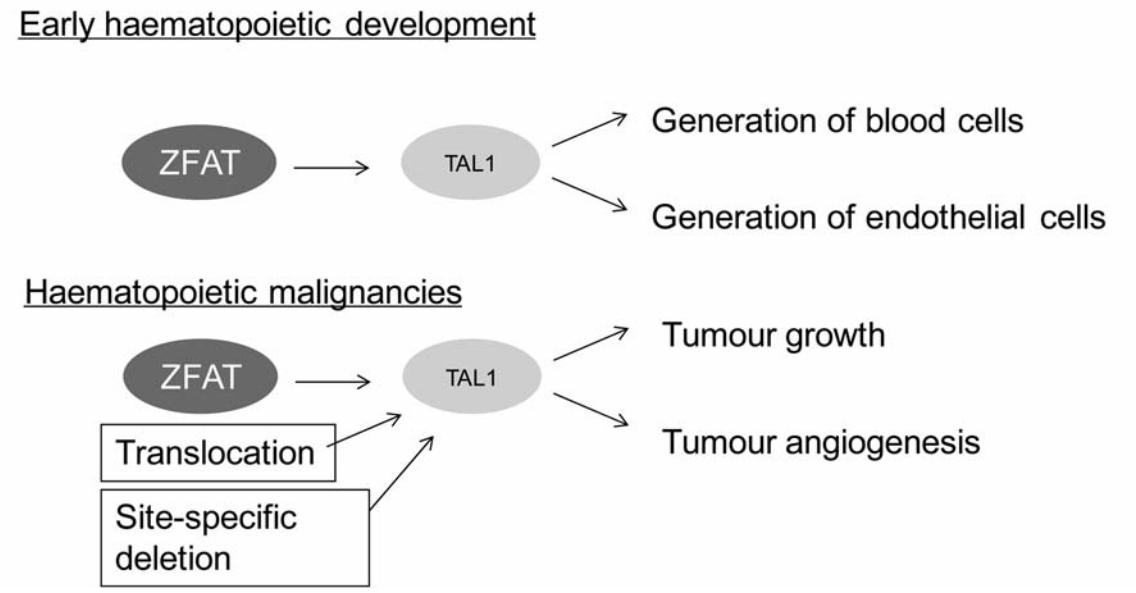

Figure 3. *Differential role of A zinc-finger gene in autoimmune thyroid disease susceptibility region (ZFAT)/ T cell acute lymphocytic leukemia 1 (TAL1) signalling between early haematopoietic development and haematopoietic malignancies.*

Role of ZFAT in Cancer Development

We previously showed that ZFAT regulates apoptosis in MOLT-4 human T-cell acute lymphocytic leukaemia (T-ALL) cells (8) and recently found ZFAT expression in other types of haematopoietic cancer cell lines (data not shown). Another recent study also demonstrated a correlation between *ZFAT* copy numbers and ovarian cancer (32). These studies indicate critical roles of ZFAT in cancer progression.

For example, the most frequent targets of genetic alterations in human lymphoid leukaemias are transcription factor genes with essential functions in blood cell

development. During early haematopoietic development, TAL1 is required for the generation of all blood cell lineages (17) and haemogenic endothelium (9), but it is not required for the generation and function of haematopoietic stem cells during adult haematopoiesis (43). TAL1 is overexpressed in T-ALL as a result of the t(1;14) translocation or site-specific deletions in approximately one-fourth of childhood T-ALL cases (44). However, these two mechanisms cannot account for all instances of *TAL1* overexpression in this disease, indicating that other mechanisms, such as *ZFAT* overexpression, are involved in these processes. The constitutive expression of *ZFAT* is suggested to induce subsequent tumour growth and angiogenesis through TAL1 activation (Figure 3). To confirm this hypothesis, we will investigate expression levels of ZFAT and TAL1 in different kinds of haematopoietic cancer cell lines.

Conclusion

In summary, ZFAT is an essential signalling molecule in haematopoietic development, angiogenesis and cancer. Deregulation of other ZFAT targets, such as LMO2 (45) and GATA1 (46) are also suggested to be critical in haematopoietic malignancies, and future studies will elucidate the detailed mechanism for the transcriptional regulation of ZFAT targets.

Conflicts of Interest

The Authors declare no conflict of interests.

References

1 Cleary ML: Oncogenic conversion of transcription factors by chromosomal translocations. Cell *66*: 619-622, 1991.

2 Sakai K, Shirasawa S, Ishikawa N, Ito K, Tamai H, Kuma K, Akamizu T, Tanimura M, Furugaki K, Yamamoto K and Sasazuki T: Identification of susceptibility loci for autoimmune thyroid disease to 5q31-q33 and Hashimoto's thyroiditis to 8q23-q24 by multipoint affected sib-pair linkage analysis in Japanese. Hum Mol Genet *10*: 1379-1386, 2001.

3 Shirasawa S, Harada H, Furugaki K, Akamizu T, Ishikawa N, Ito K, Ito K, Tamai H, Kuma K, Kubota S, Hiratani H, Tsuchiya T, Baba I, Ishikawa M, Tanaka M, Sakai K, Aoki M, Yamamoto K and Sasazuki T: SNPs in the promoter of a B-cell-specific antisense transcript, SAS-ZFAT, determine susceptibility to autoimmune thyroid disease. Hum Mol Genet *13*: 2221-2231, 2004.

4 Koyanagi M, Nakabayashi K, Fujimoto T, Gu N, Baba I, Takashima Y, Doi K, Harada H, Kato N, Sasazuki T and Shirasawa S: ZFAT expression in B-and T- lymphocytes and identification of ZFAT-regulated genes. Genomics *91*: 451-457, 2008.

5 Doi K, Fujimoto T, Okamura T, Ogawa M, Tanaka Y, Mototani Y, Goto M, Ota T, Matsuzaki H, Kuroki M, Tsunoda T, Sasazuki T and Shirasawa S: ZFAT plays critical roles in peripheral T-cell homeostasis and its T-cell receptor-mediated response. Biochem Biophys Res Commun *425*: 107-112, 2012.

6 Tsunoda T, Takashima Y, Tanaka Y, Fujimoto T, Doi K, Hirose Y, Koyanagi M, Yoshida Y, Okamura T, Kuroki M, Sasazuki T and Shirasawa S: Immune-related zinc finger gene ZFAT is an essential transcriptional regulator for hematopoietic differentiation in blood islands. Proc Natl Acad Sci USA *107*: 14199-14204, 2010.

7 Doi K, Fujimoto T, Koyanagi M, Tsunoda T, Tanaka Y, Yoshida Y, Takashima Y, Kuroki M, Sasazuki T and Shirasawa S: ZFAT is a critical molecule for cell survival in mouse embryonic fibroblasts. Cell Mol Biol Lett *16*: 89-100, 2011.

8 Fujimoto T, Doi K, Koyanagi M, Tsunoda T, Takashima Y, Yoshida Y, Sasazuki T and Shirasawa S: ZFAT is an antiapoptotic molecule and critical for cell survival in MOLT-4 cells. FEBS Lett *583*: 568-572, 2009.

9 Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V and Lacaud G: The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. Nature *457*: 892-895, 2009.

10 Glasker S, Li J, Xia JB, Okamoto H, Zeng W, Lonser RR, Zhuang Z, Oldfield EH and Vortmeyer AO: Hemangioblastomas share protein expression with embryonal hemangioblast progenitor cell. Cancer Res *66*: 4167-4172, 2006.

11 Palis J and Yoder MC: Yolk-sac hematopoiesis: The first blood cells of mouse and man. Exp Hematol *29*: 927-936, 2001.

12 Huber TL, Kouskoff V, Fehling HJ, Palis J and Keller G: Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature *432*: 625-630, 2004.

13 Coultas L, Chawengsaksophak K and Rossant J: Endothelial cells and VEGF in vascular development. Nature 438: 937-945, 2005.

14 Oberlin E, Tavian M, Blazsek I and Peault B: Blood-forming potential of vascular endothelium in the human embryo. Development *129*: 4147-4157, 2002.

15 Ferguson JE, 3rd, Kelley RW and Patterson C: Mechanisms of endothelial differentiation in embryonic vasculogenesis. Arterioscler Thromb Vasc Biol *25*: 2246-2254, 2005.

16 Begley CG, Visvader J, Green AR, Aplan PD, Metcalf D, Kirsch IR and Gough NM: Molecular cloning and chromosomal localization of the murine homolog of the human helix–loop–helix gene SCL. Proc Natl Acad Sci USA *88*: 869-873, 1991.

17 Robb L, Lyons I, Li R, Hartley L, Kontgen F, Harvey RP, Metcalf D and Begley CG: Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. Proc Natl Acad Sci USA *92*: 7075-7079, 1995.

18 Shivdasani RA, Mayer EL and Orkin SH: Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. Nature *373*: 432-434, 1995.

19 Visvader JE, Fujiwara Y and Orkin SH: Unsuspected role for the T-cell leukemia protein SCL/TAL1 in vascular development. Genes Dev *12*: 473-479, 1998.

20 Wilson NK, Miranda-Saavedra D, Kinston S, Bonadies N, Foster SD, Calero-Nieto F, Dawson MA, Donaldson IJ, Dumon S, Frampton J, Janky R, Sun XH, Teichmann SA, Bannister AJ and Gottgens B: The transcriptional program controlled by the stem cell leukemia gene *Scl/Tal1* during early embryonic hematopoietic development. Blood *113*: 5456-5465, 2009.

21 Warren AJ, Colledge WH, Carlton MB, Evans MJ, Smith AJ and Rabbitts TH: The oncogenic cysteine-rich LIM domain protein RBTN2 is essential for erythroid development. Cell *78*: 45-57, 1994.

22 Wadman IA, Osada H, Grutz GG, Agulnick AD, Westphal H, Forster A and Rabbitts TH: The LIM-only protein LMO2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and LDB1/NLI proteins. EMBO J *16*: 3145-3157, 1997.
23 Yamada Y, Pannell R, Forster A and Rabbitts TH: The oncogenic LIM-only transcription factor Lmo2 regulates angiogenesis but not vasculogenesis in mice. Proc Natl Acad Sci USA *97*: 320-324, 2000.
24 Patterson LJ, Gering M, Eckfeldt CE, Green AR, Verfaillie CM, Ekker SC and Patient R: The transcription factors scl and lmo2 act together during development of the hemangioblast in zebrafish. Blood *109*: 2389-2398, 2007.
25 Lecuyer E, Lariviere S, Sincennes MC, Haman A, Lahlil R, Todorova M, Tremblay M, Wilkes BC and Hoang T: Protein stability and transcription factor complex assembly determined by the SCL–LMO2 interaction. J Biol Chem *282*: 33649-33658, 2007.
26 Rodriguez P, Bonte E, Krijgsveld J, Kolodziej KE, Guyot B, Heck AJ, Vyas P, de Boer E, Grosveld F and Strouboulis J: GATA1 forms distinct activating and repressive complexes in erythroid cells. EMBO J *24*: 2354-2366, 2005.
27 Yokomizo T, Takahashi S, Mochizuki N, Kuroha T, Ema M, Wakamatsu A, Shimizu R, Ohneda O, Osato M, Okada H, Komori T, Ogawa M, Nishikawa S, Ito Y and Yamamoto M: Characterization of GATA1(+) hemangioblastic cells in the mouse embryo. EMBO J *26*: 184-196, 2007.
28 Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Lee JK, Oh B and Kim HL: A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet *41*: 527-534, 2009.
29 Takeuchi F, Nabika T, Isono M, Katsuya T, Sugiyama T, Yamaguchi S, Kobayashi S, Yamori Y, Ogihara T and Kato N: Evaluation of genetic loci influencing adult height in the Japanese population. J Hum Genet *54*: 749-752, 2009.
30 Makvandi-Nejad S, Hoffman GE, Allen JJ, Chu E, Gu E, Chandler AM, Loredo AI, Bellone RR, Mezey JG, Brooks SA and Sutter NB: Four loci explain 83% of size variation in the horse. PLoS One *7*: e39929, 2012.
31 Signer-Hasler H, Flury C, Haase B, Burger D, Simianer H, Leeb T and Rieder S: A genome-wide association study reveals loci influencing height and other conformation traits in horses. PLoS One *7*: e37282, 2012.
32 Ramakrishna M, Williams LH, Boyle SE, Bearfoot JL, Sridhar A, Speed TP, Gorringe KL and Campbell IG: Identification of candidate growth-promoting genes in ovarian cancer through integrated copy number and expression analysis. PLoS One *5*: e9983, 2010.
33 Slavin TP, Feng T, Schnell A, Zhu X and Elston RC: Two-marker association tests yield new disease associations for coronary artery disease and hypertension. Hum Genet *130*: 725-733, 2011.
34 Comabella M, Craig DW, Morcillo-Suarez C, Rio J, Navarro A, Fernandez M, Martin R and Montalban X: Genome-wide scan of 500,000 single-nucleotide polymorphisms among responders and nonresponders to interferon β therapy in multiple sclerosis. Arch Neurol *66*: 972-978, 2009.
35 Carmeliet P: Mechanisms of angiogenesis and arteriogenesis. Nat Med *6*: 389-395, 2000.
36 Gibbons GH and Dzau VJ: The emerging concept of vascular remodeling. N Engl J Med 330: 1431-1438, 1994.
37 Nassiri F, Cusimano MD, Scheithauer BW, Rotondo F, Fazio A, Yousef GM, Syro LV, Kovacs K and Lloyd RV: Endoglin (CD105): A review of its role in angiogenesis and tumor diagnosis, progression and therapy. Anticancer Res *31*: 2283-2290, 2011.
38 Linkous AG and Yazlovitskaya EM: Novel therapeutic approaches for targeting tumor angiogenesis. Anticancer Res *32*: 1-12, 2012.
39 Carmeliet P and Jain RK: Angiogenesis in cancer and other diseases. Nature *407*: 249-257, 2000.
40 Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE and Weinstein BM: Endothelial tubes assemble from intracellular vacuoles *in vivo*. Nature *442*: 453-456, 2006.
41 Yoshida Y, Tsunoda T, Takashima Y, Fujimoto T, Doi K, Sasazuki T, Kuroki M, Iwasaki A and Shirasawa S: ZFAT is essential for endothelial cell assembly and the branch point formation of capillary-like structures in an angiogenesis model. Cell Mol Biol Lett *15*: 541-550, 2010.
42 Lazrak M, Deleuze V, Noel D, Haouzi D, Chalhoub E, Dohet C, Robbins I and Mathieu D: The bHLH TAL-1/SCL regulates endothelial cell migration and morphogenesis. J Cell Sci *117*: 1161-1171, 2004.
43 Mikkola HK, Klintman J, Yang H, Hock H, Schlaeger TM, Fujiwara Y and Orkin SH: Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia *SCL/TAL1* gene. Nature *421*: 547-551, 2003.
44 O'Neil J and Look AT: Mechanisms of transcription factor deregulation in lymphoid cell transformation. Oncogene *26*: 6838-6849, 2007.
45 Oram SH, Thoms JA, Pridans C, Janes ME, Kinston SJ, Anand S, Landry JR, Lock RB, Jayaraman PS, Huntly BJ, Pimanda JE and Gottgens B: A previously unrecognized promoter of LMO2 forms part of a transcriptional regulatory circuit mediating LMO2 expression in a subset of T-acute lymphoblastic leukaemia patients. Oncogene *29*: 5796-5808, 2010.
46 Shimizu R, Kuroha T, Ohneda O, Pan X, Ohneda K, Takahashi S, Philipsen S and Yamamoto M: Leukemogenesis caused by incapacitated GATA1 function. Mol Cell Biol *24*: 10814-10825, 2004.

Received April 4, 2013
Revised June 3, 2013
Accepted June 4, 2013