Appropriate vessel development and its coordinated function is essential for proper embryogenesis and homeostasis in the adult. Defects in vessels cause birth defects and are an important etiology of diseases such as cardiovascular disease, tumor and diabetes retinopathy. The accumulative data indicate that ETV2, an ETS transcription factor, performs a potent and indispensable function in mediating vessel development. This review discusses the recent progress of the study of ETV2 with special focus on its regulatory mechanisms and cell fate determining role in developing mouse embryos as well as somatic cells.

INTRODUCTION

In consonant with a close proximity in anatomy, coordinated development of the circulatory system including vessels, blood and the heart is prerequisite for securing successful embryogenesis. In developing embryos, the growth of the circulatory system is identifiable first and abnormalities in the establishment of the system frequently cause embryonic lethality. As early as embryonic day (E) 7.5 in mice, the first structure with the signature of endothelial and hematopoietic cells is the blood islands of the extraembryonic yolk sac (Haar and Ackerman, 1971). Shortly after, the blood islands, which have erythrocytes inside the lumen circled by a layer of endothelial cells, fuse together to create primitive forms of vessels (i.e., primary plexus), which then undergo a remodeling process, generating the complex vascular network interwoven by small capillary vessels and large vessels. On the other hand, angioblasts (also known as endothelial precursor cells) initiate the formation of the vascular structures in the embryonic proper such as dorsal aorta, cardinal veins, vitelline vessels (Drake and Fleming, 2000; Flamme et al., 1997; Patan, 2004). The more elaborated vasculatures are further completed through vascular reshaping, recruitment of perivascular cells and deposition of the extracellular matrix (Carmeliet and Jain, 2011; Jain, 2003). While the blood cells that first appear in the yolk sacs are mainly erythrocytes and macrophages in the blood islands (Choi, 2002; Païs et al., 1999), hematopoietic stem cells, which can supply all types of blood cells throughout the adult life, are detected in the specialized region of the dorsal aorta (Bertrand et al., 2010; Boisset et al., 2010; Kissa and Herbomel, 2010; Zovein et al., 2008) and sequentially populate the fetal liver, spleen and bone marrow.

Transcriptional factors have been implicated in a myriad of biological processes including embryogenesis, tumor and cell proliferation. Among are the E26 transforming sequence or E-twenty-six specific sequence (ETS) transcription factors, which are categorized by the presence of the ETS DNA binding domain (Fig. 1A) (Hollerhorst et al., 2004). Extensive studies have revealed important functions of the ETS factors in endothelial and hematopoietic cell development (Bartel et al., 2000; Ciau-Uitz et al., 2013; Dejana et al., 2007; Findlay et al., 2013; Randi et al., 2009). For example, compound knockout of $Ets1$ and $Ets2$ show abnormal endothelial cell branching (Wei et al., 2009). While Fli1 null mouse embryos develop vascular leakage due to enhanced endothelial cell death (Hart et al., 2000; Spyropoulos et al., 2000), the lack of $Te1$ in mice leads to defective vascular remodeling in the yolk sac and is accompanied by considerable apoptosis (Wang et al., 1997). However, emergence of the vascular structures is not blocked by the absence of these ETS factors and the inactivation of $Ets1$ does not cause vascular defects (Barton et al., 1998). This fact suggests the redundant functions of the ETS factors for at least some members in vessel development (Craig et al., 2015; Pham et al., 2007; Wei et al., 2009). In contrast, recent studies have discovered the non-redundant and indispensable role of one of the ETS factors, ETV2 in vessel as well as blood cell development (Ferdous et al., 2009; Kataoka et al., 2011; Lee et al., 2008). In this review, we will discuss the functional significance of ETV2 in embryonic vessel development, postnatal angiogenesis and direct cell reprogramming.

ETV2 IS ESSENTIAL FOR VASCULAR ENDOTHELIAL AND HEMATOPOIETIC CELL DEVELOPMENT

ETV2 has drawn a great deal of attention as an important regulator for embryonic vessel and blood cell development. Structurally, ETV2 shares a conserved ETS DNA binding domain with other ETS factors but does not exhibit any similarities outside...
ETV2 and Vascular Endothelial Cells
Se-Yeong Oh et al.

Fig. 1. Regulation of the expression and function of ETV2. (A) A schematic structural diagram of the complex of the ETS domain of PU.1 in gold and DNA in purple (deposited on The RCSB PDB www.rcsb.org; DOI: 10.2210/pdb1pue/pdb) (Berman et al., 2000; Kodandapani et al., 1996). (B) In early embryos or differentiating mouse ES cells, BMP/NOTCH/WNT pathways act upstream of ETV2 expression. During this process, transcriptional activation of ETV2 is induced by at least MESP1, CREB and FOXC2. let7a functions to inhibit ETV2 protein synthesis. It is of note that the relationship between BMP/NOTCH/WNT pathways to MESP, CREB and FOXC2 is not known. Also, whether the three transcription factors interact each other in regulating ETV2 gene expression remain elucidated. (C) ETV2 can bind and activate promoters/enhancers of genes critical for endothelial and hematopoietic cell development. OVOL2, FOXC2, GATA2 are reported to interact with ETV2 in mediating these regulation. Whether the three transcription factors can form a transcriptionally active complex remains determined.

MOLECULAR MECHANISMS OF ETV2 IN REGULATING CARDIOVASCULAR DEVELOPMENT

Figures 1B and 1C summarize the findings regarding the molecular mechanisms of ETV2. The very first clue as to how ETV2 regulates cardiovascular cell lineage development was reported by our group and showed that ETV2 directly binds to the ETS consensus sequence (GGAAT) (Hollenhorst et al., 2004; 2011) in the promoter of the Flk1 gene, leading to the induction of the gene expression (Lee et al., 2008). In this study, it was also shown that overexpression of ETV2 can generate Flk1+ mesoderm as well as endothelial and hematopoietic cells in differentiating ESCs in a serum-free condition. In subsequent studies, Sox7, Lmo2, Tie2, Nfac1, were identified as direct targets of ETV2 (Behrens et al., 2014; Koyano-Nakagawa et al., 2012; Lee et al., 2011; Palencia-Desai et al., 2011). Rather than performing Chromatin Immunoprecipitation (ChIP) assay on the targeted ones, a recent study (Liu et al., 2015) performed a ChIP-sequencing analysis to reveal the direct downstream target genes of ETV2 at a genome wide analysis level and found that ETV2 can not only bind to promoters or enhancers of already known target genes including Flk1 and Cdhn5 but also other genes that perform critical roles in vascular endothelial and hematopoietic cells. Among these genes are Scl, Gata2, Meis1, Dll4, Notch1, Nrp1/2, Flk4, Flt1, Rhox, Mapk. Thus, these results strongly indicate that ETV2 regulates the endothelial and hematopoietic programs in early stage embryos through direct binding to the ETS elements present in the
aforementioned genes (Fig. 1C).

The ETS factors have been shown to interact with other proteins when regulating target genes (Dejana et al., 2007; Sharrocks, 2001; Verger and Duterque-Coquillaud, 2002). Furthermore, several studies showed that ETV2 can form a transcription complex with other proteins (Fig. 1C). In 2008, De Val et al. reported that the interaction of ETV2 and FOXC2 (forkhead transcription factor) synergistically induces the expression of endothelial and hematopoietic genes (De Val et al., 2008). Recently, we demonstrated that OVOL2, a zinc finger transcription factor, directly binds to ETV2 to cooperatively generate FLK1+ mesoderm and vascular endothelial and hematopoietic cell lineages from mouse ESCs (Kim et al., 2014). Interestingly, stability of ETV2 was significantly enhanced upon the overexpression of OVOL2, suggesting a possible mechanism for the cooperative interaction of the two proteins. Additionally, Shi et al. (2014) reported Gata2 as the interacting protein of ETV2. This interaction was cooperative in activating important genes for vascular endothelial and blood cell development. It is of note that all the identified proteins of ETV2 interacting partners have been implicated in embryonic vessel and blood cell development (Kume et al., 2001; Lucus et al., 2007; Seo et al., 2006; Tsai and Orkin, 1997; Tsai et al., 1994; Unezaki et al., 2007). All in all, it is evident that ETV2 can form a multiprotein transcription complex to control the expression of target genes. Thus, revealing more ETV2 interacting proteins in the regulation of endothelial and hematopoietic genes would be an important next step.

One of the major outstanding questions would be the mechanisms, which regulates the expression of ETV2 (Fig. 1B). In the first report conducted by our group, treatment of inhibitors of mechanisms, which regulates the expression of ETV2 (Fig. 1B). In the first report conducted by our group, treatment of inhibitors of pathways in governing ETV2 expression. Furthermore, two groups have demonstrated a positive feedback loop mechanism between ETV2 and other ETS factors, at least Fli1 (Abedin et al., 2014; Liu et al., 2015). In 2014, Abedin et al. (2014), showed using Fli1 knockout mice that while Fli1 messages were significantly decreased in Etv2 knockout mouse embryos, other ETS factors such as Ets1/2, Ets2/3 and Etv6 showed comparable levels of expression. The expression of Fli1 is directly upregulated by ETV2 as well as by Fli1 itself through the ETS binding sites on the Fli1 promoter as demonstrated by the ChIP-PCR and luciferase-based promoter assays. Interestingly, that fact that Fli1 binds to its own promoter was observed at E11.5, which is the time in which the ETV2 expression becomes extinct, but not at E9.5, which is when the ETV2 message is still abundant. Also, the ChIP-PCR analysis revealed in vivo occupancy of Fli1 on the promoters of Tie2 and Cdh5 at E11.5, but not at E9.5 embryos. Consistently, several key endothelial genes such as Tie2 and Cdh5 were not expressed. Therefore, ETV2 showed reduced levels of expression in Fli1 deficient embryos as well as Fli1 knockout primary endothelial cells.

http://molcells.org  Mol. Cells  1031

ETV2 and Vascular Endothelial Cells
Se-Young Oh et al.
These results suggest that the function of FLI1 replaces the function of ETV2 at least partly for endothelial cell survival and vascular maintenance at the midgestation stage. Subsequently, Liu et al. (2015) reported a similar feedback regulation mechanism between ETV2 and FLI1. Performing genome wide analyses with ChiP-sequencing comprehensively revealed direct targets of ETV2, which can be classified into VEGF signaling/endothelial lineage specification genes, NOTCH/MAPK signaling and RHO GTPase. The ETS factors such as Fli1, Ets1/2, Erg and notably Etv2 itself were identified as potential targets of ETV2 in differentiating mESCs. In agreement with the results, overexpression of Etv2 led to the immediate induction of Fli1, suggesting that Fli1 is a direct downstream target of ETV2. Indeed, the ChiP-PCR experiment showed in vivo occupancy of ETV2 on Fli1 genomic DNA. The findings that Fli1 null embryos and mouse ESCs showed comparable levels of expression of Etv2 compared to wild type controls but overexpression of Fli1 in mouse ESCs did not induce Etv2 message further support the argument (Liu et al., 2015). Similar to the findings discussed above (Abedin et al., 2014), key endothelial and hematopoietic genes such as Tie2, Cadh5, Lmo2 and Scl, which are the direct targets of ETV2, can also be directly regulated by Fli1 when ETV2 expression is not detected in differentiating ESCs. Given that ETV2 can activate its own promoter, these results suggest the following model; ETV2 triggers a genetic program for endothelial and hematopoietic lineage development through its transcriptional activation function (i.e. positive autoregulation and transactivation of target genes). Once the endothelial cells and hematopoietic cells are generated when the ETV2 expression is silent, other ETS factors, especially FLI1 induced by ETV2 ensure further establishment and maintenance of the vessel and blood systems. Thus, molecular and biochemical studies that uncover the functional significance of other ETS factors in the context of FLI1 in generating vessel and blood cells would be worthy areas to pursue.

**CARDIOVASCULAR CELL FATE DETERMINING ROLE OF ETV2**

The first emerging FLK1+ mesoderm in developing mouse embryos have the potential to differentiate into vascular endothelial, hematopoietic, muscle cell lineages including cardiomyocyte and smooth muscle cells (Chung et al., 2002; Ema et al., 2003; 2006; Faloon et al., 2000; Mutoike et al., 2003; Yamashita et al., 2001), it is plausible that ETV2 regulates the cell fate of FLK1+ mesoderm as a multipotent progenitor in cardiovascular cell lineages. A series of studies showed that FLK1+ mesoderm can be subdivided into two distinct cell populations; FLK1+PDGFRα+ (platelet-derived growth factor receptor α) with cardiogenic potential and FLK1+PDGFRα- cells with endothelial and hematopoietic potential (Hirata et al., 2007; Liu et al., 2012; Sakurai et al., 2006). However, mechanisms that determine the cell fate of FLK1+ mesoderm into the cell population remain to be elucidated. Given the role of transcription factors in determining cell identity (Frum and Ralston, 2015; Hatakeyama and Kageyama, 2004; Iwafuchi-Doi and Zaret, 2014; Park et al., 2013; Weintraub et al., 1991), it is plausible that ETV2 regulates the multipotency of the FLK1+ mesoderm. Indeed, the lack of Etv2 in mice and mouse ESCs leads to the failure to generate FLK1+PDGFRα+ cells (we refer this hematangiogenic FLK1+ cells) with concomitant augmentation of FLK1+PDGFRα- cells (we refer this cardiogenic FLK1+ cells) (Liu et al., 2012; 2015). Also, reduced expression of genes of vascular endothelial and blood cells and augmentation of cardiac genes were observed in Etv2 knockout embryos (Lee et al., 2008). Inversely, the overexpression of Etv2 in differentiating mouse ESCs leads to a significant increment of hematangiogenic FLK1+ cells at the expense of cardiogenic FLK1+ cells. The capability of ETV2 to induce hematangiogenic potential is further strengthened when GATA2 and SCL are co-expressed (Liu et al., 2013). Additional supporting results in this field have been reported from studies in zebrafish. In 2007, Schoenebeck et al. (2017), showed that the overexpression of etsrp together with scl leads to the expansion of hematopoietic and endothelial cell area with a reduction in the cardiac field as well as heart size. The observed phenotype was reversed upon injection of etsrp and scl morpholino in zebrafish embryos. A similar finding was reported by the knockdown of etsrp alone in zebrafish embryos (Palencia-Desai et al., 2011). In this study, the authors further showed using etsrp-gfp cells that etsrp+ cells are deficient in etsrp fated to cardiogenic cell lineages. These results suggest that ETV2 functions as an essential cell fate determinant between hematangiogenic and cardiogenic mesoderm. Likewise, transcriptional regulation of such antagonistic relationships between hematangiogenic and cardiogenic cell lineage specification is evident in other studies as well. Injection of nkd2-5 into zebrafish embryos reduced the expression of endothelial and blood cell markers such as etsrp, scl and pu.1, but significantly expanded the hand2+ cardiac boundary (Simoes et al., 2011). When Mesp1, another key cardiogenic transcription factor in differentiating mESCs, was overexpressed, the antagonistic developmental outcomes have also been reported (Bondue et al., 2008; Lindsley et al., 2008).

Further insight as to how ETV2 regulates the cell fate of FLK1+ mesoderm was suggested by Liu et al. (2012) as the study reported the first evidence of the role of ETV2 and WNT-β-catenin signaling in this process. The authors found reduced expression of genes involved in WNT-β-CATENINE signaling with decreased cardiomyocyte generation when Etv2 was overexpressed in differentiating ESCs. In sharp contrast, overexpressing β-catenin reversed the ETV2-induced hematangiogenic cell lineage generation. However, the proposed mechanism was not consonant with the knockout mouse study in which deficiency of β-catenin in FLK1+ mesoderm resulted in no obvious phenotypic defects in heart formation (Stenman et al., 2008). Therefore, further investigation to explain the molecular mechanism of ETV2 in determining the cell fate of FLK1+ mesoderm is required.

**DIRECT CELL REPROGRAMMING AND ETV2**

The monumental findings from the studies conducted by Gurdon (2006), Weintraub et al. (1989) and recently the Yamanaka group (Takahashi and Yamanaka, 2006; Takahashi et al., 2007) have challenged the longstanding consensus that the differentiation process is unidirectional and produces a progressive loss of differentiation potential like a ball rolling from the top of a mountain to the ground and thus generates cells with an irreversibly determined fate (Waddington, 1957). Fueled by the Yamanaka’s finding that four pluripotency factors (OCT4, SOX2, KLF4, and C-MYC) can generate embryonic stem like cells, such as induced pluripotent cells (iPSCs) from differentiated somatic cells (Takahashi and Yamanaka, 2006; Takahashi et al., 2007), considerable efforts have been focused on regenerative medicine, which aims to develop the generation of functional cells or even tissues for autologous cell replacement therapies. However, the clinical applicability of the iPSC-based approaches have been significantly limited due to the inefficient generation of targeted cells and tumorigenic potential (Cohen and Melton, 2011; Kneepfier, 2009), necessitating a novel means to overcome these obstacles. In agreement
with the previous studies (Johnson et al., 2008; Kim et al., 2010; Weintraub et al., 1989; Xie et al., 2004), the overexpression of cell type or tissue specific transcription factors is sufficient to directly convert or reprogram somatic cells into targeted cells such as cardiomyocytes, neuron or hepatocytes that bypass the iPSC stages in vivo (Huang et al., 2011; Ieda et al., 2010; Pang et al., 2011; Sekiya and Suzuki, 2011; Song et al., 2012; Vierbuchen et al., 2010).

Considering that cardiovascular diseases (CVDs) are the most serious diseases in both the United States and the world (Mozaffarian et al., 2015), a great number of researchers have been investigating an efficient way to generate autologous functional endothelial cells for cell therapy. Over the past 15 years, interrogating potential of PSCs (i.e., embryonic stem cells and induced pluripotent stem cells) and endothelial progenitor cells that can generate endothelial cells for therapeutic purposes has been an active research area. As discussed, the recent emergence of the direct reprogramming technology has also prompted investigators to seek novel methods to generate functional endothelial cells directly from somatic cells, which can be applied to the treatment of CVD patients. In 2012, Ginsberg et al. showed that ETV2 together with other ETS factors such as FLI1 and ERG can convert human mid gestation c-kit lineage-committed amniotic cells (ACs) into endothelial cells (Ginsberg et al., 2012). Interestingly, in analogy to embryonic vessel development, the function of ETV2 was only required in the beginning phase of the reprogramming process and the onward steps were completed by FLI1 and ERG in conjunction with the suppression of TGF-β signaling. The reprogrammed endothelial cells were able to evidence neovascularization in vivo. However, the combination of factors was only applied to the ACs, and not to adult somatic cells. In a subsequent study by Han et al. (2014), it was shown that the mixture of transcription factors crucial for vessel development (FOXO1, ETV2, KLF2, TAL1, and LMO2) was able to directly reprogram mouse adult skin fibroblast into endothelial cells. In contrast, we and another group reported direct cell reprogramming of human dermal fibroblasts into endothelial cells by overexpressing ETV2 alone (Lee et al., 2014; Morita et al., 2015). Similarly, skeletal muscles can be converted into endothelial cells in zebrafish. Collectively, these results suggest a potent determinant function of ETV2 in converting non-endothelial cells into endothelial and that ETV2 might function as a therapeutic agent for treating CVDs. However, the use of lentiviral or retroviral delivery systems in these studies, which can alter the genomic integrity, is not compatible with clinical use. Therefore, to design novel tools with non-integrating materials such as modified mRNA, small molecules or chemicals is an imperative need in the field of direct reprogramming. Additional efforts should be made to generate specific types of endothelial cells such as arterial, venous and lymphatic ECs.

CONCLUSION

By virtue of extensive studies over the past few years, we have a better understanding on the critical function of ETV2 in the genesis of the vessel, blood and heart in developing mouse embryos. As discussed, one of the salient observations in ETV2 biology is its transient expression in vessel and blood cells (Ferdous et al., 2009; Kataoka et al., 2011; Lee et al., 2008). The intricate interplay between ETV2 and FLI1 is proposed as a means to maintain functional vessels and hematopoietic cells throughout embryogenesis and perhaps in adults (Abdelin et al., 2014; Liu et al., 2015). However, the mechanisms, in which the ETV2 expression is off, remain to be determined. The switch-off of the proposed upstream signals as previously discussed could be one possible explanation. Additional means of regulation would be active ways to restrict the expression of ETV2 in a certain narrow window of time to ensure proper development of vessel and hematopoietic cells. Indeed, let7-a miRNA is capable of targeting zebrafish etsp, resulting in the reduction of expression of both vascular endothelial and hematopoietic markers (Moore et al., 2013). Given the recent report that Kdm1a, histone demethylase, in zebrafish promotes hematopoietic cell development by suppressing etsp function (Takeuchi et al., 2015), epigenetic modifications of the ETV2 genomic loci would be another possible mechanism. In addition, it was reported that the sustained expression of ETV2 in endothelial and hematopoietic cells caused abnormal development and endothelialization, respectively (Hayashi et al., 2012). Thus, future studies on the safe-guard mechanisms of the ETV2 expression are warranted. We and others unequivocally proved the potent vasculogenic function of ETV2 in developing mouse embryogenesis. As previously stated, the message becomes extinct once the vessel and hematopoietic cells develop. This raised a question as to the functional significance of ETV2 in post-natal life. In this regard, Lee et al. (2011) found the enriched expression of ETV2 in BM HSCs and reported that Mxt1-cre driven deletion of Etv2 led to the decrease in the number and repopulating capacity of BM HSCs. The authors claimed the increased death of BM HSC in the absence of Etv2 as an etiology of the observed phenotypes. Mechanistically, they showed that ETV2 can directly regulate Tie2 expression, but failed to link how the reduced Tie2 expression is related to the death of BM HSCs. Recently, we have found that the endothelial ETV2 acts as a critical regulator in neovascularization in response to injury (Park et al., 2015). The Etv2 expression in endothelial cells is reactivated after injury. Mice deficient in Etv2 in endothelial cells exhibited a significant impairment of new vessel formation upon injury such as wounding, eye injury and hindlimb ischemia. Interestingly, single delivery of lentiviral Etv2 not only promotes the recovery of blood perfusion, but also augments proliferation of endothelial cells as well as smooth muscle cells, leading to neovascularization and tissue repair in the injured hindlimbs. These results suggest that ETV2 in adults plays an important role in vessel and blood construction systems under physiological conditions. Extending from these findings (Park et al., 2015), investigation of the function of ETV2 in pathological settings such as tumor angiogenesis and diabetes related vessel defects would be of significant interest from a therapeutic stand point. Last, but not least, ETV2 alone or together with other endothelial transcription factors were reported to directly convert non-endothelial cells into endothelial cells (Ginsberg et al., 2012; Han et al., 2014; Lee et al., 2014; Morita et al., 2015; Veldman et al., 2013). As reviewed above, the identity of the converted endothelial cells is not clear: are they venous, arterial or lymphatic endothelial cells, functionally and (epi) genetically? What are the mechanism of the direct reprogramming by ETV2? Based upon our results as previously discussed, can ETV2 in clinically compatible forms be developed with high efficiency for clinical use in vivo?

In summary, ETV2 is an indispensable transcription factor and plays a crucial role in vessel development and function. Recent findings have revealed additional capabilities of ETV2 in cell reprogramming. Thus, deciphering the mechanisms by which ETV2 is regulated in governing these processes would provide a novel research venue for the basic and translational aspects of endothelial cell biology.

http://molcells.org Mol. Cells 1033
ETV2 and Vascular Endothelial Cells
Se-Yeong Oh et al.

ACKNOWLEDGMENTS
This work was supported by the American Heart Association, 11SDG7390074, National Institutes of Health (NIH) R01 HL119291 and the National Center for Advancing Translational Sciences of the NIH under Award Number UL1TR000454. The content is solely the responsibility of the authors and does not necessarily represents the official views of the NIH.

REFERENCES
Abedin, M.J., Nguyen, A., Jiang, N., Perry, C.E., Shelton, J.M., Watson, D.K., and Ferdous, A. (2014). Flt1 acts downstream of Etv2 to promote cell survival and vascular homeostasis via positive autoregulation. Circ. Res. 114, 1690-1699.
Batel, F.O., Higuchi, T., and Spyropoulos, D.D. (2000). Mouse models in the study of the Ets family of transcription factors. Oncogene 19, 6443-6454.
Barton, K., Muthusamy, N., Fischer, C., Ting, C.N., Walunas, T.L., Lanier, L.L., and Leiden, J.M. (1998). The Ets-1 transcription factor is required for the development of natural killer cells in mice. Immunity 9, 555-563.
Bethrens, A.N., Zierold, C., Shi, X., Ren, Y., Koyano-Nakagawa, N., Garry, D.J., and Martin, C.M. (2014). Sox7 is regulated by ETV2 during cardiovascular development. Stem Cells Dev. 23, 2004-2013.
Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyakov, I.N., and Bourne, P.E. (2000). The Protein Data Bank. Nucleic Acids Res. 28, 235-242.
Bertrand, J.Y., Chi, N.C., Santosco, B., Teng, S., Stainer, D.Y., and Traver, D. (2010). Haematopoietic stem cells derive directly from aortic endothelium during development. Nature 464, 108-111.
Boisset, J.C., van Cappellen, W., Andrieu-Soler, C., Galjart, N., Behrens, A.N., Zierold, C., Shi, X., Ren, Y. Koyano-Nakagawa, N., Bartel, F.O., Higuchi, T., and Spyropoulos, D.D. (2000). Mouse gene (Etsrp71) and initial characterization of its promoter. Nucleic Acids Res. 28, 1653-1664.
Brown, T.A., and McKnight, S.L. (1992). Specificities of protein-protein and protein-DNA interaction of GABP alpha and two newly defined ets-related proteins. Genes Dev. 6, 2502-2512.
Caprioli, A., Koyano-Nakagawa, N., Iacovino, M., Shi, X., Ferdous, A., Harvey, R.P., Olson, E.N., Kyba, M., and Garry, D.J. (2011). Nkx2-5 represses Gata1 gene expression and modulates the cellular fate of cardiac progenitors during embryogenesis. Circulation 124, 1116-1120.
Bondue, A., Lapouge, G., Paulissen, C., Semeraro, C., Iacovino, M., Kyba, M., and Garry, D.J. (2011). Nkx2-5 acts as a master regulator of multipotent cardiovascular progenitor specification. Cell Stem Cell 3, 69-84.
Choi, K. (2002). The hemangioblast: a common progenitor of hematopoietic and endothelial cells. J. Hematother. Stem Cell Res. 11, 179-195.
Chung, Y.S., Zhang, W.J., Arentson, E., Kingsley, P.D., Palis, J., and Cohen, D.E. (2011). Turning straw into gold: Fli1 acts downstream of Etv2 on vascular and hematopoietic development in the mouse. Genes Dev. 17, 380-393.
De Val, S., Chi, N.C., Meadows, S.M., Minovitsky, S., Anderson, J.P., Harris, I.S., Ehlers, M.L., Agarwal, P., Visel, A., Xu, S.M., et al. (2008). Combinatorial regulation of endothelial cell gene expression by etv1 and forkhead transcription factors. Cell 135, 1053-1064.
Dejana, E., Taddei, A., and Randi, A.M. (2007). Foxes and Ets in the transcriptional regulation of endothelial cell differentiation and angiogenesis. Biochim. Biophys. ACTA 1775, 298-312.
Drake, C.J., and Fleming, P.A. (2000). Vasculogenesis in the day 6.5 to 9.5 mouse embryo. Blood 95, 1671-1679.
Ema, M., Falocon, P., Zhang, W.J., Hirashima, M., Reid, T., Stanford, W.L., Orkin, S., Choi, K., and Rossant, J. (2003). Combinatorial effects of Fik1 and Tal1 on vascular and hematopoietic development in the mouse. Genes Dev. 17, 380-393.
Ema, M., Takahashi, S., and Rossant, J. (2006). Deletion of the selection cassette, but not cis-acting elements, in targeted Fik1-Iac allele reveals Fk1 expression in multipotent mesodermal progenitors. Blood 107, 111-117.
Falocon, P., Arentson, E., Kazarov, A., Deng, C.X., Porcher, C., Orkin, S., and Choi, K. (2000). Basic fibroblast growth factor positively regulates hematopoietic development. Development 127, 1931-1939.
Ferdous, A., Caprioli, A., Iacovino, M., Martin, C.M., Morris, J., Richardson, J.A., Latif, S., Hammer, R.E., Harvey, R.P., Olson, E.N., et al. (2009). Nkx2-5 transactivates the Ets-related protein 71 gene and specifies an endothelial/endoocardial fate in the developing embryo. Proc. Natl. Acad. Sci. USA 106, 814-819.
Findlay, V.J., LaRue, A.C., Turner, D.P., Watson, P.M., and Watson, D.K. (2013). Understanding the role of ETS-mediated gene regulation in complex biological processes. Adv. Cancer Res. 119, 1-61.
Flamme, I., Frolich, T., and Risau, W. (1997). Molecular mechanisms of vasculogenesis and embryonic angiogenesis. J. Cell Physiol. 173, 206-210.
Frun, T., andRalston, A. (2015). Cell signaling and transcription factors regulating cell fate during formation of the mouse yolk sac. Trends Genet. 31, 402-410.
Ginsberg, M., James, D., Ding, B.S., Nolan, D., Geng, F., Butler, J.M., Schachtler, W., Pulliaj, V.R., Mathew, S., Chasen, S.T., et al. (2012). Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGFbeta suppression. Cell 151, 559-575.
Gurdon, J.B. (2006). From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation. Ann. Rev. Cell Dev. Biol. 22, 1-22.
Haas, J.L., and Ackerman, G.A. (1971). A phase and electron microscopic study of vasculogenesis and erythropoiesis in the yolk sac of the mouse. The Anatomical Record 170, 199-223.
Han, J.K., Chang, S.H., Cho, H.J., Choi, S.B., Ahn, H.S., Lee, J., Jeong, H., Youn, S.W., Lee, H.J., Kwon, Y.W., et al. (2014). Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. Circulation 130, 1168-1178.
Hatakeyama, J., and Kageyama, R. (2004). Retinal cell fate determination and bHLH factors. Semin. Cell Dev. Biol. 15, 83-89.
Hayashi, M., Puchinotta, M., Momiyama, A., Tanaka, Y., Nishikawa, S., and Kataoka, H. (2012). Endothelialization and altered endotheliosies by persistent Etv2 expression in mice. Exp. Hematol. 40, 738-750 e711.
Hirata, H., Kawamata, S., Murakami, Y., Inoue, K., Nagahashi, A., Tosaka, M., Yoshishita, I., Miyamoto, Y., Iwana, H., Ashahara, T., et al. (2007). Coexpression of platelet-derived growth factor receptor alpha and fetal liver kinase 1 enhances cardiogenic potential in embryonic stem cell differentiation in vitro. J. Biosci. Bioeng. 103, 412-419.
Hollenstein, P.C., Jones, D.A., and Graves, B.J. (2004). Expression profiles frame the promoter specificity dilemma of the ETS family of transcription factors. Nucleic Acids Res. 32, 5693-5702.
Hollenstein, P.C., McIntosh, L.P., and Graves, B.J. (2011). Genomic and biochemical insights into the specificity of ETS transcription factors. Ann. Rev. Biochem. 80, 437-471.
Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X., and Hui, L. (2011). Induction of functional hepatocyte-like cells
from mouse fibroblasts by defined factors. Nature 475, 386-389.
Ieda, M., Kataoka, H., Hayashi, M., Nakagawa, R., Tanaka, Y., Izumi, N., Nishikawa, S., Jakt, M.L., Tarui, H., and Nishikawa, S. (2011). ETV2/ETSrp/Etv2 function. Development 138, 6975-6986.
Kim, H., Nguyen, V.P., Petrova, T.V., Cruz, M., Altalb, K., and Dumont, D.J. (2010). Embryonic vascular endothelial cells are malatable to reprogramming via Prox1 to a lymphatic gene signature. Dev. Biol. 344, 156-166.
Kim, J.Y., Lee, R.H., Kim, T.M., Kim, D.W., Jeon, Y.J., Huh, S.H., Oh, S.Y., Kyba, M., Kataoka, H., Choi, K., et al. (2014). OVOL2 is a critical regulator of ER71/ETV2 in generating Flk1+, hematopoietic, and endothelial cells from embryonic stem cells. Cell Stem Cell 5, 293-306.
Kissa, K., and Herbomel, P. (2010). Blood stem cells emerge from aortic endothelium by a novel type of cell transition. Nature 464, 112-115.
Knoepfler, P.S. (2009). Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. Stem Cells 27, 1050-1056.
Kodandapani, R., Pio, F., Ni, C.Z., Piccialli, G., Klersm, M., McKercher, S., Maki, R.A., Ely, K.R. (1996). A new pattern for helix-turn-helix recognition revealed by the PU.1 ETS-domain-DNA complex. Blu. Dev. 112, 456-460.
Koyano-Nakagawa, N., Kwoen, J., Iacovino, M., Shi, X., Rasmussen, T.L., Borges, L., Zirbes, K.M., Li, T., Perlino, R.C., Kyba, M., et al. (2012). Etv2 is expressed in the yolk sac hematopoietic and endothelial progenitors and regulates Lmo2 gene expression. Stem Cells 30, 1611-1623.
Kume, T., Jiang, H., Topczewska, J.M., and Hogan, B.L. (2001). The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and arterial specification. Dev. Cell 1, 375-386.
Lee, D., Kim, T.M., and Malik, A.B. (2013). Transcriptional functions at multiple steps in hemangioblast development and differentiation. Development 140, 553-564.
Lee, S., Park, C., Han, J.W., Kim, J.Y., Cho, K., Kim, E.J., Kim, S., Lee, S.-J., An, H.J., Sin, M.Y., et al. (2014). Abstract 18205. Direct Reprogramming of Human Dermal Fibroblasts into Endothelial Cells Using a Single Transcription Factor. Circulation 130, A18205.
Lindsley, R.C., Gill, J.G., Murphy, T.L., Langer, E.M., Cai, M., Mashayekhi, M., Wang, W., Niwa, N., Nuberolle, J.M., Kyba, M., et al. (2005). ER71/Mob1 promotes cardiovascular development by regulating cardiovascular fate restriction and epithelial-mesenchymal transition in differentiating ESCs. Cell Stem Cell 3, 55-68.
Liu, F., Kang, I., Park, C., Chang, L.W., Wang, W., Lee, D., Lim, D.S., van der Neerbonne, J.M., and Cho, K. (2012). ER71 specifies Flik+ hematopoietic progenitor by inhibiting cardiac medus and Wnt signaling. Blood 119, 3295-3305.
Liu, F., Bhang, S.H., Arentson, E., Sawada, A., Kim, C.K., Kang, I., Yu, J., Sakurai, N., Kim, S.H., Yoo, J.J., et al. (2013). Enhanced hematopoietic and vascular development and regeneration from embryonic stem cells by defined transcription factors. Stem Cell Rep. 1, 166-182.
Liu, F., Li, D., Yu, Y.Y., Kang, I., Cha, M.J., Kim, J.Y., Park, C., Watson, D.K., Wang, T., and Choi, K. (2015). Induction of hematopoietic and endothelial cell program orchestrated by ETS transcription factor ER71/ETV2. EMBO Rep. 16, 654-669.
Lugus, J.J., Chung, Y.S., Mills, J.C., Kim, S.J., Grass, J., Kyba, M., Doherty, J.M., Bresnick, E.H., and Choi, K. (2007). GATA2 functions at multiple steps in hemangioblast development and differentiation. Development 134, 393-405.
Lyons, I., Parsons, L.M., Hartley, L., Li, R., Andrews, J.E., Robb, L., and Harvey, R.P. (1995). Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5. Genes Dev. 9, 1654-1666.
Meadows, S.M., Myers, C.T., and Krieg, P.A. (2011). Regulation of endothelial cell development by ETS transcription factors. Semin. Cell Dev. Biol. 22, 976-984.
Moore, J.C., Sheppard-Tindell, S., Shestopalov, I.A., Yamasoe, S., Chen, J.K., and Lawson, N.D. (2013). Post-transcriptional mechanisms contribute to Etv2 repression during vascular development. Dev. Biol. 384, 128-140.
Monta, R., Suzuki, M., Kasahara, H., Shimizu, N., Shichita, T., Sekiya, T., Kimura, A., Sasaki, K., Yasukawa, H., and Yoshimura, A. (2015). ETS transcription factor ETv2 directly converts human fibroblasts into functional endothelial cells. Proc. Natl. Acad. Sci. USA 112, 160-165.
Motoike, T., Markham, D.W., Rossant, J., and Sato, T.N. (2003). Evidence for novel fate of Flk1+ progenitor: contribution to muscle lineage. Genesis 35, 153-159.
Mozaffarian, D., Benjamion, E.J., Go, A.S., Arnett, D.K., Blaha, M.J., Cushman, M., de Ferranti, S., Despres, J.P., Fullerton, H.J., Howard, V.J., et al. (2015). Heart disease and stroke statistics—2015 update: a report from the American Heart Association. Circulation 131, e29-322.
Neuhau, H., Muller, F., and Hollemann, T. (2010). Xenopus er71 is involved in vascular development. Dev. Dyn. 239, 3436-3445.
Palencia-Dessai, S., Kohli, V., Kang, J., Chi, N.C., Black, B.L., and Summanus, S. (2011). Vascular endothelial and endocardial progenitors differentiate as cardicocytes in the absence of Etsrp/ETv2 function. Development 138, 4721-4732.
Palis, J., Robertson, S., Kennedy, M., Wall, C., and Keller, G. (1999). Development of erythroid and myeloid progenitors in the yolk sac embryo proper of the mouse. Development 126, 5073-5084.
Pang, Z.P., Yang, N., Vierbuchen, T., Ostermeier, A., Fuentes, D.R., Yang, T.Q., Citri, A., Sebastiano, V., Marro, S., Sudhof, T.C., et al. (2011). Induction of human neuronal cells by defined transcription factors. Nature 476, 220-223.
Park, C., Kim, T.M., and Malik, A.B. (2013). Transcriptional regulation of endothelial cell and vascular development. Circ. Res. 112, 1380-1400.
Park, C., Lee, T.J., Bhang, S.H., Liu, F., Nakamura, R., Olatuppu, S.P., Pitha-Rowe, I., Shi, X., Ferdous, A., Choi, H.S., Kim, T.M., et al. (2015). Injury-Mediated Vascular Regeneration Requires Endothelial ER71/ETV2. Arteriosclerosis, thrombosis, and vascular biology. Nov 19. pii: ATVBAHA.115.306430. [Epub ahead of print].
Patan, S. (2014). Vasculogenesis and angiogenesis. Cancer Treat. Rev. 117, 3-32.
Pham, V.N., Lawson, N.D., Mugford, J.W., Dye, L., Castranova, D., Lo, B., and Weinstein, B.M. (2007). Combinatorial function of ETS transcription factors in the developing vasculature. Dev. Biol. 303, 772-783.
Randi, A.M., Sperone, A., Dryden, N.H., and Birdseye, G.M. (2009). Regulation of angiogenesis by ETS transcription factors. Biochim. Soc. Trans. 37, 1248-1253.
Rasmussen, T.L., Kweon, J., Diekmann, M.A., Belema-Bedada, F., Song, O., Bowlin, K., Shi, X., Ferdous, A., Li, T., Kyba, M., et al. (2011). ER71 directs mesodermal fate decisions during embryonic development. Development 138, 4801-4812.
Sakurai, H., Era, T., Jakt, L.M., Okada, M., Nakai, S., Nishikawa, S., and Nishikawa, S. (2006). In vitro modeling of paraxial and lateral mesoderm differentiation reveals early reversibility. Stem Cells 24, 575-586.
Schoenebeck, J.J., Keegan, B.R., and Yelon, D. (2007). Vessel and blood specification override cardiac potential in anterior Nkx2-5 null mice. Dev. Cell 13, 254-267.
Sekiya, S., and Suzuki, A. (2011). Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature 475, 390-393.
Seo, S., Fujita, H., Nakano, A., Kang, M., Duarte, A., and Kume, T. (2006). The forkhead transcription factors Foxc1 and Foxc2 are required for arterial specification and lymphatic sprouting during vascular development. Dev. Biol. 294, 458-470.
Sharrocks, A.D. (2001). The ETS-domain transcription factor family. Nat. Rev. Mol. Cell. Biol. 2, 827-837.

Shi, X., Richard, J., Zibres, K.M., Gong, W., Lin, G., Kyba, M., Thompson, J.A., Koyano-Nakagawa, N., and Garry, D.J. (2014). Cooperative interaction of Etv2 and Gata2 regulates the development of endothelial and haemangioblast lineages. Dev. Biol. 389, 208-218.

Shi, X., Zibres, K.M., Rasmussen, T.L., Ferdous, A., Garry, M.G., Koyano-Nakagawa, N., and Garry, D.J. (2015). The transcription factor Mesp1 interacts with cAMP-responsive element binding protein 1 (Cebpa) and coactivates Ets variant 2 (Etv2) gene expression. J. Biol. Chem. 290, 9614-9622.

Simoes, F.C., Peterkin, T., and Patient, R. (2011). Fgf differentially controls cross-antagonism between cardiac and haemangioblast regulators. Development 138, 3235-3245.

Sot, F.K., Nakamura, T., Qiu, X., Qi, X., Tan, W., Huang, G.N., Achiya, A., Smith, C.L., Tallquist, M.D., Neilson, E.G., et al. (2012). Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature 485, 599-604.

Spyropoulos, D.D., Pharr, P.N., Lavenburg, K.R., Jackers, P., Papas, T.S., Ogawa, M., and Watson, D.K. (2000). Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the Flt1 transcription factor. Mol. Cell. Biol. 20, 5643-5652.

Stainier, D.Y., Weinstein, B.M., Detrich, H.W., 3rd, Zon, L.I., and Stennert, D.Y. (1999). The cardiac homeobox gene Csx/Nkx2.5 lies genetically upstream of multiple genes essential for heart development. Genes Dev. 13, 1269-1280.

Takayama, N., Eto, K., Nishikawa, S., and Yamashita, J.K. (2012). Etsrp/Etv2 is directly regulated by PKA/CREB signaling triggers initiation of endothelial and hematopoietic cell differentiation. Blood 89, 3636-3643.

Tsai, F.Y., Keller, G., Kuo, F.C., Weiss, M., Chen, J., Rosenblatt, M., Alt, F.W., and Orkin, S.H. (1994). An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature 371, 221-226.

Umezaki, S., Horai, R., Sudo, K., Iwakura, Y., and Ito, S. (2007). Ox2/Mvo, a homologue of Drosophila ov, is required for angiogenesis, heart formation and placental development in mice. Genes Cells 12, 773-785.

Veldman, M.B., and Lin, S. (2012). Etsrp/Etv2 is directly regulated by Foxc1/1b in the zebrafish angioblast. Circ. Res. 110, 220-229.

Veldman, M.B., Zhao, C., Gomez, G.A., Lindgren, A.G., Huang, H., Yang, H., Yao, S., Martin, B.L., Kimmelman, D., and Lin, S. (2013). Transdifferentiation of fast skeletal muscle into functional endothelium in vivo by transcription factor Etv2. PLoS Biol. 11, e1001590.

Verger, A., and Duterque-Couillaud, M. (2002). When Ets transcription factors meet their partners. Bioessays 24, 362-370.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kobuku, Y., Sudhof, T.C., and Nernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463, 1035-1041.

Waddington, C.H. (1957). The strategy of the genes; a discussion of some aspects of theoretical biology. (London: Allen & Unwin).

Wei, G., Srivivasan, R., Cantermire-Stone, C.Z., Sharma, S.M., Santhanam, R., Weinstein, M., Muthusamy, N., Man, A.K., Oshima, R.G., Leone, G., et al. (2009). Ets1 and Ets2 are required for endothelial cell survival during embryonic angiogenesis. Blood 114, 1123-1130.

Weintrab, H., Tapscott, S.J., Davis, R.L., Thayer, M.J., Adam, M.A., Lassar, A.B., and Miller, A.D. (1989). Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. Proc. Natl. Acad. Sci. USA 86, 5434-5438.

Wei, G., Srivivasan, R., Cantermire-Stone, C.Z., Sharma, S.M., Santhanam, R., Weinstein, M., Muthusamy, N., Man, A.K., Oshima, R.G., Leone, G., et al. (2009). Ets1 and Ets2 are required for endothelial cell survival during embryonic angiogenesis. Blood 114, 1123-1130.

Weintrab, H., Davis, R., Tapscott, S., Thayer, M.J., Adam, M.A., Lassar, A.B., and Miller, A.D. (1989). Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. Proc. Natl. Acad. Sci. USA 86, 5434-5438.

Weintrab, H., Davis, R., Tapscott, S., Thayer, M.J., Krause, M., Benezra, R., Blackwell, T.K., Turner, D., Rupp, R., Hollenberg, S., et al. (1991). The myoD gene family: nodal point during specification of the muscle cell lineage. Science 251, 761-766.

Xie, H., Ye, M., Feng, R., and Graf, T. (2004). Stepwise reprogramming of B cells into macrophages. Cell 117, 663-676.

Yamamizu, K., Matsunaga, T., Katayama, S., Kataoka, H., Takayama, N., Els, K., Nishikawa, S., and Yamashita, J.K. (2012). PKA/CREB signaling triggers initiation of endothelial and hematopoietic cell differentiation via Etv2 induction. Stem Cells 30, 687-696.

Yamashita, J., Itoh, H., Hirashima, M., Ogawa, M., Nishikawa, S., Yurugi, T., Naito, M., Nakao, K., and Nishikawa, S. (2000). Fkh-like positive cells derived from embryonic stem cells serve as vascular progenitors. Nature 408, 92-96.

Zovein, A.C., Hofmann, J.J., Lynch, M., French, W.J., Turjo, K.A., Yang, Y., Becker, M.S., Zanetta, L., Dejana, E., Gasson, J.C., et al. (2008). Fate tracing reveals the endothelial origin of hematopoietic stem cells. Cell Stem Cell 3, 625-636.